M

Mad Cow Disease *See* Bovine Spongiform Encephalopathy (Mad Cow Disease).

Magnesium

Russell Barbare

- Representative Chemicals: Magnesium sulfate (Epsom salts); Magnesium hydroxide (in suspension: milk of magnesia); Magnesium citrate
- Chemical Abstracts Service Registry Number: CAS 7439-95-4
- Chemical/Pharmaceutical/Other Class: Alkaline earth metal
- Chemical Formula: Mg^{2+}

Uses

The elemental form of magnesium is used in light metal alloys, some aspects of metallurgy, and in the production of precision instruments and flares. Many foods contain magnesium and vitamins are often supplemented with it. Magnesium sulfate may be used topically as a soak, internally as a laxative, or intravenously during pregnancy to control eclamptic seizures and uterine activity. Many antacids contain magnesium oxide or trisilicate as active ingredients.

Background Information

Magnesium is the most abundant divalent cation in cells, where it is essential for a wide range of cellular functions. Magnesium is the sixth most abundant metal on earth and dissolved magnesium constitutes 0.13% of seawater. It is found naturally only in the form of its salts. First obtained in metallic form in 1808, it is an essential nutrient necessary for human, animal, and plant health as it is an important component of red blood cells, a cofactor in over 300 cellular processes, and central to the chlorophyll molecule. The physiological role of magnesium was essentially ignored until recently. With the development of new technologies to measure the intracellular free concentration of magnesium ($[Mg^{2+}]_i$), the biologically important fraction, there has been a large increase of interest in the molecular, biochemical, physiological, and pharmacological functions of magnesium. Moreover, improved methods for assessing magnesium status in the clinic have contributed to the further understanding of magnesium regulation in health and disease. Magnesium deficiency is now considered to contribute to many diseases and the role for magnesium as a therapeutic agent is being tested in numerous large clinical trials. Specific clinical conditions in which magnesium deficiency has been implicated to play a pathophysiological role include hypertension, ischemic heart disease, arrhythmias, preeclampsia, asthma, and critical illness. There are two conditions where magnesium is now considered the therapeutic agent of choice, preeclampsia and torsades de pointes. Future research at the fundamental and clinical levels will lead to further increases in the understanding of how magnesium contributes to pathological processes and under what circumstances it should be used therapeutically.

Exposure Routes and Pathways

The primary route of exposure is ingestion. Secondary routes can include intravenous, ocular, or inhalation.

Toxicokinetics

Homeostasis of magnesium is tightly regulated and depends on the balance between intestinal absorption and renal excretion. Thirty-to-forty percent of ingested magnesium is absorbed from the gastrointestinal system, mostly by the small bowel. Most of the magnesium in the body is stored intracellularly or

in the skeleton; $<1\%$ is extracellular. In plasma, $\sim 65\%$ is in ionic form, with the rest being bound in proteins. The primary route of excretion is through the kidneys, but it is also excreted in sweat and breast milk. Various hereditary disorders of magnesium handling have been clinically characterized, and genetic studies in affected individuals have led to the identification of some molecular components of cellular magnesium transport.

Mechanism of Toxicity

Magnesium levels outside of the normal range alter cellular ion balances and activity, especially Ca^{2+} activity, which directly affects neural and muscular functions. One study found magnesium in relatively high amounts in about half of human colon cancers, but the relationship is unknown and animal studies have found that magnesium actually reduces sarcoma incidence in some nickel- and cadmium-induced tumors.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute animal toxicity resembles acute human toxicity. A unique effect of magnesium when introduced in small amounts into the skin of animals has been called 'gas gangrene' or 'magnesiogenous pneumagranuloma'. Necrosis and tumor-like formation are caused by the production of hydrogen and magnesium hydroxide when metallic magnesium reacts with water of body fluids.

Human

Magnesium is a skin, eye, and pulmonary irritant. Inhalation of fumes can cause metal fume fever. Acute systemic toxicity, defined as serum concentrations $>2.8\,\mathrm{mEq\,l^{-1}}$, is almost always caused by both overingestion and reduced renal excretion together. Hypotension starts around $3\,\mathrm{mEq\,l^{-1}}$ and significant prolongation of cardiac intervals occurs between 4 and $6\,\mathrm{mEq\,l^{-1}}$. Higher serum levels lead to coma and paralysis and heart stoppage occurs around $14–15\,\mathrm{mEq\,l^{-1}}$.

Chronic Toxicity (or Exposure)

Animal

Chronic animal toxicity resembles human toxicity.

Human

There is no hormonal regulation of systemic magnesium levels, so toxic effects occur frequently with both hypermagnesemia and hypomagnesemia but systemic toxicity is rare in adults unless there is impaired renal function. Hypomagnesemia is most commonly associated with alcoholism or small bowel disease and is often accompanied by other electrolyte deficiencies, mostly hypokalemia (K deficit) and hypocalcemia (Ca shortage). The symptoms most commonly include tremor, neuromuscular irritability, and widening of the QRS complex. Human hypermagnesemia is generally caused by either increased ingestion or renal impairment. The symptoms of moderate increases include hypotension, sedation, and somnolence. The possible association between the risk of ovarian cancer and the levels of calcium and magnesium in drinking water from municipal supplies was investigated in a matched case–control study in Taiwan. The results of the study show that there may be a significant protective effect of magnesium intake from drinking water on the risk of ovarian cancer death. Another study has produced data supporting a protective role of higher intake of magnesium in reducing the risk of developing type 2 diabetes, especially in overweight women.

Clinical Management

Hypomagnesemia is treated initially with oral, intramuscular, or intravenous administration of magnesium salts. Immediate control of the symptoms of acute hypermagnesemia is obtained with doses of intravenous calcium repeated hourly but extreme toxicity may require cardiac support or mechanical ventilation. Calcium gluconate and calcium chloride can also be administered as antidotes. Serum levels are lowered by reducing intake and by normal methods of excretion, with diuretics given to patients with normal renal function. Other accompanying electrolyte imbalances should be treated concurrently, followed by treatment of the condition(s) that lead to the imbalances.

Environmental Fate

Elemental magnesium oxidizes and joins the natural environmental reserve.

Ecotoxicology

Magnesium and its compounds are not significantly ecotoxic.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value, 8 h time-weighted average, is 10 mg m^{-3}.

See also: Calcium Channel Blockers; Metals; Vitamin A; Vitamin D; Vitamin E.

Further Reading

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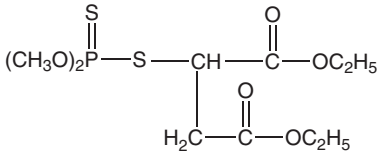
Relevant Website

http://ods.od.nih.gov – US National Institutes of Health (NIH) Magnesium (from NIH's Office of Dietary Supplements).

Malathion

Kevin N Baer

- Chemical Abstracts Service Registry Number: CAS 121-75-5
- Synonyms: *O,O*-Dimethyl-*S*-(1,2-dicarbethoxyethyl)phosphorodithioate; Chemathion; Karbophos; Cythion; Malaspray; Malathiozol
- Chemical Class: Organophosphorus insecticide
- Chemical Structure:



Uses

Malathion is an insecticide and acaricide for control of mosquitoes, household insects, and human head and body lice.

Exposure Routes and Pathways

Poisonings have occurred mainly from accidental or intentional ingestion, although dermal exposure has resulted in systemic symptoms.

Toxicokinetics

Malathion is absorbed through the skin, lungs, and gastrointestinal tract. However, skin absorption is fairly low. Most organophosphate insecticides require activation by oxidation of the P = S bond to the more toxic P = O compound by microsomal enzymes of the liver and other organs, including the brain. However, the carboxyethyl ester groups in malathion are rapidly hydrolyzed by malathion esterases. This action effectively detoxifies malathion and is the reason for the relatively low mammalian toxicity compared with many other organophosphates. The liver and kidney are primary sites of distribution and reflect the rapid detoxification and clearance of malathion. Malathion is rapidly excreted in the urine ($\geq$90%) after 24 h. The half-life following intravenous administration in human volunteers was approximately 3 h.

Mechanism of Toxicity

Malathion is converted to the toxic oxygen analog (replacement of covalent sulfur with oxygen) by microsomal enzymes. The oxygen analog then inhibits acetylcholinesterase as do other organophosphates. As a result, acetylcholine accumulates at cholinergic nerve endings with subsequent hyperstimulation of postsynaptic cells.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute oral and dermal LD_{50} values in rats and mice range from 1 to 12 g kg^{-1}. Domestic animals exhibit similar signs of cholinergic toxicity as seen in humans. Chickens may be somewhat more sensitive to acute toxicity from malathion exposure, but delayed neurotoxicity is not caused by this agent.

Human

Malathion exhibits very low toxicity compared with other organophosphates. The lethal dose in a 70-kg man is estimated to be $\geq$60 g. However, commercial preparations of malathion may contain organophosphate impurities that can lead to increased toxicity by interference with the detoxification systems. Signs and symptoms of severe malathion poisonings are similar to those of parathion and other organophosphates. They include an increase in secretions, gastrointestinal cramps, diarrhea, urination, slow pulse, uncontrollable muscle twitches followed by muscle weakness, paralysis, confusion, dizziness, ataxia, cyanosis, convulsions, and coma. However, life-threatening respiratory or cardiac involvement typical in parathion poisoning is usually not associated with malathion.

Chronic Toxicity (or Exposure)

Animal

As with most organophosphorus insecticides, acute toxicity is predominant. However tolerance to repeated exposures can occur. The no-observed-adverse-effect level (NOAEL) established from a rabbit developmental toxicity study was 50 mg kg^{-1} day^{-1} based on maternal toxicity (i.e., reduced body weight gain). Developmental toxicity studies were negative in rats and rabbits. A two-generation reproductive toxicity study in rats showed no increased sensitivity in pups compared to dams. Repeated exposure to malathion does not cause delayed neurotoxicity. The NOAEL of 2.4 mg kg^{-1} day^{-1} was established based on plasma cholinesterase inhibition in a long-term dosing study in rats.

Human

Generally, the onset and course of toxicity is rapid. However, a number of poisoning cases have shown prolonged symptoms including weakness of proximal limb muscles, cranial nerve palsies, and respiratory depression. As with other organophosphorus anticholinesterases, it is possible to accumulate acetylcholinesterase inhibition with repeated exposures, leading to signs of acute cholinergic toxicity.

Clinical Management

For exposure to eyes, eyelids should be held open and the eyes flushed with copious amounts of water for 15 min. For exposure to skin, affected areas should be washed immediately with soap and water. The victim should receive medical attention if irritation develops and persists.

For exposure through inhalation, the victim should be moved to fresh air and, if not breathing, given artificial ventilation. The victim should receive medical attention as soon as possible.

First aid for ingestion victims would be to induce vomiting, keeping in mind the possibility of aspiration of solvents. Gastric decontamination should be performed within 30 min of ingestion, to be the most effective. Initial management of acute toxicity is the establishment and maintenance of adequate airway and ventilation. Atropine sulfate in conjunction with pralidoxime chloride can be administered as an antidote. Atropine by intravenous injection is the primary antidote in severe cases. Test injections of atropine (1 mg in adults and 0.15 mg kg^{-1} in children) are initially administered, followed by 2–4 mg (in adults) or 0.015–0.05 mg kg^{-1} (in children) every 10–15 min until cholinergic signs (e.g., diarrhea, salivation, and bronchial secretions) decrease. High doses of atropine over several injections may be necessary for effective control of cholinergic signs. If lavage is performed, endotracheal and/or esophageal control is suggested. At first signs of pulmonary edema, the patient should be placed in an oxygen tent and treated symptomatically.

Exposure Standards and Guidelines

The acute population adjusted dose is 0.5 mg kg^{-1} day^{-1}. The chronic population adjusted dose is 0.024 mg kg^{-1} day^{-1}.

See also: Carboxylesterases; Cholinesterase Inhibition; Neurotoxicity; Organophosphates; Pesticides; Veterinary Toxicology.

Further Reading

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rats. *Journal of Toxicology and Environmental Health, Part A* 67(4): 331–356.
Gallo MA and Lawryk NJ (1991) Organic phosphorus pesticides. In: Hayes WJ Jr. and Laws ER Jr. (eds.) *Handbook of Pesticide Toxicology*, vol. 3, pp. 976–985. San Diego: Academic Press.

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Malathion.
http://www.epa.gov – United States Environmental Protection Agency.

Male Reproductive System *See* Reproductive System, Male.

Mancozeb

Mona Thiruchelvam

- Chemical Abstracts Service Registry Number: CAS 8018-01-7
- Synonyms: Manganese–zinc ethylenebis(dithiocarbamate); Carbamic acid ethylenebis(dithio) manganese–zinc complex; Dithane; Manzeb; Manzate; Zimaneb
- Chemical/Pharmaceutical/Other Class: Ethylene(bis)dithiocarbamate
- Chemical Formula: $C_4H_6N_2S_4 \cdot Mn \cdot Zn$
- Chemical Structure:

$$[-SCSNHCH_2CH_2NHCSSMn-]_x(Zn)_y$$

Uses

Mancozeb is an ethylene(bis)dithiocarbamate fungicide. Mancozeb is classified as a contact fungicide with preventive activity. It is widely used to control fungal diseases in conifer and fir trees. It is also used to control blight in potatoes. It is also used to protect many other fruit, vegetable, nut, and field crops against a wide spectrum of fungal diseases. It is also used for seed treatment of cotton, potatoes, corn, safflower, and cereal grains.

Mancozeb is available as dusts, liquids, water-dispersible granules, wettable powders, and as ready-to-use formulations. It is commonly found in combination with maneb and zineb.

Exposure Routes and Pathways

Exposure routes and pathways to mancozeb are similar to the other commonly used ethylene(bis)-dithiocarbamates, maneb. Mancozeb has been shown to cross sensitize with zineb and maneb. Inhalation exposure can lead to upper respiratory tract irritation. Ingestion of mancozeb can lead to nausea, dizziness, headache and diarrhea. Severe overexposure can lead to convulsions and coma.

Toxicokinetics

The absorption and metabolism of mancozeb is similar to maneb. Mancozeb does not accumulate at high levels in most organs due to its rapid turnover rate. In experiments where rats were dosed with ^{14}C-mancozeb repeatedly for 7 days and sacrificed 1 day after the last dose, radioactivity was detected in various organs, with highest levels found in the liver, followed by the kidney and thyroid glands, with traces found in all other organs.

Mechanism of Toxicity

Mancozeb has been classified as a contact fungicide with preventive activity. It inhibits enzyme activity in fungi by forming a complex with metal-containing enzymes including those that are involved in the production of ATP.

Mancozeb has effects on various organ systems. Its primary mechanism of toxicity is via skin contact, leading to contact dermatitis and dermal sensitization. Mancozeb has also been shown to have teratogenic and reproductive effects. Mancozeb exposure also alters the reproductive and endocrine structures, leading to decreased fertility. Animals orally exposed to mancozeb showed thyroid hyperplasia, probably via its ability to inhibit the synthesis of thyroxin. Additionally, mancozeb exposure produces neurotoxicity via yet an unknown mechanism.

Similar to maneb, mancozeb also has chelating properties, allowing it to possibly interfere with a number of enzyme systems that contain metals, such as zinc, copper, and iron (e.g., dopamine β-hydroxylase).

Acute and Short-Term Toxicity (or Exposure)

The acute toxicity of mancozeb is rather low both in humans and experimental animals. Thus acute poisoning is highly unlikely unless large amounts are ingested. Mancozeb is slightly toxic via the dermal route. Contact with mancozeb leads to inflammation and/or irritation of the skin, eyes, and respiratory tract. Acute exposure to mancozeb may lead to effects such as hyperactivity, incoordination, loss of muscular tone, nausea, vomiting, diarrhea, loss of appetite, weight loss, drowsiness, slowed reflexes, and respiratory paralysis.

Animal

In general, mancozeb is not very toxic acutely unless high levels of exposure occur. The acute LD_{50} for mancozeb is $4500\,mg\,kg^{-1}$ in laboratory animals. The acute dermal LD_{50} is greater than $5000\,mg\,kg^{-1}$ in rodents. Dermal exposure to mancozeb leads to mild irritation to the skin. Exposure to the eye also leads to moderate irritation. Inhalation of mancozeb leads to irritation of the respiratory tract, with LC_{50} of greater than $5.14\,mg\,l^{-1}$.

A single exposure to mancozeb to relatively high doses at day 11 of gestation produced substantial malformations in the surviving animals. The malformations observed were cleft palate, hydrocephaly, and other serious defects. There was also an increase in the rate of resorption.

Human

Since the acute toxicity of mancozeb is relatively low as is with most dithiocarbamates, acute intoxication in humans is unlikely to occur unless large amounts are ingested. Mostly mancozeb is known for its irritant and allergic potential in occupational exposures. Skin irritation and sensitization has been studied in humans and have shown mild erythema and itching.

Chronic Toxicity (or Exposure)

Animal

There is limited information regarding the chronic toxicity of mancozeb. It has been indicated that mancozeb has low toxicity in most experimental animals. Its major metabolite, ethylenethiourea (ETU), has been shown to produce carcinogenic and teratogenic effects in laboratory animals at high dose levels.

Studies in dogs and mice indicate that mancozeb does not have carcinogenic effects; however, in rats there was an increase in thyroid tumors. The tumors as well as the inhibition of thyroid function due to these tumors are thought to be due to its metabolite, ETU.

Inhalation exposure of rats to mancozeb, exposed everyday for 4 months indicated an increase in irritation of the mucous membrane of the upper respiratory tract and concentration-related nonspecific changes to the liver and kidneys. Exposure was in the form of dispersed aerosols at concentrations ranging from 2 to $135\,mg\,m^{-3}$. At the lower concentrations, there were no observable effects. In animals exposed repeatedly to high doses of mancozeb (dust) equivalent to 150–250 times the acceptable exposure limit (AEL), reduced body weight, inflammation of the lungs, and abnormal thyroid function were observed.

Toxic effects in animals from repeated ingestion of high doses include reduced body weight and thyroid effects. Increased incidences of thyroid tumors and ocular lesions (retinopathy) were observed in rats administered 750 ppm (equivalent to $\sim 35\,mg\,kg^{-1}\,day^{-1}$) of mancozeb in their diet for 2 years. This compound is considered to show weak carcinogenic activity. Tests in some animals indicate that the compound may produce embryo and fetal toxicity, but only at maternally toxic doses. Multigeneration studies in animals demonstrate no reproductive toxicity. Although there have been isolated reports in the scientific literature of mutagenic activity of mancozeb, in general mancozeb is not genotoxic in animals or in cell cultures. Mancozeb has not been tested for heritable gene mutation. It has been shown to exert a dose-dependent adverse effect to gonads of male and female rats, with reproductive and endocrine structures being affected leading to decreased fertility. The exposure paradigm utilized here was twice a week for 4.5 months. Mancozeb also has been shown to produce teratogenic effects, with gross malformations observed in surviving rats of exposed dams.

ETU, a breakdown product and a minor metabolite of mancozeb, was shown to induce liver tumors in mice but not in rats or hamsters, and caused thyroid tumors in rats. ETU is not genotoxic. ETU has been categorized as a probable human carcinogen by the International Agency for Research on Cancer and as group B carcinogen by the National Toxicology Program. At sufficiently high doses, ETU also causes birth defects in laboratory animals.

Human

Exposure of mancozeb to humans can occur via absorption through the gastrointestinal tract, absorption through the skin or lungs. Human exposure to mancozeb, similar to maneb, has been calculated for

the population of the United States on the basis of estimated consumption of dietary residues of ETU in treated crops. Please refer to the maneb entry for more specifics on mancozeb human toxicity.

Most human exposure to mancozeb is via occupational exposure. Cases of diffuse erythema and eczematoid dermatitis have been observed among agricultural workers. Overexposure to mancozeb by skin contact may initially include skin irritation with discomfort or rash. The compound has been infrequently associated with skin sensitization in humans. Significant skin permeation and systemic toxicity after contact appears unlikely. Eye contact may initially include eye irritation with discomfort, tearing, or blurring of vision. Based on animal studies, long-term exposure to high levels of mancozeb may cause abnormal thyroid function. Individuals with preexisting diseases of the thyroid may have increased susceptibility to the toxicity of excessive exposures.

In Vitro Toxicity Data

In vitro systems have been developed to try and understand the mechanism of action of mancozeb, similar to other dithiocarbamates. The genotoxic, cytotoxic, and neurotoxic effects of mancozeb have been studied using a variety of primary cultures as well as cell-lines.

Clinical Management

Mancozeb can be absorbed into the body by inhalation, though the skin, and by ingestion.

If swallowed, large amounts of water should be ingested, only if person is conscious, to dilute the concentration of the compound and a physician should be called immediately. Vomiting can also be induced. Upon inhalation exposure, the exposed individual should be removed to fresh air, away from the contamination site. If skin contact occurs, all contaminated clothing should be removed and the area exposed should be washed with copious amounts of water and soap. If the product is present in the eyes, large amounts of water should be used to flush the eye for at least 15 min.

Environmental Fate

Mancozeb is generally not active in the soil. It rapidly degrades in the soil into numerous secondary products, principally ETU and eventually CO_2. Plants however can absorb ETU. Because it degrades so quickly, very little mancozeb gets adsorbed by the soil and its breakdown products are highly soluble and do not get adsorbed to soil particles.

Its persistence is very low in soil. One study recovered only 1.16% of mancozeb 7 days after application to silt loam soils, while the half-life was measured as only 3 days in fine sand. Lots of soil microorganisms readily break down mancozeb.

Ecotoxicology

Mancozeb is generally of low toxicity to most wildlife. It is practically nontoxic to birds and honey bees. It has a relatively high toxicity to fish. The 48 h LC_{50} for goldfish is $9\,mg\,kg^{-1}$, and for rainbow trout it is $2.2\,mg\,kg^{-1}$.

Mancozeb has been shown to reduce the population of soil organisms, and in soil nitrification has been reported at concentrations ranging from normal to 10 times the normal field application rates. These changes have tended to be temporary and reversed within 3 months.

Mancozeb is toxic to some plants such as marigold at normal field application rates. Some genetic effects were seen in onion cells exposed to mancozeb.

Exposure Standards and Guidelines

- Occupational Safety and Health Administration: $5\,mg\,m^{-3}$ ceiling.
- American Conference of Governmental Industrial Hygienists: $5\,mg\,m^{-3}$ time-weighted average (TWA).
- National Institute for Occupational Safety and Health: $1\,mg\,m^{-3}$ recommended TWA; $3\,mg\,m^{-3}$ recommended short-term exposure limit.
- Threshold limit value: 5 mg (Mn) m^{-3}.

Miscellaneous

Mancozeb is a grayish-yellow powder with a musty odor, which is practically insoluble in water as well as most organic solvents. It is a polymer of maneb combined with zinc. While it is relatively stable and noncorrosive under normal, dry storage conditions, it is decomposed at high temperatures by moisture and by acid. Mancozeb may produce flammable products upon decomposition. It is also unstable in acidic conditions.

See also: Dithiocarbamates; Maneb; Pesticides.

Further Reading

Belpoggi F, Soffritti M, Guarino M, Lambertini L, Cevolani D, and Maltoni C (2002) Results of long-term experimental studies on the carcinogenicity of ethylene-bis-dithiocarbamate (Mancozeb) in rats. *Annals of New York Academy of Sciences* 982: 123–136.

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US Environmental Protection Agency (1992) Substance Registry System – Mancozeb. Washington, DC: US EPA.

World Health Organization, International Program on Chemical Safety (1988). Dithiocarbamate Pesticides, Ethylenethiurea, and Propylenethiourea: A General Introduction. Environmental Health Criteria No. 78. Geneva, Switzerland: World Health Organization.

Maneb

Mona Thiruchelvam

- Chemical Abstracts Service Registry Number: CAS 12427-38-2
- Synonyms: Manganese ethylenebis(dithiocarbamate); Ethylene bis(dithiocarbamic acid)-manganese salt; Farmaneb; Manesan; Manex; Manzate; Nereb; Newspor
- Chemical/Pharmaceutical/Other Class: Ethylene(bis)dithiocarbamate
- Chemical Formula: $C_4H_6N_2S_4 \cdot Mn$
- Chemical Structure: $[–SCSNHCH_2CH_2NHCSS-Mn–]_x$

Uses

Maneb is an ethylene(bis)dithiocarbamate fungicide used in the control of early and late blights on potatoes, tomatoes and many other diseases on various fruits, vegetables, field crops, and ornamentals. Maneb has been shown to be effective on a wider spectrum of fruit, vegetable, and turf diseases caused by fungi compared to other fungicides. It is available as granular, wettable powder, flowable concentrate, and ready-to-use formulations.

Maneb is also used for the protection of wheat because of its growth inhibition properties and in the plastics and rubber industries as accelerators and catalysts.

Exposure Routes and Pathways

Exposure to maneb can occur via several routes, including dermal, oral, and inhalation. Skin contact with maneb can result in contact dermatitis and in some cases lead to sensitization. Besides dermal exposure, maneb can also be absorbed when inhaled or ingested.

Occupational exposure during manufacturing, mixing/loading, spraying, and harvesting to this compound can occur via dermal deposition and inhalation. Numerous studies have examined the effects of long-term occupational exposure to maneb at various steps in the manufacturing and application process of maneb. These studies have led to the implementation of preventive measures to reduce occupational exposure to maneb. Human exposure can also occur via consumption of treated crops. Residues of maneb and its metabolites have been found in and/or on treated crops. The residue levels change during storage, processing, and cooking due to environmental factors and during these processes the parent compound may be transformed.

Toxicokinetics

Maneb is absorbed via the skin, mucous membrane, respiratory, and gastrointestinal tracts. Its absorption through the skin and the gastrointestinal tract are poor due to its metal-complexed state. Maneb is metabolized to ethylene thiourea (ETU), ethylenediamine, ethylenebisisothiocyanate sulfide (EBIS), and carbon disulfide. ETU is further broken down to molecules that can be incorporated into compounds such as oxalic acid, glycine, urea, and lactose. Due to its rapid metabolism, maneb does not accumulate at high levels in most organs. Most of what is excreted in the urine and feces is in the form of the metabolite, ETU, with very little of the parent compound being eliminated unchanged.

Mechanism of Toxicity

Maneb has effects on various organ systems. Its primary mechanism of toxicity is via skin contact, leading to contact dermatitis, erythema, and even dermal sensitization. Maneb has also been shown to have teratogenic and reproductive effects. Exposure to pregnant animals has been shown to have adverse effects on the fetus. Maneb exposure has also been

shown to alter the reproductive and endocrine structures, leading to decreased fertility. Animals orally exposed to maneb showed thyroid hyperplasia, probably via its ability to inhibit the synthesis of thyroxin. Additionally, maneb exposure produces neurotoxicity via yet unknown mechanism. Humans exposed to maneb show signs of parkinsonism with tremors and slowed movement and gait, developing after years of unprotected handling of exceptionally large amounts of this compound.

Maneb possesses chelating properties, allowing it to possibly interfere with a number of enzyme systems that contain metals such as zinc, copper, and iron (e.g., dopamine β-hydroxylase). It is also capable of inhibiting sulfhydryl-containing enzymes and some other enzyme systems involved in glucose metabolism.

Acute and Short-Term Toxicity (or Exposure)

The acute toxicity of maneb is rather low, and thus acute intoxication is unlikely to occur.

Maneb is practically nontoxic by ingestion. Via the dermal route, it is slightly toxic. Contact with maneb leads to inflammation and/or irritation of the skin, eyes, and respiratory tract. Acute exposure to maneb may lead to effects such as hyperactivity, incoordination, loss of muscular tone, nausea, vomiting, diarrhea, loss of appetite, weight loss, drowsiness, slowed reflexes, and respiratory paralysis.

Animal

In general the acute oral and dermal toxicity of maneb for most mammals is relatively low. The acute oral LD_{50} for rats is $>5000\,mg\,kg^{-1}$. The acute dermal LD_{50} for rabbits is $>5000\,mg\,kg^{-1}$ and for rats is $>10\,000\,mg\,kg^{-1}$. It is a moderate skin and eye irritant.

Rats exposed to maneb produced dose-dependent signs of decreased movement, disturbances of coordination, lack of appetite, and general weakness. Teratogenic and embryogenic toxicity has been observed with single exposures to maneb. In rats given a single dose of $770\,mg\,kg^{-1}$ maneb on the 11th day of gestation, early fetal deaths occurred. Fetal abnormalities of the eye, ear, body, central nervous system, and musculoskeletal system were seen in rats given this single dose. In mice a single oral toxic dose of $1420\,mg\,kg^{-1}$ during gestation caused toxicity to the fetus. Relatively high acute doses of maneb are required to observe adverse consequences.

Human

Since the acute toxicity of maneb is relatively low as is with most dithiocarbamates, acute intoxication in humans is unlikely to occur. A case was reported where a 62-year-old man suffered acute kidney insufficiency following maneb application; however, the precise contribution of maneb exposure was unclear as the patient had other health complications.

Maneb is primarily known for its irritant and allergic potential in occupational exposures. Skin irritation and sensitization has been studied in humans: mild erythema and itching are common.

Chronic Toxicity (or Exposure)

Animal

Chronic exposure to maneb has been related to reproductive, embryotoxic teratogenic, and neurotoxic effects. Although the toxicity associated with maneb exposure is low, it has been shown that in combination with other toxicants such as metals, other fungicides and herbicides the effects of maneb may be more pronounced, leading to more severe deficits.

Rats fed maneb for 2 years at a dose of $12.5\,mg\,kg^{-1}$ showed no adverse effects; however, when fed with $67.5\,mg\,kg^{-1}$ maneb for only 97 days, rats showed reduced growth rate and increased thyroid weight. Dogs treated orally with $200\,mg\,kg^{-1}\,day^{-1}$ maneb for 3 or more months developed tremors, lack of energy, gastrointestinal disturbances, and incoordination. Additionally, spinal cord damage was observed. Rats exposed to $1500\,mg\,kg^{-1}\,day^{-1}$ for 10 days showed evidence of weight loss, weakness of hind legs, and increased mortality.

Inhalation exposure to maneb in rats produced irritation to the upper respiratory tract, and led to nonspecific changes to the liver and kidneys.

Chronic exposure to maneb also affects reproductive abilities. Rats fed maneb for 3 months before mating showed decreased fertility, and changes to reproductive and endocrine structures.

Teratogenic effects of maneb are observed at relatively high levels of exposure. Progeny of albino rats treated with either 700 or $1400\,mg\,kg^{-1}$ maneb twice a week for 4.5 months showed congenital deformities in the caudal vertebrae, palates, limbs, and tail. However, in the mouse the teratogenic effects of maneb exposure were much milder, with almost no deformities observed.

Little or no mutagenic potential has been detected in any assays with maneb.

Most dithiocarbamates have neurotoxic effects, including maneb. Rats exposed orally to maneb twice a week for 4 months at doses of 350 and $1750\,mg\,kg^{-1}$ produced high mortality and paresis in the hind limb progressing to complete paralysis. Exposure to maneb in combination with some

known dopaminergic neurotoxicants (e.g., MPTP and paraquat) has been shown to potentiate changes to the dopaminergic system even though exposure to maneb alone showed no significant alterations. In combination with these other toxicants, signs reminiscent of Parkinson's disease have been observed.

Human

Exposure of maneb to humans can occur via absorption through the gastrointestinal tract, and through the skin or lungs. Human exposure to maneb (and other ethylenebisdithiocarbamates) has been calculated for the population of the USA on the basis of estimated consumption of dietary residues of ETU in treated crops. Upper and lower limits of exposure have been assigned by the US EPA. Food residues have been detected and usually are analyzed as a collective level because analysis is accomplished by measuring carbon disulfide levels. Residues are regularly detected in fruit and vegetables, but mostly at levels below the maximum residue level. However, repeated exposure via ingestion can lead to a chronic exposure state, potentially leading to cumulative toxic effects.

Most human exposure to maneb is via occupational exposure. Cases of diffuse erythema and eczematoid dermatitis have been observed among agricultural workers. Studies on maneb production workers showed elevated levels of ETU in the urine and high blood levels of manganese. Very slight alterations to thyroid function were observed.

In Vitro Toxicity Data

In vitro systems have been developed to try and understand the mechanism of action of maneb. In particular, the mechanism of toxicity of maneb on the central nervous system using synaptosomal and mitochondrial preparations from brain tissue has been utilized. These studies have shown that maneb has adverse effects on the dopaminergic system, via mechanisms that relate to mitochondrial inhibition and altered neurotransmitter uptake. The genotoxic, cytotoxic, and neurotoxic effects of maneb have been studied using a variety of primary cultures as well as cell lines, including human lymphocytes. As noted above, maneb has little mutagenic potential.

Clinical Management

The extent of exposure will determine the initial treatment. On skin contact, contaminated clothing should be removed immediately followed by washing contaminated skin with soap and water to remove the chemical from the body. Similarly, if exposure to eyes occurs, large amounts of water or isotonic saline for at least 15 min should be used to flush the eye, occasionally lifting upper and lower lids.

If inhalation exposure occurs, the person should be removed from the exposure area to an area with fresh air. If needed, rescue breathing should be administered and medical attention sought immediately.

Upon ingestion, vomiting should be induced in the conscious patient. Activated charcoal should be administered to adsorb the remaining fungicide, followed by a sodium or magnesium cathartic.

Environmental Fate

Maneb has low persistence, with a reported field half-life of 12–36 days. It is readily transformed to ETU, which is much more persistent. Maneb strongly binds to most soils and is not highly soluble in water; therefore, it is not very mobile. It therefore does not represent a significant threat to groundwater. However, its breakdown product, ETU, may be more mobile. Maneb breaks down under both aerobic and anaerobic soil conditions. In one particular study, it was shown that maneb does not leach below the top 5 in. of soil.

Maneb degrades very quickly in water, with a half-life less than 1 h. Its main breakdown product is ETU. Significant amounts of ETU have been found in vegetables treated with maneb. Vegetables such as spinach, carrots, and potatoes that are treated with maneb after harvest produce a significant amount of ETU in the cooking process. Washing the vegetables or fruits before cooking or eating eliminated a majority of the residues.

Ecotoxicology

Maneb is practically nontoxic to birds. A 5 day dietary LC_{50} for maneb in bobwhite quails and mallard ducklings is greater than 10 000 ppm.

Maneb is however highly toxic to fish and other aquatic species. The 96 h LC_{50} for maneb is 1 mg l^{-1} in bluegill sunfish. The reported 48 h LC_{50} is 1.9 mg l^{-1} in rainbow trout and 1.8 mg l^{-1} in carp. Maneb-treated crop foliage may also be toxic to livestock.

Exposure Standards and Guidelines

- OSHA ceiling limit is 5 mg m^{-3}.
- ACGIH TWA is 1 mg m^{-3} (NIOSH recommended TWA).
- NIOSH recommended STEL is 3 mg m^{-3}.

- Mine Safety and Health Administration (MSHA) Standard air ceiling concentration is 5 mg (Mn) m^{-3}.
- Occupational Safety and Health Administration (OSHA) permissible exposure limit (general industry, construction, shipyards, federal contractors) ceiling concentration is 5 mg (Mn) m^{-3}.

Miscellaneous

Maneb is a yellow powder with a faint odor. It is a polymer of ethylenebisdithiocarbamate units linked with manganese. It is highly insoluble. Its water solubility is 6 mg l^{-1} and is practically insoluble in common inorganic solvents.

See also: Dithiocarbamates; Manganese.

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Manganese

Shayne C Gad

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- Chemical Abstracts Service Registry Number: CAS 7439-96-5
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formula: Mn^{2+}

Uses

Manganese is used in ceramics, glass, dyes, dry-cell batteries, and special high-carbon steels. It is also added to fertilizers and animal food. Potassium permanganate is used as an oxidizing agent, and several antioxidant drugs now under development incorporate manganese in an organic matrix. Manganese is an essential trace element, and its concentrations are highest in tissues rich in mitochondria, where it forms stable complexes with ATP and inorganic phosphate. Manganese functions as a constituent of metalloenzymes and an activator of enzymes.

Exposure Routes and Pathways

Ingestion is the primary exposure pathway for the general population; sources of exposure include grains, nuts, fruits, and tea. Inhalation is a significant exposure pathway in industrial settings. Air and water pollution are minor sources in most areas. Manganese is a ubiquitous constituent in the environment, occurring in soil, air, water, and food. Thus, all humans are exposed to manganese, and manganese is a normal component of the human body. Food is usually the most important route of exposure for people, with typical daily intakes of 2.5–5 mg day^{-1}.

Toxicokinetics

Less than 5% of ingested manganese is absorbed from the gastrointestinal tract. Manganese is carried in blood serum by a β-globulin, which may be specific for this metal. Manganese is a cofactor for enzymes related to synthesis of cholesterol and also fatty acids. It is necessary for phosphorylation reactions. In some cases it can substitute for magnesium. Manganese is excreted in the bile, but systematic loads are slowly cleared.

Mechanism of Toxicity

Brain extracellular concentrations of amino acids and divalent metals (e.g., manganese) are primarily regulated by astrocytes. Adequate glutamate homeostasis is essential for the normal functioning of the central nervous system (CNS), for example, glutamate is important for nitrogen metabolism and, along with aspartate, is the primary mediator of the excitatory

pathways in the brain. Similarly, the maintenance of proper manganese levels is important for normal brain functioning. *In vivo* and *in vitro* studies have linked increased manganese concentrations with alterations in the content and metabolism of neurotransmitters, for example, dopamine, γ-aminobutyric acid, and glutamate. Rat primary astrocytes exposed to manganese display decreased glutamate uptake, thereby increasing the excitotoxic potential of glutamate. Furthermore, decreased uptake of glutamate has been associated with decreased gene expression of glutamate–aspartate transporter in manganese-exposed astroctyes. Other studies suggest that attenuation of astrocytic glutamate uptake by manganese may be a consequence of reactive oxygen species generation. These data suggest that excitotoxicity may occur due to manganese-induced altered glutamate metabolism, representing a proximate mechanism for manganese-induced neurotoxicity.

Acute and Short-Time Toxicity (or Exposure)

Human

Available human toxicity data are limited to the industrial setting, where adverse health effects have resulted from inhalation of manganese (primarily as manganese dioxide). Inhalation of particulate manganese compounds such as manganese dioxide (MnO_2) or manganese tetroxide (Mn_3O_4) can lead to an inflammatory response in the lung.

Acute inhalation exposure produces manganese pneumonitis; the incidence of respiratory disease among exposed workers is higher than that of the general population.

Chronic Toxicity (or Exposure)

Human

In workers with chronic inhalation exposure, iron deficiency and liver cirrhosis are commonly observed. Chronic inhalation exposure also affects the CNS, resulting in Parkinsonian-like symptoms. Mental aberrations are also observed. The psychiatric disturbance has been called 'manganese madness'. Symptoms include confusion, unusual behavior, and sometimes hallucinations. Apathy, difficulty with speech, and loss of balance are most common. Other symptoms include difficulty with fine motor movement, anxiety, and pain. Manganese intoxication can result in a syndrome of parkinsonism and dystonia. If these extrapyramidal findings are present, they are likely to be irreversible and may even progress after termination of the exposure to manganese. Clinical features are usually sufficient to distinguish these patients from those with Parkinson's disease. The neurological syndrome does not respond to levodopa. Imaging of the brain may reveal magnetic resonance imaging signal changes in the globus pallidus, striatum, and midbrain. Positron emission tomography reveals normal presynaptic and postsynaptic nigrostriatal dopaminergic function. The primary site of neurological damage has been shown by pathological studies to be the globus pallidus. The mechanism of toxicity is not clear. The US Environmental Protection Agency (EPA) lists manganese as category D, that is, it is not classifiable as to human carcinogenicity. While rare in occurrence, manganese deficiency in humans has been reported. It is characterized by skeletal abnormalities and seizure activity, probably due to decreased MnSOD and glutamine synthetase activities.

Clinical Management

Many symptoms of manganese toxicity disappear after the victim is removed from the source of exposure. L-Dopa (levodopa) can reverse some symptoms, but complete recovery is not expected. Calcium-EDTA (the calcium disodium salt of ethylenediaminetetraacetic acid) will help improve an acute manganese-induced psychosis.

Environmental Fate

Higher levels of environmental exposures to manganese are most likely to occur in or near a factory or a waste site that releases manganese dust into air. Manganese is also released into air by combustion of unleaded gasoline that contains manganese as an antiknock ingredient. Some manganese compounds are readily soluble, so significant exposures can also occur by ingestion of contaminated drinking water. However, manganese in surface water may oxidize or adsorb to sediment particles and settle out. Manganese in soil can migrate as particulate matter in air or water, or soluble compounds may be dissolved by water and leach from the soil. Elemental manganese and inorganic manganese compounds have negligible vapor pressures, but may exist in air as suspended particulate matter derived from industrial emissions or the erosion of soils. The half-life of airborne particles is usually on the order of days, depending on the size of the particle and atmospheric conditions.

The transport and partitioning of manganese in water is controlled by the solubility of the specific chemical form present, which in turn is determined

by pH, Eh (oxidation–reduction potential), and the characteristics of available anions. The metal may exist in water in any of four oxidation states (2+, 3+, 4+, or 7+). Divalent manganese (Mn^{2+}) predominates in most waters (pH 4–7), but may become oxidized at pH greater than 8 or 9. The principal anion associated with Mn^{2+} in water is usually carbonate (CO_3^{2-}), and the concentration of manganese is limited by the relatively low solubility (65 mg l^{-1}) of $MnCO_2$. In relatively oxidized water, the solubility of Mn^{2+} may be controlled by manganese oxide equilibria, with manganese being converted to the (3+) or (4+) valence states. In extremely reduced water, the fate of manganese tends to be controlled by the formation of the poorly soluble sulfide.

Manganese in water may be significantly bioconcentrated at lower trophic levels.

Manganese is a natural component of most foods. The highest manganese concentrations (up to 40 ppm) are found in nuts and grains, with lower levels (up to 4 ppm) found in milk products, meats, fish, and eggs. Concentrations of manganese in infant formulas range from 34 to 1000 ppb, compared to concentrations of 10 ppb in human milk and 30 ppb in cow's milk.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value, 8 h time-weighted average (TWA), is 0.2 mg m^{-3} for elemental manganese and inorganic compounds. The (US) Occupational Safety and Health Administration permissible exposure limit, 8 h TWA, is 5 mg m^{-3} for manganese as a fume and 0.2 mg m^{-3} for manganese as particulate matter. The US EPA recommends a concentration of manganese in drinking water not in excess of 0.05 ppm. The US Food and Drug Administration has set the same level for bottled water.

See also: Metals.

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http://www.inchem.org – International Programme on Chemical Safety. Manganese (Environmental Health Criteria 17). See also: Manganese and its Compounds (Concise International Chemical Assessment Document, CICAD).

Margin of Exposure (MOE)

Udayan M Apte and Harihara M Mehendale

Definition

Margin of exposure (MOE) is defined as the ratio of the no-observed-adverse-effect level (NOEAL) to the estimated exposure dose:

$$\text{MOE} = \frac{\text{NOEAL}}{\text{Estimated exposure dose}}$$

Introduction

The determination of MOE is a part of the risk characterization process of a compound. MOE is a way to express the risk of noncarcinogenic effects of a compound. It utilizes the NOEAL determined in animals and estimated exposure dose to human population. NOEAL is the highest dose level of a chemical that does not produce a significantly elevated increase in an adverse response. NOEAL is determined in test animals such as rats and is expressed in milligram per kilogram per day. The estimated exposure dose is determined by estimating amounts of the chemical in the sources of contamination (e.g., water supply) and is expressed in milligram per kilogram per day. MOE indicates how close the estimated exposure of the toxicant is to the dose, which produces no observable adverse effect in a test animal. Low values of MOE indicate that the human exposure of the chemical in the target population is close to the NOEAL in the animals. MOE values below 100 are considered unacceptable and generally demand further investigation. Higher

values of MOE indicate that the exposure of the chemical is much lower than the NOEAL in animals. It should be noted that the MOE calculation does not take into account the differences in animal-to-human susceptibility and/or the extrapolation of dose from animals to humans.

Example of MOE

Consider that the human exposure of a chemical X calculated via drinking water supply is 2 ppp, that is, $2\,mg\,l^{-1}\,day^{-1}$. Suppose a 70 kg man consumes 2 l of drinking water per day then the estimated exposure dose would be $2\,mg\,kg^{-1}\,day^{-1} \times 2\,l\,day^{-1}$ divided by 70 kg (body weight), which is equal to $0.057\,mg\,kg^{-1}\,day^{-1}$. Suppose that the NOEAL of chemical X is $150\,mg\,kg^{-1}\,day^{-1}$, then the MOE would be more than 2600. This indicates that the exposure of chemical X is much below its NOEAL and the risk to public health is very low.

See also: Risk Assessment, Human Health.

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Marijuana

Christopher P Holstege

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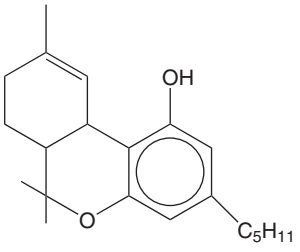
- Chemical Abstracts Service Registry Number: CAS 7663-50-5
- Synonyms: Tetrahydrocannabinol (THC); Bhang; Dronabinol; Cannabis; Ganja; Grass; Hashish; Hemp; Honey oil; Marihuana; Marinol; Mary Jane; Pot; Refeer; Weed
- Chemical/Pharmaceutical/Other Class: Psychoactive substance
- Chemical Structure:



Uses

Dronabinol is prescribed for its antiemetic and appetite stimulant properties. Marijuana is primarily a drug of abuse, although it is currently used by patients for the same purposes as dronabinol.

Exposure Routes and Pathways

Inhalation of marijuana smoke is the most common method of use followed by ingestion. Parenteral use is uncommon. Dronabinol is an oral capsule.

Toxicokinetics

After smoking, 18–50% of the available THC is absorbed, the onset of clinical effects occurs within 10 min, and effects continue for 2–4 h. Peak plasma levels occur within 5–12 min of smoking with peak clinical effects noted at 20–30 min later, after distribution into brain and other tissues. Following ingestion, only 5–20% of THC is bioavailable, the onset of effects begins within 30–60 min, and effects persist for 4–6 h. Gastrointestinal absorption is increased by fatty foods or a lipid vehicle. Peak plasma levels occur 2–3 h after THC ingestion. THC is 97–99% protein bound with a volume of distribution of $\sim 10\,l\,kg^{-1}$. THC undergoes substantial first-pass metabolism by the liver. THC is metabolized primarily to 11-hydroxy-delta-9-THC. The 11-hydroxy-delta-9-THC is pharmacologically active, but is further metabolized to inactive metabolites, primarily 11-nor-delta-9-THC carboxylic acid. Less than 1% of THC is excreted unchanged in the urine. The high lipid solubility results in an initial short plasma half-life, but this adipose storage produces a biologic half-life of 25–30 h. THC may be detectable in plasma for up to 15 days. With chronic high-dose use of marijuana, the presence of metabolites of THC in the urine can be detected for 6–8 weeks.

Mechanism of Toxicity

The mechanisms involved in THC's central nervous system (CNS) and cardiovascular effects have not been well delineated. Specific cannabinoid receptors in the cerebral cortex may be responsible for the pharmacologic effects of THC. THC also has immunosuppressive effects and results in depression

of both B- and T-cell activity and depression of tumor necrosis factor levels by macrophages. The antiemetic effect appears to involve the CNS vomiting control center.

Acute and Short-Term Toxicity (or Exposure)

Animal

The clinical effects of marijuana in animals are similar to those observed in humans. Clinical effects may be more pronounced after ingestion of marijuana than those seen with inhalation exposure.

Human

Toxicity primarily involves the CNS and cardiovascular system. Euphoria, increased apparent visual and auditory sensory perception, and altered perceptions of time and space are common with mild intoxication. Larger doses can impair memory, decrease attention and cognition, and result in lethargy. Impaired sensory interpretation and performance of complicated mental tasks increases the risk of trauma with activities such as operating a motor vehicle. Decreased balance, ataxia, and muscle incoordination can occur. Anxiety, panic attacks, paranoia, depression, confusion, and hallucinations can occur with high doses; these effects are more common in less experienced, younger users. Cardiovascular effects include increased heart rate and cardiac output and decreased exercise tolerance. Bronchodilation and, less frequently, bronchoconstriction may be seen. The pupils will constrict slightly and the conjunctiva will become red secondary to congestion of the blood vessels. A dry mouth is common. The intravenous administration of marijuana has been associated with severe multiple organ system failure, including renal failure, rhabdomyolysis, increased hepatic enzymes, shortness of breath, headaches, and hypotension.

Chronic Toxicity (or Exposure)

Animal

Nonhuman primates have displayed behavioral signs of withdrawal after chronic administration of THC. Chronic administration of THC via gavage over 2 years found no evidence of carcinogenic effect in rats and equivocal findings in mice at higher doses. Chronic use of THC has been shown to induce tumor regression in rodents.

Human

Chronic use can result in an amotivational state, paranoid behavior, worsening muscle incoordination, slurred speech, and delusions. Smoking marijuana is implicated in both chronic lung disease and the development of lung cancer. Fertility can be impaired in both males (decreased sperm count and activity) and females (decreased ovulation and abnormal menses). Prenatal marijuana use by the mother correlates with increased hyperactivity, impulsivity, and delinquency in the child. Tolerance to some CNS effects may develop with chronic use, and a withdrawal syndrome is possible after chronic high-dose use.

In Vitro Toxicity Data

The active moieties of marijuana have been studied for medicinal purposes in a variety of models. Some canabinoids have displayed effects on neuronal transmission and alterations of calcium homeostasis. Other cannabinoids have been shown to stimulate cell death (apoptosis), which may help explain observed antitumor effects in some animal models.

Clinical Management

Clinical management is primarily supportive. Reassurance is generally effective in treating alterations in thought process, although benzodiazepines may be necessary in uncommon, severe toxicity. If large amounts of marijuana are ingested, activated charcoal administration may be considered for recent exposures.

See also: Drugs of Abuse.

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Marine Organisms

William R Kem

A wide variety of natural toxins, from small heterocyclic molecules to large proteins, occurs in marine organisms. The phyletic diversity of plants in the ocean is far less than on land, while the number of marine animal phyla significantly exceeds that on land. Thus, it is not surprising that many of the known marine toxins are of animal origin. In this article, we will not only focus upon the toxins of unicellular organisms and marine animals, but also consider a few seaweed toxins.

What is a toxin? First, the word denotes a single chemical entity or compound which possesses a defined chemical composition and covalent structure. Generally, this word is reserved for molecules that occur naturally within an organism. Vertebrate (and human) toxins include the complement system and defensin peptides which serve as one of our chemical defenses against infectious bacteria. Toxic substances made with human hands (and minds) are generally referred to as poisons. A venom is a mixture of substances secreted together by an animal to either defend itself and/or capture prey. Animal venoms usually are mixtures of enzymes and toxins that, acting together, are more effective than when acting separately. For instance, phospholipases are commonly present in venoms because they facilitate the distribution of the toxins in the venom by digesting lipids in lipid membranes which act as barriers to the distribution of toxins throughout the body. Conversely, some membrane-disrupting toxins also enhance lipid digestion by phospholipases. Enzymes, hyaluronidase and collagenase, break down macromolecules responsible for holding cells together, also enhance the distribution of venoms in the body.

Many toxins act rapidly on their victims. This certainly makes sense if the toxin is being used to immobilize prey or to escape from predators. Rapidly acting toxins generally affect excitable cells such as nerves and muscles (including the heart myocardium) which allow movement. Their targets (receptors) include voltage-gated ion channels involved in the generation of nerve and muscle action potentials, which share many of the same characteristics. These ion channels are membrane-penetrating proteins which open in response to a change in the electrical potential across the membrane, allowing sodium or calcium ions to diffuse inwards, causing a rapid (millisecond timescale) depolarization of the membrane sufficient to serve as an electrical stimulus for the adjacent membrane and thereby causing the conduction of an electrical signal called an action potential. This depolarizing wave rapidly propagates down the length of the cell, ultimately causing contraction (muscle) or release of a neurotransmitter (nerve). Either process activated by an action potential involves the opening of calcium-selective ion channels, which allows calcium ions to rush into the cell and trigger either contractile proteins or release of packets of neurotransmitter at the nerve terminal. Many toxins, aquatic and terrestrial, attack the sodium or calcium channels involved in these processes, since their alteration usually causes paralysis and possibly death of the affected organism.

A neurotransmitter diffuses a very short distance to reach its receptor on a nearby cell which has formed a synapse with it; there the neurotransmitter activates what is called a ligand-gated ion channel which also usually generates a smaller electrical signal which can be excitatory (depolarizing, causing another action potential to be generated on the other side of the synapse) or inhibitory (suppressing action potential generation in the postsynaptic cell). There are many toxins which affect the release of neurotransmitters from their presynaptic sites or the subsequent effect of the neurotransmitter on its receptor. These effects also can cause a very rapid paralytic effect on a victim. In the following discussion of marine toxins we will at least briefly consider what is known about the sites and modes of action of a toxin.

Dinoflagellate Toxins

Single-celled organisms (formerly referred to as protozoans but more recently as prokaryotes) abound in aquatic environments including the seas and oceans. Much is known about their biology as they can often be cultured in the laboratory and their unicellular nature makes them excellent subjects for many cell biology studies. While most prokaryotes do not contain toxins, some marine dinoflagellates can secrete or release upon death very potent toxins capable of causing harm to a variety of animals including humans. The most cosmopolitan type of toxic dinoflagellate (genus *Gonyaulax*) contains a toxin called saxitoxin which blocks voltage-gated sodium channels in nerve and skeletal muscle and thereby inhibits excitability. Saxitoxin is concentrated by clams and mussels as well as other filter-feeding animals which feed upon *Gonyaulax*. Although these animals are relatively insensitive to this toxin (otherwise they could not feed upon this

dinoflagellate!), animals which feed upon bivalves containing sufficient amounts of this or closely related saxitoxin analogs can be paralyzed by sodium channel blockade caused by this toxin. In many ways saxitoxin acts like a local anesthetic (e.g., lidocaine) on the nerve impulse, blocking the sodium channels and causing paralysis. However, there are two obvious differences. First, saxitoxin much more selectively blocks the sodium channels and at over 1000-fold lower concentrations. Second, since saxitoxin is a much more polar molecule, it does not enter the brain readily from the systemic circulation, and thus acts primarily on the peripheral neuromuscular system causing relaxation of skeletal muscles. Depression of breathing by inhibiting the intercostals and diaphragm skeletal muscles can be fatal! Fortunately, our myocardial sodium channel is less sensitive to saxitoxin and thus cardiac depression is rare. Shellfish beds which are harvested for human consumption are monitored by federal agencies for dinoflagellate toxin levels to assure their safe consumption. When saxitoxin or related intoxication occurs, symptomatic treatment in a hospital setting is used to get the patient through the critical period of respiratory weakness.

Besides paralytic shellfish poisoning (PSP) there is also neurotoxic (NSP) and diarrhetic (DSP) shellfish poisoning due to other dinoflagellates in the marine environment. NSP is relatively rare, but in 1987 received considerable attention when there was an occurrence of this type of poisoning in Nova Scotia. NSP victims showed central nervous system cognitive deficits such as amnesia. The causative agent was later found to be domoic acid, which is known to be toxic to excitatory synapses in the brain which involve the neurotransmitter glutamic acid. This toxin is a chemical analog of glutamic acid, which is not readily removed from the nervous system and thus causes persistent stimulation of such synapses, which results in a massive calcium elevation which proves lethal to neurons expressing glutamate receptors. Again, this dinoflagellate toxin was only retained and concentrated by the bivalve.

DSP is not as life-threatening as PSP and NSP. The main toxin, called ciguatera toxin, is actually a group of very similar polyether molecules which, like PSP, also affects voltage-gated sodium channels. However, ciguatoxin stimulates the opening of a small fraction of sodium channels and this causes an increase in nerve excitability in contrast with saxitoxin's depressant action on excitability. Gastrointestinal cramps and diarrhea are the major effects. Ciguatoxin is made by a bacterium but because it is very lipophilic it is concentrated as it is passed up the food chain. Another chemically related marine bacterial toxin, maitotoxin, also causes ciguatera symptoms but acts by a different mechanism, enhancement of resting membrane calcium ion permeability. Thus predatory animals at the very top of the chain can accumulate the highest toxin concentrations. These include fish like barracuda. The highly lipophilic ciguatera toxins leave the victims very slowly, sometimes over months or a year, thus prolonging the misery.

There are several other marine dinoflagellates which secrete toxins into the sea water primarily when their high concentrations (blooms) cause a population crash, and the dead cells then release their toxins. In the United States, a very common organism causing massive fish mortalities is *Karenia* (formerly *Gymodinium*) *breve*. The so-called brevetoxins, like ciguatoxin, are large polyether molecules which tightly bind to voltage-gated sodium channels in excitatory cells and enhance their excitability. Because fish sodium channels are very sensitive to these toxins, they usually die before they are caught and consumed by humans. Thus this toxin is primarily injurious to marine ecosystems due to massive mortalities of fish and other animals. The only common human effect is bronchoconstriction of the airways resulting from inhalation of brevetoxins which can be airborne in coastal regions experiencing this red tide.

Although red tides occurred before human population density became high, the frequency and widespread occurrence of particular red tides is often attributed to eutrophic conditions along coasts caused by runoff of agricultural fertilizers and animal wastes. Unfortunately, the spores of these organisms are readily transported from one sea to another in the ballast waters of ships. It is thought that red tide dinoflagellates are now widely distributed around the oceans of our planet because of these human influences.

Increases in environmental pollution or nutrient levels, reduced oxygen levels, and other factors can change conditions significantly in marine environments, especially in protected coastal areas where tidal flushing currents may be slow. Sometimes when this occurs, different organisms that thrive under these altered conditions begin to emerge as do health concerns for both people and other species coming in contact with these species and the toxins they produce. One fairly recent example of this is a major outbreak of finfish kills and some human health problems (respiratory and eye irritation, skin rashes, gastrointestinal and neurological symptoms) reported along the middle Atlantic seaboard of the United States in the early 1990s. The cause of this appears to be exposure to dinoflagellate *Pfiesteria* sp. (including *Pfiesteria piscicida* and *Pfiesteria* shumwayae) and to

the yet unidentified substances that they produce. Such toxicity had not previously been detected in this region. This is just one illustration of how important it is to be aware of the impact of human activity on marine environments and the unintended changes our species may be bringing about.

Invertebrate Toxins

Sessile marine animals such as encrusting sponges, bryozoans, and tunicates are known to harbor a variety of toxins which may serve as chemical defenses against predators. These are filter feeding animals and thus many of the toxins and repellant substances obtained from these organisms may originally have been made by bacteria or other planktonic organisms which are concentrated by these animals. Certain sponges (the genus was originally *Haliclona*, but has been changed to *Amphimedon*) make pyridinium polymers called halitoxins which lyse blood and other cells which have been tested. Sponges containing high concentrations of this polymeric toxin are generally avoided by most predatory fish. The Carribbean Fire Sponge (*Tedania* sp.) possesses toxins which cause a delayed hypersensitivity as well as acute inflammatory reaction whose unpleasant nature the author has experienced. The active constituents of this and other inflammatory sponges have not yet been characterized.

Bryozoans look more like plants than animals and are common coastal animals growing on docks and boats in addition to the natural surfaces. A family of heterocyclic molecules aptly called bryostatins has been identified and is being tested as potential treatments for certain cancers. Similarly, tunicates, representing some of our most primitive chordate (backbone) ancestors, produce cyclic peptides which preferentially kill certain types of cancer cells. Vast numbers of sponge, bryozoan, and tunicate and other encrusting marine species are being extracted and tested for antineoplastic activity by a screening program sponsored by the National Cancer Institute and many lead compounds have already been identified.

The phylum Cnidaria consists of hydrozoans (including Portuguese Man O'War medusae and fire corals), scyphozoans (jellyfish), and anthozoans (soft corals, hard corals, and sea anemones). All of these animals are covered with stinging capsules (the cnidae) which are used to paralyze prey and defend against predators. The cnidae are located in cnidocytes, the epidermal cells which make the stinging capsules and eventually control their discharge. The wall of the stinging capsule has been shown to be impermeant to molecules larger than about 800. Since all of the known cnidocyst toxins are peptides or proteins exceeding this mass, they can be kept within the capsule without expenditure of energy. Jellyfish and hydrozoan toxins are relatively large, unstable proteins which form large pores in cell membranes, which cause their cells to swell up and burst due to the osmotic imbalance. The toxins of sea anemones are smaller and generally stable after isolation. The amino acid sequences of several sea anemone toxins are known. The toxins which affect excitable membranes are generally called neurotoxins, although they may be even more potent on heart sodium channels. These peptides of about 50–55 amino acid residues are known to prolong the repolarization phase of the action potential by delaying the process of sodium channel inactivation which is important for returning the nerve membrane to its resting state. This leads to an abnormally large release of neurotransmitters at nerve endings, and results in spastic paralysis of the victim. The other sea anemone toxins are larger peptides which form large ion channels pores in cell membranes, causing depolarization, loss of osmotic balance, and cell death (cytolysis). Particularly common are the 'actinoporins', which are $\sim$20 000 Da proteins, which, like the bacterial porins, possess large amounts of B-pleated sheet structures. A third, more recently discovered group of sea anemone peptide toxins block voltage-gated potassium channels at extremely low concentrations. One can imagine that when these three toxins act together on a nerve membrane that it will be depolarized much of the time! Soft corals, in contrast to the above-mentioned cnidarians, seem to rely upon small, repellant terpene molecules to deter predators.

Of the 25 animal phyla, almost half are worms. Thus, it is not at all surprising that some worms contain toxins. The nemertines are a phylum of over 800 known species which resemble flatworms but are active predators on crustaceans and other worms. This phylum is exceptionally toxic among the various worm phyla. The Heteronemertine side possesses peptide toxins which appear to be only defensive, as these animals have no means of injecting a venom. The peptides include neurotoxins, which enhance excitability of nerve membranes, and cytolysins, which permeabilize and destroy cell membranes. Members of the Hoplonemertine class inject a venom into their prey using a mineralized stylet located in their proboscis, which is also used to immobilize the prey. Their toxins are alkaloids similar to nicotine which in minute amounts paralyze crustaceans and annelid worms and primarily activate nicotinic acetylcholine receptors. Another well-known worm toxin is nereistoxin, a disulfide-containing alkaloid which also binds to nicotinic

receptors but is largely inhibitory to their normal functioning. This toxin was isolated after fisherman noticed that flies which ate the flesh of the dead worms were paralyzed. It later became an important agricultural insecticide because it is particularly effective on rice-stem boring insects.

Starfishes and sea urchins usually contain toxins serving as a chemical defense against predators and potential settling animals. Starfishes make saponins (diterpene glycosides) that are chemically similar to the saponins found in unripe tomatoes and in potato spuds. These enter the lipid bilayer part of the cell membrane and form complexes with cholesterol, a membrane-stabilizing lipid. This makes the membrane leaky to ions and water, causing cyolysis. Among the spines of sea urchins are found small flower-like appendages, pedicellariae, some of which are venomous. Their toxins are peptides and none have yet been characterized chemically. They can paralyze small animals which might otherwise attach (settle) to the surface of the urchin.

While most mollusks possess a protective shell, some also possess powerful venoms which can be used as a further defense against predators and also for paralysis of their prey. Undoubtedly, the best known group is one of marine snails known as 'cone' snails because their shells are often nearly perfectly conical. The genus *Conus* actually contains more than 300 species, and it is likely that all possess a venom harmful to some animal. Only $\sim$10% of the species are thought to be harmful to vertebrates and these are species that usually prey upon fish. Venoms of the others may also contain peptide toxins affecting vertebrates but are unlikely to be lethal. Most cones actually prey upon annelid worms or nonpoisonous snails (sometimes the cones battle as well, in a chemical warfare without backbones). Their venoms tend to be specialized for their molluscan or vermiferous prey rather than us vertebrates. Nevertheless, when scuba diving or snorkeling, it is best not to handle cones unless your skin is protected by gloves and wet suit. Since the venom is emitted from a tiny harpoon shot out with considerable force, it is also advised not to place the snail in a pocket! Octopuses are also venomous. Although the Australian blue-ringed octopus uses tetrodotoxin (TTX, see next section), most octopuses inject a salivary gland venom containing a protein (cephalotoxin) which paralyzes crabs in very small amounts. This toxin does not seem very potent when injected into vertebrates.

Vertebrate Toxins

Sea snakes (family Hydrophiidae) are close relatives of the cobra, coral, and other snakes belonging to the family Elapidae. While these snakes are usually not very aggressive, they are potentially dangerous, possessing venoms that on a unit weight basis are amongst the most potent of all snakes. Sea snakes are confined to the Pacific Ocean and contiguous tropical seas including the Red Sea. They use their venom to paralyze prey, primarily fish. Two peptide toxins and phospholipase A2 are generally present in these snake venoms. The most life-threatening toxin is the so-called α-neurotoxin, a peptide composed of $\sim$60 amino acid residues that is held together in a three-fingered loop structure by three disulfide bonds; the longer, middle loop binds to the nicotinic acetylcholine receptor on neuromuscular synapses and blocks the ability of the neurotransmitter acetylcholine to activate skeletal muscle. This sea snake toxin acts essentially like curare alkaloids and modern nondepolarizing muscle relaxants, but it binds more tightly to the receptor and thus the neuromuscular block takes more time to be reversed as the toxin disappears from the systemic circulation.

The second sea snake peptide toxin, cardiotoxin, is homologous (common ancestral gene) with the α-neurotoxin, but lacks the particular amino acid residues favorable for binding of the latter peptide to the nicotinic receptor. Cardiotoxin binds rather indiscriminantly to cell membranes including those of the heart and disrupts their normal structure such that they become more permeable to sodium, calcium, and other ions, which depolarizes the normal resting membrane sufficient to cause systolic arrest of the heart. It acts synergistically with phospholipase since it makes the membrane phospholipids more accessible to attack by the phospholipase A2 which is also a major enzymatic constituent of this venom. The most common means of treatment of sea snake envenomation involves intravenous injection of sea snake antivenin containing antibodies directed toward the various toxic constituents. When antivenin is unavailable cholinesterase inhibitors might be useful therapy when muscular paralysis is not complete. Artificial ventilation must be maintained until the victim is able to breathe spontaneously.

There are many poisonous fishes in the oceans of the world. Perhaps the most notorious is the puffer fish (family Tetrodontidae). Besides being able to inflate itself, thereby directing the spines on its skin toward a potential predator and becoming a large oval shape, this fish contains a heterocyclic toxin which, like saxitoxin, blocks some voltage-gated sodium channels at very low (nanomolar) concentrations. TTX was initially purified from a puffer fish prized as food in Japan, where chefs must pass a rigorous test demonstrating their ability to remove

the poisonous viscera and skin from the edible flesh. Puffers apparently use TTX only as a chemical defense against predators. TTX has been demonstrated to be produced by a bacterium which lives within the poisonous tissues of the fish. This may also explain why it also occurs in a wide variety of other animals including the California newt (an amphibian), the blue-ringed octopus, marine crabs, and worms. Fortunately, our myocardial (heart) sodium channels are relatively resistant to this toxin, as are the nerves of the puffer fish. Also, being ionized and very polar, the toxin does not readily penetrate across the blood brain barrier into the brain.

There are many fishes with poisonous spines, most notably the stone fishes and scorpion fishes occurring in Pacific and contiguous seas. The stone fish is an ugly fish that quietly sits upon the rocky substrate of shallow coastal waters waiting for its prey. Unlike other species it does not move when a human intruder appears, but rather holds its 'ground'. Thus, people who are wading in shallow waters sometimes step on these fishes with their upright dorsal fin spines which can puncture the skin readily and produce extremely painful stings that are usually not life threatening. Recent research has yielded several protein toxins which are currently being investigated. Scorpion fish have large pectoral and dorsal fins which have numerous poisonous spines also possessing protein toxins which depress neurotransmitter release from nerve terminals. Small scorpion fish are sometimes found in marine aquarium shops. Perhaps the most commonly encountered fishes with poisonous spines are sting rays. Unlike stone fish, sting rays usually swim away when disturbed. Waders in waters infested with these bottom dwelling fishes are advised to walk in a shuffling gait to provide the rays with enough advance notice of their presence and to wear boots when possible, to avoid being stuck by the 'whiplashing' tail spine. Some species of catfish also have stinging spines containing a venom which has not yet been characterized. Therapeutic treatments of individuals envenomated by poisonous fish spines are still largely symptomatic since antivenins are not usually available.

Treatment of Marine Envenomations and Intoxications

Relative to treatment of snake, spider, scorpion, and other terrestrial animal envenomations, the treatment of most envenomations due to marine animals is rather primitive. This is primarily due to our knowledge of these venoms being less complete. The incidence of jellyfish envenomation amongst swimmers is undoubtedly much higher than for stings of some of the above mentioned terrestrial serpents, but rarely are jellyfish stings life threatening unless the swimmer is stung over a large surface area by the Australian box jellyfish (*Chironex fleckeri*) or the hydrozoan Portuguese Man O'War (genus *Physalia*). However, marine 'toxinology' has made steady progress in the past two decades and one can expect antivenins for common marine envenomations to eventually become available. Antivenins are primarily useful for neutralizing proteinaceous venom constituents. If the effect of a venom is largely due to a single type of toxin, one can anticipate future treatments to be based on counteracting the effects of the toxin on its receptor target.

Toxins as Molecular Models for Development of New Drugs

Centuries ago the Swiss physician Paracelsus stated that all drugs are poisons and all poisons are drugs. While the first portion of this statement is generally considered valid, not all poisons are drugs. Nevertheless, there is a long tradition of developing materia medica from natural sources, generally plant extracts, which were used to treat a variety of disease conditions. An example would use of powdered leaves of the foxglove plant (and later purified digitalis alkaloids) to treat congestive heart failure. Toxins and other substances, because they often are potent modulators of particular ion channels or receptors, also can serve as 'lead' compounds for designing new drugs. Manipulation of the molecular structure frequently improves selectivity for a particular target (receptor) and thereby reduces the likelihood of adverse effects in therapeutic use. Toxic natural products isolated from several phyla of marine organisms have led to new drug candidates in recent years and there will likely be more in the not too distant future.

See also: Algae; Animals, Poisonous and Venomous; Saxitoxin; Shellfish Poisoning, Paralytic; Tetrodotoxin.

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Relevant Website

http://www.marine-medic.com.au – Marine-medic.com

Material Safety Data Sheets *See* Chemical Hazard Communication and Material Safety Data Sheets.

Maximum Allowable Concentration (MAC)

Shayne C Gad

Maximum allowable concentrations (MACs) are the maximum airborne concentrations that can be justified consistent with the objective of maintaining unimpaired health or comfort of workers or both. The criteria on which the standard is established are the avoidance of (1) undesirable changes in body structures or biochemistry, (2) undesirable functional reactions that may have no discernible effects on health, and (3) irritation or other adverse sensory effects.

MACs in the United States were established by The American National Standards Institute (ANSI); however, permissible exposure levels have superceded the use of MACs in the United States. Based on recommendations issued by the American Conference of Governmental Industrial Hygienists and ANSI, they serve the same function.

See also: American Conference of Governmental Industrial Hygienists; Exposure; Exposure Assessment; Occupational Toxicology.

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Maximum Tolerated Dose (MTD)

Shayne C Gad

The maximum tolerated dose (MTD) is commonly estimated to be the maximum dose that can be administered for the duration of a specific study that will not compromise the survival of the animals by causes other than carcinogenicity. If the MTD has been chosen appropriately, there should be no adverse effect on survival, only a modest decrement in body weight gain and minimal overt signs of toxicity. The MTD has been exceeded if there is increased mortality, severe body weight decrement, or marked signs of toxicity. It should be noted that another meaning for MTD has sometimes been 'minimum toxic dose'.

The information used for dose selection usually comes from subchronic toxicity studies, but other information about the pharmacological effects of a drug and its metabolism and pharmacokinetics may also be considered. The maximum recommended human dose (MRHD) of the drug might be an additional criterion, if this is known when the carcinogenicity studies are being designed.

For most pharmaceutical companies, the doses selected are as follows: The highest dose is selected to be the estimated MTD, the lowest dose is usually a small multiple of the MRHD (one to five times), and the mid-dose approximates the geometric mean of the other two doses.

The procedures for dose selection described previously are generally consistent with major regulatory

guidelines for carcinogenicity and other studies, for example, the *Redbook* from the US Food and Drug Administration. Earlier versions of the *Redbook* focused on direct food additives and color additives used in food. The *Redbook 2000* provides guidance for the safety assessment of food ingredients, including direct food additives, color additives used in food, Generally Recognized as Safe substances, food contact substances and constituents, or impurities of any of the above. There are, however, exceptions to the general approach described previously. For example, for nontoxic drugs, the difference between the high and the low doses may be many orders of magnitude if the high dose is set at the estimated MTD and the low dose is a small multiple of the clinical dose. Some guidelines require that the low dose be no less than 10% of the high dose. In this situation, it may be acceptable to set the high dose at 100 times the MRHD, even if the MTD is not achieved. Similarly, when a drug is administered in the diet, the highest concentration should not exceed 5% of the total diet, whether or not the MTD is achieved.

Metabolism and/or pharmacokinetic data, when available, should also be considered in the dose selection process. It is desirable that a drug not be administered at such a high dose that it is excreted in a different manner than at lower doses, such as the MRHD. Similarly, the high dose should not lead to the formation of metabolites other than those formed at lower (clinical) doses. If data show that a given dosage produces maximum plasma levels, administration of higher doses should be unnecessary. These considerations may be very useful when interpreting the results of the study or attempting to extrapolate the results to humans.

The dose range-finding study is necessary in most cases, but the suppression of body weight gain is a scientifically questionable benchmark when dealing with the establishment of safety factors. Physiologic, pharmacologic, or metabolic markers generally serve as better indicators of systemic response than body weight. A series of well-defined acute and subchronic studies designed to determine the 'chronicity factor' and to study onset of pathology can be more predictive for dose setting than body weight suppression.

Also, the MTD may well be at a level where the metabolic mechanisms for handling a compound at real-life exposure levels have been saturated or overwhelmed, bringing into play entirely artifactual metabolic and physiologic mechanisms. The regulatory response to questioning the appropriateness of the MTD as a high level has been to acknowledge that occasionally an excessively high dose is selected, but to counter by saying that using lower doses would seriously decrease the sensitivity of detection.

See also: Dose–Response Relationship; Food and Drug Administration, US; Investigative New Drug Application; LD_{50}/LC_{50} (Lethal Dosage 50/Lethal Concentration 50); Pharmacokinetics/Toxicokinetics; Redbook.

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Mechanisms of Toxicity

Sanjay Chanda and Harihara M Mehendale

Toxicity is mostly caused by alterations in normal cellular physiology and biochemistry, eventually leading to cell death and tissue damage. Although many toxic responses are ultimately from cell death and loss of critical organ function, other responses may be the result of biochemical and pharmacological imbalances in normal physiological processes (genetic alterations) that do not result in cell death. Understanding of how different chemicals can affect these phenomena at biochemical and molecular level is essential to avert or prevent the toxicity. Despite a common outcome of toxicity, from all chemical-induced injury, the extent of tissue damage necessary to cause a life-threatening response varies depending on the tissue type and rate at which the injury is caused. Epithelial tissues (e.g., liver, kidney, lung, and intestine) and DNA have a great capacity to repair or regenerate in response to a loss of tissue mass or DNA architecture. Other tissues (e.g., neuronal tissues) either have a very poor capacity to regenerate

or do not regenerate at all. It is also true that organs have a capacity for function that exceeds the requirements for the normal homeostasis and is referred to as functional reserve capacity. Reserve capacity allows the body to survive severe toxic insults that lead to significant loss of organ functions. Humans functioning with one kidney, a part of the lung removed, only a portion of liver, or only half of the normal amount of hemoglobin are examples of the functional reserve capacity.

There are many ways in which a chemical can interfere with the normal biochemistry and physiology of the cells and a chemical may cause toxicity to multiple tissues by multiple mechanisms. The following general categories of the mechanisms are neither comprehensive nor mutually exclusive but represent the major mechanisms of toxicity of many drugs, chemicals, and environmental agents.

Covalent Binding to Macromolecules

Many toxic substances exert their toxic effects by covalently binding to proteins, thiols, and nucleic acids. The binding can be either very tight (e.g., covalent binding by shared electrons) or loose through other labile bonds. Covalent binding can lead to longer lasting toxic effects. Proteins constitute many enzymes and regulate many functions and structural components of membranes that are critical to cellular function. Binding of hydrogen cyanide to the ferric atom of cytochrome oxidase and thus preventing the electron transport and, as a result, blocking the transport of oxygen by hemoglobin in the blood is a classic example of toxicity caused through protein binding. Carbon monoxide, on the other hand, principally blocks delivery of oxygen to tissues by taking the place of oxygen on hemoglobin, the oxygen carrying protein of the red blood cells. Chemically induced porphyria caused by halogenated hydrocarbons (e.g., hexachlorobenzene) and metals (e.g., lead and mercury) in part is also caused by inhibition of specific enzymes (by protein binding) of the heme biosynthetic pathway. Many toxic trace metals (e.g., arsenic, cadmium, mercury, and lead) also bind to proteins with free sulfhydryl groups (also known as thiols of proteins, amino acids, etc.), resulting in toxicity.

Many chemicals form reactive, electrophilic intermediates and free radicals during their metabolism in the body. These can be formed via enzyme-mediated reactions (many of which are oxidations) or from autoxidation of small molecules like flavins and thiols. These electrophilic intermediates covalently react with nucleophilic sites in the cell, including glutathione (GSH) and thiol-containing proteins, causing cellular dysfunction and oxidative stress to the cell. Acetaminophen is one such drug that forms a metabolite, *N*-acetyl-*p*-aminobenzoquinoneimine, which first depletes GSH and then covalently binds to protein thiols to cause toxicity. Binding to protein thiols results in the loss of activity of thiol-containing enzymes. Calcium transporting ATPase is a thiol-containing enzyme that is affected by covalent binding with many electrophilic intermediates of chemical toxicants. Binding of this enzyme results in loss of adenosine triphosphate (ATP), important for cell survival, or excessive accumulation of extracellular calcium inside the cells and this results in cell death.

Ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) contain numerous nucleophilic sites that react readily with electrophilic chemicals. Binding to the nucleophilic sites of RNA leads to perturbed synthesis of proteins, which are very critical for the normal functioning of the body. Production of somatic mutations through DNA–adduct formation by certain chemicals may be responsible for chemical carcinogenesis. Adduct formation with RNA and DNA can also alter the expression of certain genes, thereby affecting the formation of certain gene products resulting in disruption of normal functions and life cycle of cells. N7, N2, C2, and O6 positions of guanine appear to be important in DNA–adduct formation and are known to cause mutagenicity and carcinogenicity.

Some chemicals may also enter normal cellular pathways of metabolism and cause perturbations in cellular metabolism. Fluoroacetate is a well-known example. Cell death occurs via interference with energy production. Fluoroacetate enters the citric acid cycle (Krebs cycle) with the formation of fluorocitrate. Isocitrate dehydrogenase, the normal enzyme in the sequential energy-producing pathway, is unable to handle the substrate, consequently blocking the energy production pathway. Another example is galactosamine, a naturally occurring amine derivative (present in high amounts in lobster shells) of sugar glucose. Galactosamine enters the normal galactose metabolism pathway of the liver cells because one of the enzymes accepts galactosamine in place of its normal substrate galactose. The impostor makes its way to form uridine diphosphogalactosamine (UDP-galactosamine), but the next enzyme (epimerase) detects the impostor and cannot act on this abnormal substrate. This results in the trapping of uridine into a useless intermediate causing cellular depletion of uridine, thereby causing depletion of UTP, inhibition of RNA and protein synthesis, and glycogen formation. This 'uridyl trapping' leads to cellular death, causing liver toxicity. The toxicity can be reversed by administering orotic acid, a precursor of uridine.

Interference with Calcium (Ca^{2+}) Homeostasis

Disruption of intracellular Ca^{2+} homeostasis can result from excessive Ca^{2+} influx or release of Ca^{2+} from intracellular stores or from inhibition of extrusion of Ca^{2+} by the plasma membrane. Cellular Ca^{2+} is closely regulated by living cells. Ca^{2+} concentration is ~5000- to 10 000-fold higher outside the cells. If higher amounts of Ca^{2+} enter the cells (e.g., due to perturbation of plasma membrane) the Ca^{2+} ATPase pumps in the plasma membrane pump the excessive Ca^{2+} out of the cell. Additional finer regulation of Ca^{2+} inside the cell is accomplished mainly by two mechanisms – mitochondria sequester larger fraction of Ca^{2+} and endoplasmic reticulum can sequester smaller amounts of Ca^{2+}. Interference with the normal processes responsible for regulation of intracellular Ca^{2+} plays a critical role in chemical-mediated cell injury and necrotic cell death. One or more of these regulatory mechanisms may be perturbed by toxic chemicals. Accumulation of Ca^{2+} in the cells has been correlated with necrotic cell injury and cell death from ischemia and a variety of toxic agents. Blebing or development membrane abnormalities with disruption of cytoskeletal structure (disruption of actin microfilaments by the activation of phospholipases and proteases) have been found *in vitro* after increased intracellular Ca^{2+}. Nitrophenols, quinones, peroxides, aldehydes, dioxins, halogenated alkenes, alkanes, and some metal ions cause toxicity by disrupting Ca^{2+} homeostasis.

Disruption of Ca^{2+} homeostasis has also been implicated in 'programmed cell death' or apoptosis. An increased Ca^{2+} level in the nucleus activates some endonucleases, which result in DNA fragmentation and chromatin condensation.

Lipid Peroxidation

Carbon tetrachloride (CCl_4) toxicity is a typical example of toxicity due to lipid peroxidation. The cleavage of a carbon–chlorine bond in CCl_4 by the cytochrome P450 mixed function oxidase system generates a trichloromethyl free radical ($^{\bullet}CCl_3$), which reacts rapidly with oxygen to form trichloromethyl peroxy radical ($^{\bullet}CCl_3O_2$). These free radicals can initiate a process of autocatalytic lipid peroxidation by attacking the bridges of unsaturated fatty acid side chains of microsomal lipids. Also, unsaturated fatty acids in other cellular membranes are affected. Once organic free radicals are generated in this manner, a self-propagating runaway series of reactions leads to rapid destruction of cellular membranes causing cell death.

Oxyradicals and oxyradical stress may also cause lipid peroxidation. Free radical forms of oxygen include superoxide anion (O_2^-), hydroxyradical ($OH^{\bullet}$), and hydrogen peroxide (H_2O_2). These radicals are formed by a stepwise one-electron reduction of O_2. One-electron reduction of H_2O_2 leads to the formation of water (H_2O). Superoxide anion (O_2^-) is dismutated by a cellular enzyme known as superoxide dismutase, resulting in the formation of H_2O_2. Cellular iron can metabolize O_2^- to $OH^{\bullet}$ and hydroxy anion (OH^-) radicals. Under certain circumstances a singlet O_2 (O_2) can also be formed from O_2. In the normal cellular metabolism small amounts of oxyradicals are generated. However, these are of no consequence since cellular defense mechanisms (e.g., superoxide dismutase, catalase, glutathione peroxidase, and vitamin E) mitigate these oxyradicals. Chemical toxicants may disrupt this balance to either produce excessive O_2 radicals and/or to compromise the cellular defense mechanisms.

Certain toxic chemicals may form organic radicals by being reduced by one electron, a reaction mediated by the flavin enzyme, cytochrome P450 reductase. These organic one-electron reduction products (semiradicals) can donate this electron to O_2 in the cells to form O_2^-. The organic toxic (parent) chemical is now free to be reduced again by the reductase and generate additional O_2^- radical. This reduction–oxidation cycle can continue as long as the chemical, cellular O_2, and reducing equivalents (NADPH) are available in the cell. Thus, redox cycling can generate a virtually unending supply of oxyradicals. The herbicide paraquat, anticancer agent bleomycin, and antibiotics nitrofurantoin and mitomycin are a few examples of chemicals that can undergo redox cycle.

Reperfusion injury is also thought to be associated with oxyradicals. After hypoxia during the cessation (or reduction) of blood supply to tissues (as in surgical procedures) the cells may shut down the normal defense mechanisms. When blood supply is restored after surgery, the tissue encounters a normal amount of O_2, leading to formation of oxygen free radicals. Regardless of how they are formed, free radical forms of O_2 can also initiate and propagate lipid peroxidation leading to tissue injury.

Interference with Endogenous Pathways

Many chemicals produce toxicity by interfering with different endogenous pathways (e.g., cellular energy production and excitable membrane functions). ATP is the main form of energy utilized by the living cells. ATP is thus necessary to maintain the normal functions of the cells and significant depletion will

lead to loss of cell function and cell death. Cyanide, hydrogen sulfide, and azides bind to cytochrome oxidase and thus block utilization of oxygen by different tissues and thus inhibit ATP production. Rotenone and antimycin A interfere with specific enzymes in the electron transport chain necessary to generate ATP, while sodium fluoroacetate blocks Krebs cycle.

Different ion channels maintain the stability of excitable membranes that are necessary for normal functioning of the body. Saxitoxin, tetrodotoxin, and DDT all cause toxicity by blocking the sodium channel in excitable membranes. On the other hand, organic solvents cause toxicity by changing the membrane fluidity of the neurones in the central nervous system.

Stimulation and Blockade of Cell Cycle Progression

Regardless of the mechanisms of cell and tissue injury, toxic or physical injury elicits an endogenous cell proliferative and tissue repair response in the affected tissues and organs in the body. This is a parallel but opposing response to tissue injury. Chemicals vary in their ability to induce the compensatory tissue repair response. This response also varies depending on the strains and species. The human body is also capable of this tissue repair response. Simultaneous with the initiation of tissue injury, the surviving cells respond by receiving/sending appropriate cellular and molecular signals that lead to cell cycle progression beginning with G_0 to G_1 and G_1 to S phase synthesis. The first line of defense of tissue, however, occurs by the release of normally occurring small population of G_2 cells to divide. This occurs within a few hours of exposure to toxic chemicals. When these cells divide, a host of molecular messages are expressed that facilitate surviving cells to divide. Thus, this tissue repair response usually takes the form of a biphasic cell proliferation response. At low to moderately toxic doses, tissue repair response shows a classic dose–response relationship. This allows the body to repair the injured tissue and restore the structure and function of the tissue, thereby permitting complete regression of injury, recovery, and survival. At high doses, two events occur which lead to unrestrained progression of injury initiated by the mechanisms that initiate injury. First, the tissue repair response is significantly diminished. Second, it is delayed considerably. Delay leads to unrestrained progression of injury, and diminished response is too little to cope with the progression of tissue injury and destruction. There is significant interest in understanding the biological events and the molecular regulation of these events. If tissue repair response is stimulated through specific methods of stimulation (e.g., partial hepatectomy, prior exposure to a low dose of a toxic chemical, nutritional supplementation with energy sources that facilitate cell division, and activation of molecular signals), complete protection can be demonstrated from even lethal doses of toxic chemicals, even though normally lethal massive injury may be inflicted by any of the mechanisms described earlier (Harihara M Mehendale).

Cell cycle progression is very important for the body; in newborns and young adults it helps in the normal development of the body, while in adults it is essential to replace cells that are dead or dying (either by normal aging or chemical-induced necrosis). Usually, most of the cells in an adult are in resting or G_0 phase of cell division. As a response to cell injury or impeding cell death, the cells go to G_1, S, G_2, and then M or mitotic phase of cell division to produce newly divided daughter cells. Cancerous growth is a result of abnormal and uncontrolled cell division. Chemical carcinogenicity (like cancers of unknown or viral etiology) leads to unregulated tumor growth due to uncontrolled cell division. Normally, each phase of cell division is finely regulated by growth factors, cytokines, and many other products of gene expression (e.g., cyclines). Cellular transduction mechanisms are also involved in the regulation of cell cycle progression. This finely regulated balance is perturbed by the cancer-causing chemicals. Many anticancer drugs cause toxicity by blocking the progression of cells through the cell cycle. They can either arrest the cells in different phases of cell division or can block specific enzymes by binding with the enzymes needed for cell cycle progression. Colchicine, a common anticancer drug, not only blocks the M phase of cell division but also inhibits the enzyme activity of thymidylate synthetase and thymidine kinase to arrest the cells in S phase of cell division. Taxol is another example of anticancer agent that works by blocking cell division. In contrast to colchicine, which blocks cell division by inhibition of S-phase synthesis and by preventing microtubular function, taxol works by interfering with microtubule aggregation.

Genetic Alterations

Chemicals that cause toxicity by genetic alterations of the somatic cells are called genotoxic carcinogens. Covalent interactions with DNA do not always lead to cell death. The vast majority of lesions in DNA are repaired; but in some cases the repair is incorrect or

incomplete, leading to a mutated DNA. From this time point on, all the daughter cells produced by the mutated cell(s) are also mutated. If this mutation occurs in a somatic cell then this may eventually lead to cancer but cannot be passed to future generations. It is believed that genotoxic chemicals induce cancer by altering the protooncogenes. Protooncogenes carry a cancerous phenotype. Many of the gene products are actually responsible for determining cells' response to growth factors and cytokines.

See also: Carcinogen–DNA Adduct Formation and DNA Repair; Carcinogenesis; Lipid Peroxidation; Modifying Factors of Toxicity; Molecular Toxicology–Recombinant DNA Technology; Tissue Repair; Toxicity Testing, Mutagenicity.

Further Reading

Hayes AW (ed.) (1994) *Principles and Methods of Toxicology*, 3rd edn. New York: Raven Press.

Klaassen CD (ed.) (1996) *Casarett and Doull's Toxicology. The Basic Science of Poisons*, 5th edn. New York: McGraw-Hill.

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Medical Surveillance

Christopher P Holstege

This article is a revision of the previous print edition article by William Halperin, volume 2, pp. 279–283, © 1988, Elsevier Inc.

The term *medical surveillance* is vague, but commonly utilized. There is no generally accepted definition of medical surveillance. Medical surveillance involves performing an observational study of either an individual or a population and includes collection, collation, analysis, and dissemination of data. The data acquired varies, and may be on exposures, disease, injury, death, and/or disability. There are numerous examples of medical surveillance with vastly different models. For example, the United States has formed the Army Medical Surveillance Activity (AMSA). Its main functions are to analyze, interpret, and disseminate information regarding the status, trends, and determinants of the health and fitness of America's Army and to identify and evaluate obstacles to medical readiness. AMSA is the central epidemiological resource for the Army.

In order to adequately develop a medical surveillance plan, one must perform a thorough assessment of the physical, biologic, and/or chemical hazards to which personnel may be exposed and which have the potential to cause adverse health effects. For certain chemical substances, medical surveillance is prescribed by law. The standards are set by the Occupational Safety and Health Administration (OSHA). Attention to sensitivity, specificity, and predictive value is particularly important in occupational screening programs. The primary purpose of medical surveillance is the elimination of exposures that cause disease. Medical surveillance's ultimate goal is prevention.

Prevention

Primary Prevention

Primary prevention pertains to actions that are taken before disease develops. The first line of defense against toxin-induced disease is the recognition that specific exposures are hazardous; this distinction is based on either prior human experience or experimental evidence. Recognized hazards may then be eliminated from the environment through substitution, through engineering controls, or through personal protective equipment.

Secondary Prevention

Secondary prevention occurs when a disease is detected early when the individual is asymptomatic. *Medical screening* is the periodic examination of an individual in order to detect preclinical disease. Examples of medical screening include use of urinary cytology in workers exposed to bladder carcinogens and mammography for asymptomatic cancer of the breast.

Tertiary Prevention

Tertiary prevention involves detecting clinical signs and symptoms of disease early before significant adverse consequences of that disease occur. Tertiary prevention is the delivery of optimal clinical care to minimize the consequences of symptomatic disease.

Biological Monitoring

Biological monitoring involves the examination of a sample from an individual (i.e., urine or blood) to look for evidence of exposure to chemical hazards.

Depending on the chemical of interest, biologic monitoring may evaluate the unchanged chemical in body fluids, a metabolite of the original chemical, an enzymatic alteration, a physiologic effect, or a secondary clinical finding. Examples of biological monitoring include obtaining a blood lead level and/or zinc protoporphyrin level in a worker with known lead exposure, obtaining a urinary phenol level in a worker with benzene exposure, and obtaining a red blood cell cholinesterase level in a worker with organophosphate pesticide exposure. The American Conference of Governmental Industrial Hygienists publishes guidelines and reference values (biologic exposure indices) for biologic monitoring.

Sentinel Events

Medical surveillance also includes monitoring for a single case of new disease caused by a chemical agent that triggers an alarm in an astute clinician. Such cases, called *sentinel events*, result in further study and analysis to determine a true cause and effect relationship. Historically, numerous chemical agent induced disease processes have been determined through medical surveillance and sentinel event determination. For example, the relationship between vinyl chloride exposure and hepatic angiosarcoma development was determined by astute medical surveillance.

Public Health Surveillance

Public health surveillance pursues a number of goals. The first is the estimation of the magnitude of disease occurrence and its trends over time. The second is the identification of new opportunities of prevention including entirely new diseases as well as old diseases in new circumstances. Cases of well-established occupational disease, with known preventable etiologies, are known as Sentinel Health Events, Occupational (SHEO). Each case represents a failure of prevention. Investigation of SHEOs and intervention may lead to the identification of root causes of the failure of prevention and hence to improved prevention. A third goal is identifying epidemic clusters, or epidemics of diseases, so that resources can be targeted toward their prevention.

Conclusion

The goal of medical surveillance is prevention. However, medical surveillance is an ambiguous term that includes surveillance of individuals (medical surveillance) as well as populations (public health surveillance). It includes interest in early disease (medical screening) as well as in detecting evidence of exposure (biological monitoring). Medical surveillance is most likely to lead to prevention. It is most important that medical surveillance work in conjunction with environmental monitoring.

Contact Details

- Occupational Safety and Health Administration (OSHA)
  Permissible exposure limits (PELs) and a guide to OSHA standards for screening and surveillance
  200 Constitution Ave. NW
  Washington, DC 20210, USA
  Tel.: + 1-202-693-2300
  URL: http://www.osha.gov

- American Conference of Government Industrial Hygienists
  Threshold limit values (TLVs) and biological exposure indices (BEIs)
  1330 Kemper Meadow Dr., Suite 600
  Cincinnati, OH 45240, USA
  Tel.: + 1-513-742-2020
  URL: http://www.acgih.org

- National Institute for Occupational Safety and Health (NIOSH)
  Recommended exposure limits (RELs)
  1600 Clifton Rd. NE
  Atlanta, GA 30333, USA
  Tel.: + 1-800-356-4674
  URL: http://www.cdc.gov/niosh

See also: American Conference of Governmental Industrial Hygienists; Biomonitoring.

Further Reading

Baur X (1998) Medical surveillance programs in Germany. *International Archives of Occupational and Environmental Health* 71(1): 64–78.

Krieger GR, Brailsford CS, Balge M, and Harrison MC (2001) Medical surveillance and medical screening for toxic exposure. In: Sullivan JB and Krierger GR (eds.) *Clinical Environmental Health and Toxic Exposures*, 2nd edn., pp. 107–117. Philadelphia: Lippincott Williams and Wilkins.

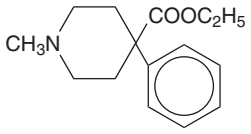
Stewart P and Stenzel M (2000) Exposure assessment in the occupational setting. *Applied Occupational and Environmental Hygiene* 15(5): 435–444.

Meperidine

Michael Hiotis

This article is a revision of the previous print edition article by Lisa Scheuring-Mroz, volume 2, pp. 238–285, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 50-13-5
- Synonyms: Pethidine hydrochloride; Demerol; Sonipeccaine hydrochloride; Pethadol; Centralgion; Dolantin
- Chemical/Pharmaceutical/Other Class: Synthetic opioid analgesic; a phenylpiperidine derivative
- Chemical Formula: $C_{15}H_{21}NO_2$
- Chemical Structure:



Uses

Meperidine is used as analgesic for acute and severe pain, as a preoperative medication, as an obstetrical analgesic, and for support of anesthesia.

Exposure Routes and Pathways

Meperidine is commercially available in parenteral solutions for intravenous and intramuscular administration and in oral tablets and solution.

Toxicokinetics

Meperidine is well absorbed from all routes of administration. Following oral administration, meperidine undergoes extensive metabolism on first pass through the liver. Meperidine is less than one-half as effective when given orally as when given parenterally. Following oral administration peak analgesia occurs within 1 h with duration of 2–4 h. Peak analgesia occurs 30–50 min after parenteral administration with duration of 2–4 h. Meperidine is metabolized primarily in the liver. It is demethylated to form normeperidine, which is then hydrolyzed along with meperidine to normeperidinic acid and meperidinic acid. The acid metabolites are less active than the meperidine and are further metabolized through conjugation. Normeperidine is pharmacologically active. Meperidine distributes widely into the liver, kidneys, and muscle. The volume of distribution is $3.84\,l\,kg^{-1}$. Protein binding is 65–75%. Meperidine plasma half-life is 2.4–4.0 h. The half-life of normeperidine is 15–30 h. Meperidine is excreted in the urine, ~5% as unchanged drug. The elimination half-life is biphasic: $T_{1/2}$ (alpha) is 12 min and $T_{1/2}$ (beta) is 3.2 h in individuals with normal renal and hepatic functions.

Mechanism of Toxicity

Meperidine's chief pharmacological action is interacting with opioid receptors in the central nervous system (CNS). The highest concentration of stereospecific binding sites is in the limbic system, thalamus, striatum, hypothalamus, midbrain, and spinal cord. Meperidine's effects may result from mimicking the actions of enkephalins and endorphins and also from altering the release of neurotransmitters. Accumulation of the metabolite normeperidine can result in the toxic effects secondary to CNS stimulation such as seizures, agitation, irritability, nervousness, tremors, twitching, and myoclonus. Patients with decreased renal function are at a higher risk for developing seizures and other toxic effects of the metabolite normeperidine.

Acute and Short-Term Toxicity (or Exposure)

Animal

Dogs act similarly to humans when exposed to opiates. Symptoms may include drowsiness, ataxia, respiratory depression, miosis, coma, seizures, and hypotension. Opiates and their derivatives have an excitatory effect on the CNS of cats and horses. Naloxone can be used at $0.02\,mg\,kg^{-1}$ if needed.

Human

When greater than therapeutic amounts are administered, CNS and respiratory depression, which may progress to cessation of respirations, may be seen. Pulmonary edema has been reported following therapeutic and toxic exposures to opiates. Peripheral vasodilation can cause hypotension and possibly circulatory collapse. In addition to the usual opiate toxicities, the course may be complicated by development of seizures. With daily doses of ≥ 3 g meperidine, convulsions may be seen due to metabolism of meperidine to normeperidine. Myoclonus will usually precede convulsions. Mydriasis may be present secondary to anoxia. Meperidine may also produce tachycardia through a vagolytic action, which increases ventricular response.

Semiquantitative and qualitative immunoassays can measure high concentrations of meperidine in the urine. Meperidine toxicity is reported with serum levels of 10–30 μg ml^{-1}; however, drug levels do not guide treatment. Normeperidine toxicity occurs with serum levels from 450 to 800 ng ml^{-1}.

Chronic Toxicity (or Exposure)

Animal

Chronic administration of meperidine in dogs at up to six times the maximum recommended therapeutic dose resulted in minor anorexia and weight loss.

Human

Addicts usually demonstrate symptoms of twitching, tremors, confusion, hallucinations, and convulsions at high doses. Meperidine use can produce physiologic dependence. An abstinence syndrome can begin within 3 h after use and peak at 8–12 h. The abstinence syndrome of meperidine consists of more severe muscular twitching and restlessness and fewer autonomic symptoms than other opiates.

In Vitro Toxicity Data

Studies of rat skeletal muscle mu 1 voltage dependent sodium channels have shown blockage of sodium channels; meperidine acts pharmacologically like a local anesthetic.

Clinical Management

Basic life-support measures should be instituted as necessary. Intensive support therapy may be required to correct respiratory failure and shock. Patients with mild to moderate toxicity may present with lethargy, miosis, decreased blood pressure, heart rate, and muscle flaccidity. With severe toxicity coma, respiratory depression, seizures, noncardiogenic pulmonary edema, apnea, and sudden death may occur. If taken orally, administration of activated charcoal is recommend as soon as possible to minimize absorption of meperidine. Emesis is contraindicated due to potential for seizures, and significant CNS and respiratory depression. The specific antagonist naloxone is used to counteract respiratory depression and coma. A dose of 0.4–2.0 mg is given intravenously and can be repeated at intervals of 2 or 3 min. The therapeutic effect of naloxone maybe of shorter duration than that of the opiate activity; therefore, a naloxone continuous infusion may then be of benefit. Nalmefene and naltroxone are other opioid antagonists. These antagonists are similar to naloxone but with longer half-life and may be considered as alternatives to naloxone. Naloxone does not antagonize the tremors or seizures caused by normeperidine. It will, however, antagonize the opiate effects. Seizures may be treated with intravenous benzodiazepines. Arterial blood gases, vital signs, and level of consciousness should be monitored continuously until cessation of symptoms. Patient should be monitored for at least 6–8 h after the last dose of naloxone is administered to prevent relapse of respiratory and CNS depression. Mepergan is a combination drug that contains meperidine and promethazine and when managing an overdose, the toxicity of both components should be considered.

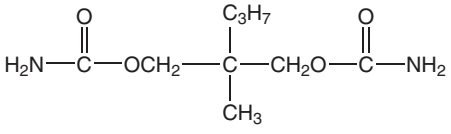
Further Reading

Goetting MG and Thirman MJ (1985) Neurotoxicity of meperidine. *Annals of Emergency Medicine* 14: 1007–1009.

Meprobamate

David Eldridge and Christopher P Holstege

- Chemical Abstracts Service Registry Number: CAS 57-53-4
- Synonyms: Miltown; Equanil; Meprobam; Meprobamatum; Procalmadiol; 2,2-Di(carbamoyloxymethyl)pentane; Carbamic acid 2-methyl-2-propyltrimethylene ester; 2-Methyl-2-propyl-1,3-propanediol dicarbamate; 2-Methyl-2-propyltrimethylene carbamate
- Chemical/Pharmaceutical/Other Class: Carbamate derivative
- Chemical Formula: $C_9H_{18}N_2O_4$
- Chemical Structure:



Uses

Meprobamate is used as a minor tranquilizer and as an anxiolytic agent.

Exposure Routes and Pathways

Meprobamate is available as tablets, an oral liquid, and extended-release capsules (Meprospan-200). Meprobamate is also the active metabolite of carisoprodol (Soma) an oral drug that is used as a muscle relaxant.

Toxicokinetics

Meprobamate is well absorbed by the gastrointestinal tract and when taken orally, at therapeutic dosing, has a peak serum concentration within 2–3 h. When taken in overdose, absorption can be prolonged (reported up to 13 h) and clinical signs and symptoms may be delayed. Meprobamate is rapidly metabolized in the liver to inactive hydroxy and glucuronide metabolites.

After absorption, meprobamate can be found throughout the body and has a volume of distribution of $0.75\,l\,kg^{-1}$. Plasma protein binding is 15%. It is excreted by the kidneys either in its unchanged form (10%) or as inactive metabolites.

Mechanism of Toxicity

Meprobamate's chief toxicity is through central nervous system depression. Its precise mechanism of action is unknown. It appears to inhibit or affect neurotransmission in the thalamus, hypothalamus, limbic system, and spinal cord. In high doses, meprobamate can act as a general anesthetic with respiratory depression and cardiovascular collapse thought to be from a combination of vascular muscle and direct cardiac effects.

Acute and Short-Term Toxicity (or Exposure)

Animal

The clinical effects of meprobamate in animals are similar to those observed in humans, although animals appear to be less sensitive to meprobamate than humans.

Human

No reliable toxic dose range of meprobamate is known. Death has resulted following the ingestion of 12 g, and survival has been reported at 40 g. Due to this variability, clinical symptoms should be the guide to intervention. Signs and symptoms of acute toxicity include central nervous system depression ranging from mild stupor to deep coma, slurred speech, weakness, vertigo, and ataxia. Profound hypotension and shock can occur. The most common pulmonary toxicity is respiratory depression, but pulmonary edema has been reported.

Chronic Toxicity (or Exposure)

Human

Prolonged use of high doses of meprobamate can cause slurred speech, ataxia, and vertigo. One of the most concerning problems with chronic use is the physical and psychological dependence that can both develop with regular use. Chronic use of high doses followed by abrupt cessation can produce a severe withdrawal syndrome. This syndrome can appear clinically similar to barbiturate withdrawal and can produce anxiety, tremors, insomnia, nausea, vomiting, delirium, hallucinations, and seizures. Meprobamate withdrawal is potentially life-threatening.

In Vitro Toxicity Data

Several mutagenicity studies in *Drosophila* models have been either inconclusive or negative. Studies of meprobamate activity from cultured rat hippocampal neurons have described meprobamate enhanced GABA-evoked responses in a concentration-dependent manner.

Clinical Management

Basic and advanced life-support measures are the most important component of clinical management. Serum levels of meprobamate are not routinely available and are therefore not useful in clinical management decisions. The administration of activated charcoal may be considered for substantial recent ingestions and a second dose may be used in patients who demonstrate continued drug absorption. The patient's level of consciousness and vital signs should be monitored closely. There is no antidote for meprobamate. If hypotension occurs, intravenous fluids should be infused. If hypotension is refractory to intravenous fluids, then vasopressors may be considered. Forced diuresis is ineffective and potentially harmful as it may lead to fluid overload. Hemodialysis has been reported to be efficacious at enhancing elimination of meprobamate, but should be considered only in severe cases when supportive care is insufficient to stabilize the patient. In chronic meprobamate users who suddenly stop therapy, withdrawal may be severe and life threatening. This withdrawal can be minimized by gradually weaning meprobamate over 2 weeks. If a

patient develops severe withdrawal symptoms, such as seizures, phenobarbital or benzodiazepines should be used to control symptoms.

See also: Barbiturates, Long-Acting; Barbiturates, Short-Acting; Benzodiazepines.

Further Reading

Allen MD, Greenblatt DJ, and Noel BJ (1977) Meprobamate overdosage: A continuing problem. *Clinical Toxicology* 11: 501–515.

Eeckhout E, Huyghens L, and Loef B (1988) Meprobamate poisoning, hypotension and the Swan–Ganz catheter. *Intensive Care Medicine* 14: 437–438.

Mercaptans

Lee R Shugart

- Representative Chemical: Methyl mercaptan
- Chemical Abstracts Service Registry Number: CAS 74-93-1
- Synonyms: Methanethiol; Mercaptomethane; Thiomethyl alcohol
- Chemical/Pharmaceutical/Other Class: Sulfur-containing organic chemical substances of the type R–SH (R = an alkyl group)
- Chemical Formula: CH_3SH

Uses

Used as an intermediate in the manufacture of jet fuels additives, pesticides, fungicides, plastics, and in the synthesis of methionine.

Background Information

Mercaptans are colorless and odorous gases linked to animal facilities, wastewater treatment plants, and paper and pulp manufacturing. It is released from decaying organic matter in marshes and is present in the natural gas of certain regions of the United States, in coal tar, and in some crude oils. If mercaptans are in the air, even at low concentrations, they are very noticeable.

Exposure Routes and Pathways

The main route of exposure is via inhalation.

Toxicokinetics

There are no toxicokinetic studies for methyl mercaptan via inhalation, oral, or dermal routes of exposure. *In vitro* studies in both humans and rats show that methyl mercaptan is oxidized (the carbon–sulfur bond is split) in blood by erythrocytes resulting in formic acid, sulfite, and sulfate ions.

Mechanism of Toxicity

Mercaptans act mainly as an irritant affecting the mucous membranes of the nose and respiratory tract.

Acute and Short-Term Toxicity (or Exposure)

Human

Mercaptans are considered to be slightly toxic if inhaled. Short-term exposure to slight amounts may result in coughing and irritation of the respiratory tract. Typically, these symptoms remit after a short period of time.

Chronic Toxicity (or Exposure)

Human

Exposure to high concentration (severe exposure) of mercaptans may produce central nervous system effects such as headache, staggering gait, muscular weakness, tremors, lung edema, convulsions, and paralysis of the respiratory center. Long-term health effects are not well documented.

Clinical Management

If inhaled and breathing is difficult, the person should be moved to fresh air and administered oxygen.

Environmental Fate

Relatively little is known about the fate of mercaptans once they are released to the environment.

Ecotoxicology

Animal toxicity: LC_{50} (mortality) for male and female rats is 675 ppm for 24 h. No mortality and no compound-related histopathological change were

noted in lungs of rats exposed continuously at 57 ppm for 3 months.

Exposure Standards and Guideline

The US Environmental Protection Agency requires that discharges, spills, or accidental release of 100 pounds or more of methyl mercaptan must be reported. Occupational Safety and Health Administration has set a permissible exposure limit of 20 mg of methyl mercaptan per cubic meter of air (20 mg m^{-3}) for an 8 h workday in a 40 h workweek. National Institute for Occupational Safety and Health recommends an occupational exposure limit of 1 mg m^{-3} for methyl mercaptan over 15 min and a threshold limit value for an 8 h time-weighted average of 0.5 ppm.

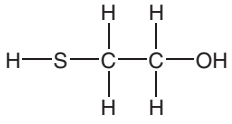
Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Mercaptans.

Mercaptoethanol, 2-

Patricia J Beattie

- Chemical Abstracts Service Registry Number: CAS 60-24-2
- Synonyms: 2-ME; Mercaptoethanol; 1-Ethanol-2-thiol; 2-Hydroxy-1-ethanethiol; 2-Hydroxyethyl mercaptan; Monothioethyleneglycol; 2-Thioethanol; Thioglycol
- Chemical/Pharmaceutical/Other Class: Thiol; Mercaptan
- Chemical Formula: $HSCH_2CH_2OH$
- Chemical Structure:



Uses

2-Mercaptoethanol (2-ME) is used as an initiator for polymeric reactions; a reagent for diagnostic bioassays; a rust inhibitor for steel; a brightening agent in copper deposition; a tarnish remover for alloys and metals; an ingredient in hair permanent chemicals, and in hair and wool dyes; a stabilizer; and a mosquito control agent.

Exposure Routes and Pathways

Skin and eye contact and inhalation are the primary routes of exposure.

Toxicokinetics

2-ME is metabolized to 2-mercaptoacetate by alcohol and aldehyde dehydrogenase. Inhibition of alcohol dehydrogenase in experimental animals, blocking the formation of 2-mercaptoacetate, eliminated the adverse liver effects typically seen after exposure to 2-ME. No metabolic data with respect to biological half-life were found.

Mechanism of Toxicity

2-ME exposure in rats induced a fatty liver condition as shown by a significant rise in liver triacylglycerol and blood-free fatty acid levels, a slight reduction of liver phospholipids, and a progressive decrease of blood triacylglycerol (25%) and blood phospholipid levels (30%), as well as a reduction of hepatic ketone body levels. 2-Mercaptoacetate induced effects similar to those produced by 2-ME on the liver and blood, consistent with the data indicating that this is the metabolite responsible for the blood and liver toxicity seen following 2-ME exposure. 2-ME has also been reported to inhibit thymus deoxycytidine formation, inhibit mitotic activity in the rat intestine, inhibit thymidine incorporation in rat DNA, and cause moderate deoxyribonuclease inhibition.

Acute and Short-Term Toxicity (or Exposure)

Animal

In a number of studies, 2-ME has been reported to cause skin and eye irritation in mice and rabbits. Acute ingestion studies in mice indicate the LD_{50} is 348 mg kg^{-1}. The animals exhibited signs of central nervous system (CNS) depression with death due to respiratory failure. Microscopic examination found lymphocyte infiltration of the liver and kidneys, destruction and hemorrhaging of the lungs, and foci inflammation of the myocardium.

Human

In humans, 2-ME is extremely irritating to skin, eyes, and mucous membranes and may cause the development of contact dermatitis and pulmonary edema. Like other thiols, 2-ME may depress the CNS and cause respiratory paralysis and death. One case of an accidental spill of 2-ME was reported to the Centers for Disease Control and Prevention, with no ill effects observed in exposed individuals.

Chronic Toxicity (or Exposure)

Animal

A 6 month inhalation study was conducted in rats in which the animals were exposed daily to 10 mg m^{-3}. At 3 months, neuromuscular depression, lymphopenia, neutrophilia, and decreased oxygen consumption were observed. In the fifth month, increased organic sulfate elimination, variation in weight, arterial pressure, liver function, and protein metabolism were observed. Liver damage was reported upon histological examination. Studies on the teratogenic, embryotoxic, and cytogenic effects of 2-ME have been inconclusive.

Human

The literature contains no information on human long-term exposure to 2-ME.

Clinical Management

If contact with 2-ME occurs, the affected areas should be flushed immediately with large amounts of water. When eye contact has occurred, the affected person should be referred to a medical facility after eye washing has been completed. Victims who are overcome with fumes should be removed to fresh air. If breathing has stopped, artificial respiration should be administered. If ingested, medical attention should be obtained immediately.

Environmental Fate

In air, 2-ME degrades rapidly by reaction with photochemically produced hydroxyl radicals with an estimated half-life of 8.7 h. 2-ME added to soil at 10 000 ppm has been reported to biodegrade at a rate of 40 kg week^{-1}. 2-ME is miscible in water, but there is insufficient data to predict the biodegradation rate in water. The bioconcentration factor for 2-ME and its miscibility in water suggest that it will not bioconcentrate in aquatic organisms.

Exposure Standards and Guidelines

American Industrial Hygiene Association: workplace environmental exposure level: 8 h; time-weighted average: 0.2 ppm, skin.

Miscellaneous

It is manufactured by reacting ethylene chlorohydrin with sodium hydrogen sulfide or by reacting ethylene oxide with hydrogen sulfide. 2-ME is a white liquid with a strong unpleasant odor.

See also: Respiratory Tract; Sensory Organs; Skin.

Further Reading

Sax NI and Lewis RJ Sr (eds.) (1987) *Hawley's Condensed Chemical Dictionary*, 11th edn., p. 740. New York: Van Nostrand Reinhold Co.

Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Mercaptoethanol.

Mercuric Chloride

Vishal S Vaidya and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 7487-94-7
- Chemical/Pharmaceutical/Other Class: Mercurial compounds
- Chemical Formula: $HgCl_2$

Uses

Mercuric chloride is used in preservatives for wood and anatomical specimens, embalming solutions, disinfectants, photographic intensifiers, leather tanning, seed treatments, analytical reagents for organic syntheses, and the manufacture of other mercury-containing compounds. Pharmaceuticals containing mercuric chloride have also been used therapeutically as topical antiseptics and disinfectants.

Exposure Routes and Pathways

In its elemental form, mercury is a heavy silvery liquid at room temperature and has a very high vapor pressure. Mercury vapor is more soluble in plasma, whole blood, and hemoglobin than in distilled water, where it dissolves only slightly. The major natural sources of mercury are degassing of the earth's crust, emissions from volcanoes, and evaporation from natural bodies of water. (The worldwide mining of mercury is estimated to yield $\sim$10 000 tons per year. The activities lead to some losses of mercury and direct discharges to the atmosphere.) Other important sources are fossil fuel combustion, metal sulfide ore smelting, gold refining, cement production, refuse incineration, and industrial applications of metals.

Occupational exposure to inorganic mercury has been investigated in chloralkali plants, mercury mines, thermometer factories, refineries, and in dental clinics. High mercury levels have been reported for all these occupational exposure situations, although levels vary according to work environment conditions.

Toxicokinetics

Gastrointestinal absorption of mercuric chloride from food is less than 15% in mice and $\sim$7% in a study of human volunteers. In humans and other mammals, the kidneys are the primary targets where mercuric ions accumulate. Renal uptake and accumulation of mercury *in vivo* are rapid. As much as 50% of low dose of mercuric chloride ($0.5\,\mu mol\,kg^{-1}$) has been shown to be present in the kidney of rats within a few hours after exposure. Within the kidney it accumulates primarily in the cortex and outer stripe of outer medulla. Mercuric chloride does not readily cross the blood–brain barrier but will accumulate in the placenta. Urinary and fecal excretion of mercury is the principal means by which humans and other mammals eliminate the different forms of mercury from the body. Under most circumstances, a greater fraction of a dose of mercury is excreted in the feces than in the urine early after exposure to a nonnephrotoxic dose of mercuric chloride.

Mechanism of Toxicity

A reference from Middle Ages in Goldwater's book on mercury describes oral ingestion of mercury as causing severe abdominal cramps, bloody diarrhea, and suppression of urine. This is an accurate report of the effects following accidental or suicidal ingestion of mercuric chloride. Injection of mercuric chloride produces necrosis of the epithelium of the pars recta kidney. Cellular changes include fragmentation and disruption of the plasma membrane and its appendages, vesiculation and disruption of the endoplasmic reticulum and other cytoplasmic membranes, dissociation of polysomes and loss of ribosomes, mitochondrial swelling and loss of amorphous intramatrical deposits, and condensation of nuclear chromatin. Although exposure to high dose of mercuric chloride is directly toxic to renal tubular lining cells, chronic low-dose exposure may induce an immunologic glomerular disease. This form of chronic mercury injury to the kidney is clinically the most common form of mercury-induced nephropathy. Experimental studies have shown that the pathogenesis of chronic mercury nephropathy has two phases: an early phase characterized by antibasement membrane glomerular nephritis followed by a superimposed immune complex glomerulonephritis with transiently raised concentrations of circulating immune complexes.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute LD_{50} of mercuric chloride lies between 10 and $20\,mg\,kg^{-1}$ i.p. for rodents. The features of acute toxicity usually consist of shock, cardiovascular collapse, acute renal failure, and severe gastrointestinal damage. Exposure of animals to mercuric chloride primarily causes nephrotoxicity. Renal injury induced by mercuric chloride is generally expressed fully during the initial 24 h after exposure and can be induced in rats with single dose as low as $1.5\,\mu mol\,Hg\,kg^{-1}$. Rats tend to be more vulnerable to nephrotoxic effects of mercuric chloride than New Zealand White rabbits or several strains of mice. In rats, nephrotoxic doses of mercuric chloride produce selective alterations in the pars recta causing selective necrosis of the proximal tubules. *p*-Aminohippuric acid is produced in the pars recta, and its secretion is very sensitive to mercuric chloride.

A 16 day study was conducted by the National Toxicology Program in which rats and mice of each sex were administered six different concentrations (0–$20\,mg\,kg^{-1}$ for rats and 0–$80\,mg\,kg^{-1}$ for mice) of mercuric chloride in deionized water by gavage for 12 days. A significant mortality was recorded with administration of the highest dose of mercuric chloride in both rats and mice. Analysis of kidney, liver, and brain tissues for mercury residues revealed that the highest concentration was in the kidneys of rats and mice. Acute renal tubule nephropathy occurred in dosed rats and was slightly more severe in males than in females. Chemical-related lesions in mice included renal tubule necrosis, inflammation

and necrosis of the forestomach, and necrosis of the glandular stomach.

Human

Mercuric chloride is primarily a skin and mucous membrane irritant that is rapidly absorbed. Acute poisoning by ingestion or inhalation may cause severe nausea, vomiting, hematemesis, abdominal pain, diarrhea, melena, renal damage, and prostration. Ingestion of 1–2 g mercuric chloride may be fatal. Acute poisoning and death have also resulted from dermal applications of mercuric chloride solutions. In 1951, 18 cases of human poisoning were reported following oral ingestions of single dose of mercuric chloride, nine of which resulted in death. The lethal doses ranged from 29 mg kg^{-1} body weight to at least 50 mg kg^{-1}. The most common autopsy findings in these cases were gastrointestinal lesions (ranging from mild gastritis to severe necrotizing ulceration of the mucosa) and renal lesions that had resulted in renal failure.

Chronic Toxicity (or Exposure)

Animal

Groups of rats and mice ($n = 60$) of each sex received three different concentrations (0, 2.5, or 5 mg $HgCl_2$ kg^{-1} for rats and 0, 5, or 10 mg kg^{-1} for mice) in deionized water by gavage 5 days per week for 2 years. A 15 month interim evaluation suggested that the severity of nephropathy was increased in male rats and mice as compared to females. Chronic nephropathy appeared to develop at an accelerated rate and led to decreased survival in both dosed male rat groups at the end of 2 years. Secondary effects of renal dysfunction in dosed male rats resulted in increased incidences of fibrous osteodystrophy of the bone, mineralizations of various tissues, and parathyroid gland hyperplasia. Under the conditions of these 2 year gavage studies, focal papillary hyperplasia and squamous cell papillomas in the forestomach as well as thyroid follicular cell adenomas and carcinomas were observed in male rats. In the same study, evidence for increases in squamous cell papillomas in the forestomach of female rats was equivocal. An equivocal evidence for renal adenomas and adenocarcinomas was observed in male mice. This tumor type is rare in mice, and the increase in incidence was statistically significant when compared with historic controls. Two other nonpositive lifetime rodent studies were considered inadequate. Based on the absence of data in humans and limited evidence of carcinogenicity in rats and mice it has been termed as a Class 3 carcinogen. The relevance of the forestomach papillomas to assessment of cancer in humans is questionable because no evidence indicated that the papillomas progressed to malignancy. The relevance of the increase in thyroid tumors has also been questioned because these tumors are generally considered to be secondary to hyperplasia; this effect was not observed in the high-dose males. It should also be noted that the authors considered the doses used in the study to exceed the maximum tolerated dose for male rats.

Human

Most of the studies with potential mercury toxicity in humans deal with metallic mercury vapor or methylmercury. There are no adequate epidemiological studies with mercuric chloride exposure in humans.

In Vitro Toxicity Data

Results from genetic toxicity studies using a variety of assays indicate that mercuric chloride is not mutagenic in bacteria or yeast, but it may produce chromosomal damage and mitotic disruption (c-mitosis) in some plant and animal test systems.

Clinical Management

A patient airway should be established. Suction may be used if necessary. Signs of respiratory insufficiency should be watched out for and assisted ventilations provided if necessary. Oxygen should be administered by nonrebreather mask at 10–15 l min^{-1}. Pulmonary edema should be monitored and treated if necessary; shock should be monitored and treated if necessary. Seizures should be anticipated and treated if necessary. For eye contamination, eyes should be flushed immediately with available water. Each eye should be irrigated continuously with normal saline during transport. Emetics should not be used. For ingestion, the mouth should be rinsed and 5 ml kg^{-1} up to 200 ml of water for dilution should be administered if the patient can swallow, has a strong gag reflex, and does not drool. Activated charcoal should be administered.

Environmental Fate

Mercury adsorbed from mercuric chloride and 2-methoxy-ethylmercury chloride (Aretan) solutions by three contrasting soils showed a dependence on soil–solution ratio and initial mercury (Hg) concentration in soil solution. Changing the soil solution ratio from 1:10 to 1:100 but keeping the initial concentration constant resulted in an increase in initial concentration but, on the other hand, resulted in decrease in Hg adsorption. Upon manipulation of the pH of the surface soils, adsorption of mercuric

chloride at 100 mgHg l^{-1} concentration increased from ~70 to over 95 mgHg kg^{-1} when the pH was raised from 5.0 to 8.0. Precipitation of Hg may also have contributed to this trend. Removal of organic matter from soil resulted in large reductions of Hg adsorbed, as much as 95% from the mercuric chloride solutions. Mercuric compounds found in the atmosphere are likely to be transformed by chemical or physical processes. Theoretical calculations on the photodissociation of mercuric compounds have indicated that mercuric chloride and mercuric cyanide are stable, while mercuric hydroxide may dissociate in the gas phase. Exchange reactions between water and mercury compounds are likely to occur in the atmosphere. The result of these exchange reactions eventually results in the release of elemental mercury into the gaseous phase.

Ecotoxicology

The organic forms of mercury are generally more toxic to aquatic organisms than the inorganic forms. Aquatic plants are more affected by mercury in the water at concentrations approaching 1 mg l^{-1} for inorganic mercury (mercuric chloride) but at much lower concentrations of organic mercury. Aquatic invertebrates vary greatly in their susceptibility to mercury. Generally, larval stages are more sensitive than adults. The 96 h LC_{50} vary between 33 and 400 µg l^{-1} for freshwater fish and are higher for seawater fish. However, the organic mercury compounds are more toxic.

Environmental Bioconcentration

Bioconcentration factors of 10 000 and 40 000 have been obtained for mercuric chloride and methylmercury with oyster.

Atmospheric Concentrations

In the atmosphere, particulate bound mercury constitutes only ~2% of total mercury in the air and has normally been found to be less than 0.1 ng m^{-3} in regions unaffected by local sources. Some other mercury compounds, which may exist in the atmosphere, are mercuric chloride, mercuric bromide, mercuric hydroxide, mercuric sulfide, and mercuric cyanide. The rest is elemental mercury in the gaseous phase. In remote areas over the Atlantic and Pacific oceans, mercury bound to particulate matter concentrations are generally at or below the picogram per cubic meter level.

See also: Kidney; Mercury.

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Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Mercuric Chloride.

Mercury

Shayne C Gad

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- Chemical Abstracts Service Registry Number: CAS 7439-97-6
- Synonyms: Hydrargyrum; Liquid silver; Quicksilver
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formulas: Hg^{+}; Hg^{2+}

Uses

Mercury is a naturally occurring metal found throughout the environment as the result of normal breakdown of minerals in the earth's crust by weathering processes involving wind and water. With the exception of mercury ore deposits, the amount of mercury that naturally exists in any one place is usually very low. Natural phenomena such as

erosion and volcanic eruptions, and anthropogenic activities like metal smelting and industrial production and use may lead to substantial contamination of the environment with mercury.

Mercury was known in antiquity and utilized by alchemists. Its neurological effects were recognized early, and its use in the hat-making trade gave rise to the phrase 'mad as a hatter'. Mercury has been used commercially and medically for centuries. In the past it was a common constituent of many medications, for example, it was used in the treatment of syphilis. Use of mercury has been drastically reduced in recent years. Within the twentieth century, mercury used to be in every physician's or pharmacist's armamentarium, for example, calomel was commonly used in infant teething powders in the 1930s and 1940s.

The antibacterial and antifungal properties of organomercurials have resulted in their long-term use as topical disinfectants (thimerosal and merbromin) and preservatives in medical preparations (thimerosal) and grain products (both methyl and ethyl mercurials). Phenylmercury has been used in the past in paints, and dialkyl mercurials are still used in some industrial processes and in the calibration of certain analytical laboratory equipment. A major issue in recent years has been the presence of mercury in some vaccines, for example, in the vaccine preservative thimerosal – this has led to suspension of some vaccination programs and the development of preservative-free (i.e., mercury-free) vaccines as replacements.

Mercury can be used for the extraction of gold. In hospitals and homes, it is still used in thermometers and blood-pressure cuffs, can be found in batteries, switches, and fluorescent light bulbs. Large amounts of metallic mercury are employed as electrodes in the electrolytic production of chlorine and sodium hydroxide from saline. Today, exposure of the general population comes from three major sources: fish consumption, dental amalgams, and vaccines.

Despite the extensive knowledge of the dangers of mercury, it is still misused and mishandled. For example, mercury has been sold in recent years in the United States under the name Azogue in Hispanic botanicas for oral administration to treat constipation, colic, or stomachache, and there have also been cases of mercury poisoning due to the use of a beauty cream, Crema de Belleza–Manning (6–10% w/w mercury), produced in Mexico but 'commonly used among women of childbearing age'.

Exposure Routes and Pathways

Humans may be exposed to organic forms of mercury by either inhalation, oral, or dermal routes, and the effects of such exposure depend upon both the type of mercury to which exposed and the magnitude of the exposure. Most of the mercury found in the environment is inorganic mercury (metallic mercury and inorganic mercury compounds). This inorganic mercury can enter the air from deposits of ore that contain mercury, the burning of coal or garbage, and the emissions of factories that use mercury. Inorganic mercury may also enter water or soil from rocks that contain mercury, factories or water treatment facilities that release water contaminated with mercury, and the disposal of wastes. Inorganic or organic compounds of mercury may be released to the soil through the use of mercury-containing fungicides.

If mercury enters the water in any form, it is likely to settle to the bottom where it can remain for a long time. Mercury also remains in soil for a long time. Mercury usually stays on the surface of the sediments or soil and does not move through the soil to underground water. It is a liquid at room temperature, and evaporates into the air.

Inhalation and ingestion are the major routes of exposure, but it can also be absorbed through the skin. In its elemental form, mercury is the only metal that is in a liquid state at room temperature. It readily volatilizes at standard temperature and pressure, and its presence in open containers can result in biologically significant air concentrations in unventilated or poorly ventilated spaces. As an example, in a hospital laboratory study, mercury vapor levels of up to $0.71\,\mathrm{mg\,m^{-3}}$ were reported in the general air of the institutions' laboratories; this was not surprising given the approaches to mercury cleanup in nonchemical laboratory areas included 'sweeping up with Kimwipes', 'wipe up with filter paper', or 'try to retrieve it'.

Extensive studies have been conducted on the consumption of mercury (as methylmercury) from fish.

Toxicokinetics

Absorption varies significantly according to the form of exposure (elemental, inorganic, or organic). Inhaled mercury vapor crosses through the alveolar cells readily, is $\sim$75% absorbed, and is carried by the red blood cells. Catalase in these cells oxidizes elemental mercury almost at once to the divalent state. Alcohol inhibits the catalase activity; however, in the seconds it takes for a complete blood circulation cycle, a significant amount of free mercury can cross the blood–brain barrier.

Ingested or dermally applied elemental mercury is essentially not absorbed (an exception involved infants who absorbed mercury from disinfected

diapers). It is estimated that humans absorb $<10\%$ of ingested elemental mercury; whereas absorption of ingested methylmercury can be as high as 90%.

Mercury will cross the placental barrier. In mammalian tissue, organic mercury, especially alkyl mercury, is converted to inorganic forms but not vice versa. Inorganic forms of mercury (not organic forms) induce a metallothionein. Inorganic mercury concentrates mainly in the kidney. Organic mercury compounds, being lipid soluble, concentrate in adipose tissue and the brain. Elimination is primarily in the urine and the feces, with small amounts in breath, sweat, and saliva.

Mammalian tissue does not convert elemental mercury to methylmercury.

Mechanism of Toxicity

Mercury has a great affinity for sulfydryl moieties and, hence, binds and inactivates a variety of enzymes. Methylmercury also initiates lipid peroxidation, which can produce alterations in cell membranes. Mercury damages the microtubules in the brain by reacting with the protein tubilin.

Acute and Short-Term Toxicity (or Exposure)

Animal

Evidence of damage to brain, kidney, heart, and lungs have been reported in rabbits exposed acutely to metallic mercury vapor at certain concentrations. Both reversible and irreversible toxic effects may be caused by mercury and its compounds. The rabbit (inhalation) LC_{Lo} is $29\,mg\,m^{-3}$ over 30 h, and another rat (inhalation) TC_{Lo} is $4\,mg\,m^{-3}$ over 2 h a day for 11 days.

Human

Mercury is an accumulative poison. Its toxicity depends on its form. Symptoms may start rapidly after acute exposure to high air concentrations of mercury vapor, and can include fever, chills, and nausea. In severe cases (e.g., as a consequence of heating), pulmonary edema may cause death within a few days. Acute exposure to mercury vapor can also produce bronchitis and interstitial pneumonitis. The toxicity of mercuric chloride (i.e., corrosive sublimate) has been well established. Oral ingestion causes severe abdominal cramps, possible ulceration and bleeding of the gastrointestinal tract, and a bloody diarrhea. Loose teeth are noted and hepatitis has been recorded. Nephritis is common; if the renal tubes are extensively damaged, it could lead to a possible fatal uremia. Renal failure occurs rapidly and, when patients survive, they must be maintained by dialysis. Regeneration of some kidney cells is possible but the damage is usually permanent. Mercurous chloride is relatively insoluble and, thus, much less toxic than the soluble mercuric chloride.

Chronic Toxicity (or Exposure)

Animal

The carcinogenicity and mutagenicity of mercury have been claimed; however, further verification is needed. Although mercury is fetotoxic, the teratological aspect also needs further study. The rat inhalation TC_{Lo} is $1\,mg\,m^{-3}$ over 24 h a day for 5 weeks, $8\,mg\,m^{-3}$ over 6.5 h a day for 41 weeks, and $17\,mg\,m^{-3}$ over 2 h a day for 31 days.

Human

The neurotoxicity of mercury is devastating, especially for the central and peripheral nervous systems of children. Central nervous system defects and erethism as well as arrythmias, cardiomyopathies, and kidney damage have been associated with mercury exposure. The central nervous system symptoms include loss of memory, excitability, fever, and local tremors that can progress to the entire body. Necrotizing bronchitis and pneumonitis from inhalation of mercury vapor can lead to respiratory failure. Mercury is also considered a potent immunostimulant and suppressant, depending on exposure dose and individual susceptibility, producing a number of pathologic sequelae including lymphoproliferation, hypergammaglobulinemia, and total systemic hyper- and hypo-reactivities. Other clinical signs include inflammation of mouth and gums (gingivitis), tremors, loosening of teeth, jerky gait, personality change, depression, irritability, and nervousness. Other congenital abnormalities con occur with prenatal exposure to methylmercury.

The importance of workplace and home diligence in the handling and disposal of mercury is illustrated by a 9-year-old boy who developed encephalopathy and peripheral neuropathy as a result of having dismantled, 3 months before the visit to the doctor, a sphygmomanometer that had been provided by the hospital for monitoring of blood pressure at home. The family was unaware of the potential risks of mercury and when the boy informed his mother a few days after spilling the mercury in his bedroom, she attempted to dispose of the mercury by vacuuming it and then flushing it down the toilet. He was found to have a blood mercury level of $1000\,nmol\,l^{-1}$ (normal $<30\,nmol\,l^{-1}$) but slowly recovered after chelation.

Mammalian tissue does not convert elemental mercury to methylmercury; however, methylmercury can reside in the muscle and liver of fish, and has led to disasters such as the Minamata Bay and Niigata in Japan involving consumption of mercury-contaminated fish. Various ocean or river biota that had been eaten by the fish converted elemental mercury to the lipid-soluble mercury compound, methylmercury; clinical symptoms included encephalitis and disease or loss of the general senses (touch, smell, taste, hearing, and vision). Children are more sensitive to methylmercury poisoning than adults. In Iran, a local population ate bread that contained wheat seed that had been dusted with a fungicide consisting of methylmercury. The seed was intended for planting only. Symptoms of methylmercury poisoning included difficulty in walking, ataxia, paresthesia, sensory disturbance, and even deafness. A number of brain centers were damaged in the visual cortex and cerebellum.

Methylmercury crosses the placental barrier. Pregnant women who have not displayed any signs of mercury toxicity have given birth to infants with birth defects. Some infants were mentally retarded; some had palsy.

Other countries with mercury-related exposure issues include Iraq, Ghana, the Seychelles, and the Faroe Islands – the exposures and effects of which have been extensively studied.

Clinical Management

Normally British Antilewisite (2,3-dimercaptopropanol; BAL), administered intramuscularly, is used as an antidote for mercury poisoning. Oral D-penicillamine has been used for less severe cases. The I-acetyl derivative has been tested with good results. Experimentally, oral *m*-2,3-dimercaptosuccinic acid and the less toxic 2,3-dimercaptopropane-l-sulfonate are more effective than BAL.

Ecotoxicology

Mercury can be bioaccumulated in sea life and 'magnified' in the food chain.

Small fish and other organisms living in the water can take up methylmercury and inorganic forms of mercury. When larger fish eat small fish or other organisms that contain methylmercury, most of the methylmercury originally present in the small fish will be stored in the bodies of the large fish. As a result, large fish living in contaminated waters can collect a relatively large amount of methylmercury. Plants may have a greater concentration of inorganic mercury in them if they are grown in soil that contains higher than normal amounts of mercury.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value, 8 h time-weighted average is $0.025\,mg\,m^{-3}$ for mercury vapor and inorganic mercury. The (US) National Institute for Occupational Safety and Health Immediately Dangerous to Life or Health value is $10\,mg\,m^{-3}$. The US Food and Drug Administration permits zero addition to the 20 μg of mercury contained in the average daily diet. Much of this comes from consumption of fish and seafood.

When mercury does spill, a thorough cleanup is necessary, and various commercial spill kits are available. Scrubbing with an aqueous solution of sodium thiosulfate has been reported to work well. A written set of work procedures and personal protective equipment should be considered in situations where mercury spills can occur.

See also: Disc Batteries; Environmental Processes; Kidney; Metals; Methylmercury; Neurotoxicity.

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Relevant Website

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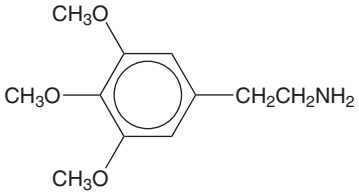
Mescaline

Christopher P Holstege

This article is a revision of the previous print edition article by William A Watcon, volume 2, pp. 289–290, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 54-04-6
- Synonyms: 3,4,5-Trimethoxyphenethylamine; Peyote; Mescal; Mescal button
- Chemical/Pharmaceutical/Other Class: A phenylethylamine derivative alkaloid hallucinogen found in the North American small, blue-green, spineless cactus *Lophophora williamsii* and in several South American cacti of the *trichocereus* species
- Chemical Structure:



Uses

Mescaline does not have a therapeutic use. It is used as a Native American religious intoxicant. It is also a drug of abuse.

Exposure Routes and Pathways

The primary exposure pathway is oral ingestion of 'buttons'. These buttons are the round, fleshy tops of the cactus that are sliced and dried. Each button reportedly contains ~45 mg of mescaline.

Toxicokinetics

Each button of peyote reportedly contains ~45 mg of mescaline, with 6–12 buttons typically ingested to attain hallucinogenic effect. Mescaline is well absorbed from the gastrointestinal tract. Peak blood levels occur 2 h postingestion. Clinical effects occur within 1 h and last for typically 6–12 h. Mescaline does not bind to plasma proteins. The exact volume of distribution is unknown, but certainly above $1\,l\,kg^{-1}$. Mescaline is metabolized by the liver to inactive metabolites. Over 90% of a dose is recovered in the urine during the first 24 h, with 60% as mescaline and the remainder as mescaline metabolites.

Mechanism of Toxicity

The exact mechanism of mescaline has not been clearly defined. The central nervous system effects of mescaline appear to involve stimulation of both serotonin and dopamine receptors. In experimental studies, these effects can be blocked by either serotonin antagonists such as methysergide or dopamine antagonists such as haloperidol. Mescaline is structurally related to the amphetamines and cathine (khat). Sympathomimetic effects can occur and are thought to be centrally mediated. Mescaline does not appear to inhibit monoamine oxidase.

Acute and Short-Term Toxicity (or Exposure)

Human

Peyote buttons taste bitter. After ingestion, a transient initial phase of nausea, vomiting, and generalized abdominal discomfort typically occurs. This is followed by a sympathomimetic phase including increased blood pressure, tremor, mydriasis, diaphoresis, and tachycardia. Approximately 4–6 h after ingestion, a phase similar to lysergic acid diethylamide (LSD) intoxication occurs. This may include euphoria, depersonalization, disorientation, anxiety, ataxia, nystagmus, and vivid visual hallucinations. Changes in taste, smell, and hearing can also be present. Larger doses can produce bradycardia, hypotension, and respiratory depression. There is an increased risk for trauma in the mescaline intoxicated abuser due to the altered perception and increased emotional lability, panic attacks, and anxiety. Symptoms usually resolve over 6–12 h.

Chronic Toxicity (or Exposure)

Animal

Like LSD, mescaline has been used in animal models of schizophrenia. Both produce effects on 5HT2 receptors.

Human

Mescaline has been linked to a specific group of fetal abnormalities when used excessively. It is considered a potential teratogen. Studies of life-long peyote users did not find evidence of increased chromosomal

abnormalities. Mescaline may be associated with the phenomenon of 'flashbacks'.

In Vitro Toxicity Data

Recent studies have demonstrated mescaline extract toxicity in mouse and human leukocyte models of immune systems.

Clinical Management

Acute mescaline toxicity can be treated with supportive care. The patient's airway, breathing, and circulation should initially be assessed and therapy provided as required. Reassurance and provision of a quiet, nonthreatening environment may be effective in decreasing anxiety. Benzodiazepines should be utilized to alleviate sympathomimetic effects, anxiety, or panic attacks that do not respond to reassurance. If additional drug therapy is required for agitation or psychosis, haloperidol should be considered.

See also: Amphetamine; Drugs of Abuse; LSD (Lysergic Acid Diethylamide).

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Metabonomics

Vishal S Vaidya, Jeremy K Nicholson, and Harihara M Mehendale

What is Metabonomics?

Metabonomics, which can provide real biological endpoints, is defined as the quantitative measurement of the time-related multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification. The word metabonomics is derived from two Greek words 'meta' – meaning change – and 'ekynomous'– the word used to describe the behavior of a complex system. Its relationship to other 'omics' is shown in **Figure 1**.

Applying metabonomics involves generating metabolic databases for control animals and humans, diseased patients, and animals used in drug testing, and the simultaneous acquisition of multiple biochemical parameters on biological samples. Metabonomics is usually conducted with biofluids, many of which can be obtained noninvasively (urine) or relatively easily (blood), but other more exotic fluids such as cerebrospinal fluid, bile, seminal fluid, cell culture supernatants, tissue extracts, and similar preparations can also be used to determine the metabolites present, both in homeostasis and when the organism has been affected by factors such as environmental exposures.

Metabonomics is a promising approach because disease, drugs, or toxins cause changes in the concentrations and fluxes of endogenous metabolites involved in key cellular pathways. For example, the response of cells to toxic or other stressors generally results in an adjustment of their intra- or extracellular environment in order to maintain a constant internal environment (homeostasis). This metabolic change/adjustment is expressed as a fingerprint of biochemical perturbations, which is characteristic of the nature or site of a toxic insult or disease process. Urine, in particular, often shows changes in metabolic profile in response to toxic or disease-induced stress; because

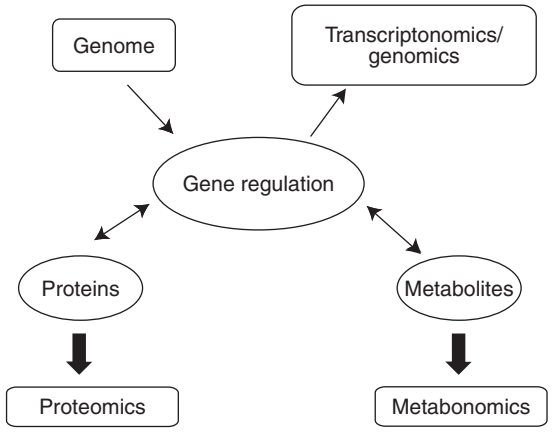

Figure 1 In the field of 'omics', the science for evaluating: protein expression changes is termed as 'proteomics'; gene expression changes is termed as 'transcriptonomics/genomics', and metabolite changes is termed as 'metabonomics'.

one way that the body's cellular systems attempt to maintain homeostasis after a toxic challenge is by modulating the composition of biofluids to eliminate substances from the body. Even when cellular homeostasis is maintained, subtle responses to toxicity or disease are expressed in altered biofluid composition. Metabonomics is also a powerful tool for investigating phenotypic abnormalities in mutant animals and human diseases and in modeling physiological variation in experimental animals and man.

Metabonomics is both systems biology and a dynamic approach, as metabonomic analysis can provide a description of the integrated physiological behavior of an entire living system across time. Metabolic profiling is a hugely complex undertaking, generating huge amounts of data to be analyzed and mined for gaining significant information about metabolic pathways and networks, novel biomarkers, and how metabolites interact not only with genes and proteins, but also with environmental, nutritional, and lifestyle factors. The integration of this array of variables holds the potential to tell researchers a great deal about human health and disease etiology.

Metabonomics versus Metabolomics

Metabolism is considered to be a phenotype. It integrates all the factors from nutrition to environment to genetics assessing one's current state of health. Metabolomics arose from metabolic control theory and was originally based on the metabolome, which is defined as the metabolic composition of a cell and is analogous to the genome or proteome. In metabonomics, static cellular and biofluid concentrations of endogenous metabolites are evaluated over a time-course.

In addition to providing molecular concentrations, metabonomics covers the study of molecular dynamic information, such as molecular reorientational correlational times and diffusion coefficients in intact tissues. Metabonomics can be regarded as a full systems biology approach; when a whole organism with separate organs and many cell types is studied, metabonomics can integrate the disparate effects that occur over both time and space. Metabolomics, which is the corresponding study in single cells, can be thought of as a subset of the systems covered by metabonomics.

Importance of Time-Course Estimations in Metabonomics

In a biological system, it is evident that the initiation of functionally connected gene expression events, cell signaling, protein synthesis changes, and metabolic responses to a stressor must be essentially sequential. Maturation and persistence of changes in gene expression, protein synthesis and posttranslational modification, and subsequent effects on metabolic processes also differ significantly. An event must therefore be evaluated in relation to time at each level of biomolecular organization if molecular responses are to be accurately associated with their macroscopic consequences in an organism. So, in metabolic studies, it is extremely important to measure time-dependent patterns of change in response to stimuli, because metabolic fluxes occur very rapidly, even in normal homeostasis, and consideration of the 'metabolite content' at only a fixed point in time can be misleading. The time-course studies are especially important in order to make a distinction between adaptive and toxic effects following exposure. This applies to the variables that change due to the initial adverse (mechanism-related) interactions, the homeostatic response to the cellular derangement (which could reflect entirely positive reactions of a healthy cell or tissue), and the changes due to cell death.

An important potential role of metabonomics is to direct the use and timing of proteomic and genomic analyses in order to maximize the probability of observing biological transitions that predict functional outcomes; this principle applies to human, animal, and microbial systems. In the case of single exposure to a toxic drug, there will be a response that takes time to complete, and the patterns that are observed in gene expression, proteins, and metabolites will therefore vary according to when the measurements are made. If the measurements are made long after dosing, it is possible that only profile changes will be due to biomarkers of recovery or cellular repair. In the case of a multidose study the second and subsequent doses of a compound might arrive before the effects of the first dose are cleared, complicating the profiles further. As the doses continue, there might be a rising curve of toxicity and there could be superimposed profile changes due to cell death and regeneration.

Quantitative Analysis of Metabonomics

To investigate the complex metabolic consequences of disease processes, toxic reactions, and genetic manipulation, nonselective but specific analytical approaches are required. Several spectroscopic methods in addition to nuclear magnetic resonance (NMR) can produce metabolic signatures of biomaterials, including mass spectrometry (MS), gas chromatography/mass spectrometry (GC/MS), liquid chromatography/mass spectrometry (LC/MS), liquid

chromatography/tandem mass spectrometry (LC/MS/MS), liquid chromatography/time-of-flight mass spectrometry (LC/TOFMS), high-performance liquid chromatography (HPLC), and optical spectroscopic techniques. Bioanalytically, NMR and MS are powerful means of generating multivariate metabolic data. NMR has the advantages of being nondestructive, applicable to intact biomaterials, and intrinsically more information rich with respect to the determination of molecular structures, especially in complex-mixture analyses. Furthermore, a technique known as magic angle spinning (MAS)-NMR can be used to carry out biochemical studies on intact tissues and cells, which, if carefully conducted, can preserve the samples for other studies and allow abnormal molecular compartmentation and interactions to be studied in intact tissues. MS is analytically more sensitive than NMR, but differential ionization suppression can make pattern quantification difficult, and extraction and derivatization might be necessary. The choice between NMR and MS approaches is ultimately matrix or problem dependent. Both technologies require further development, especially in terms of high-throughput and data processing methods, to optimize their use in complex metabolism studies.

NMR Spectroscopy of Biofluids

The successful application of ^{1}H NMR spectroscopy of biofluids to study a variety of metabolic diseases and toxic processes has now been well established and many novel metabolic markers of organ-specific toxicity have been discovered. ^{1}H NMR spectroscopy is well suited to the study of toxic events, as multicomponent analyses on biological materials can be made simultaneously, without bias imposed by expectations of the type of toxin-induced metabolic changes. This is particularly true for NMR spectra of urine in situations where damage has occurred to the kidney or liver. Quantitative changes in NMR spectroscopic metabolite patterns have also been shown to give information on the location and severity of toxic lesions, as well as insights into the underlying molecular mechanisms of toxicity.

The first studies of using pattern recognition to classify biofluid samples used a simple scoring system used to describe the levels of 18 endogenous metabolites in urine from rats that either were in a control group or had received a specific organ toxin that affected the liver, the testes, the renal cortex, or the renal medulla. This study showed that samples corresponding to different organ toxins mapped into distinctly different regions. Various refinements in the data analysis were investigated, including taking scored data at three time points after the toxin exposure for the nephrotoxins only as well as using a simple dual scoring system (the time and magnitude of the greatest change from control). The maps derived from the full time-course information provided the best discrimination between toxin classes. This study was further extended to incorporate actual metabolite NMR resonance intensities rather than simple scores. This was carried out for the nephrotoxins in the earlier group plus additional nephrotoxic compounds. A good separation of renal medullary from renal cortical toxins was achieved. In addition, it was possible to differentiate cortical toxins according to the region of the proximal tubule, which was affected, and also by the biochemical mechanism of the toxic effect.

Examples of toxicants investigated using this metabonomics approach are given below:

- *Kidney cortical toxins*: mercury chloride, *p*-aminophenol, uranyl nitrate, ifosfamide, cephaloridine.
- *Kidney medullary toxins*: propylene imine and 2-bromoethanamine hydrochloride.
- *Liver toxins*: acetaminophen, hydrazine, allyl alcohol, thioacetamide, α-naphthylisothiocyanate, and carbon tetrachloride.
- *Testicular toxin*: cadmium chloride using environmentally realistic levels.
- *Agents causing phospholipidosis*: amiodarone, chloroquine, DMP-777 (a neutrophil elastase inhibitor).
- Effect of dexamethasone on vascular lesions.
- Other studies include the toxicity of the aldose reductase inhibitor HOE-843 and lanthanum nitrate.
- Toxic stress in earthworms has also been investigated using metabonomics.

Extensions to the earlier chemometric approaches include a toxicological assessment approach based on neural network software to ascertain whether the methods provide a robust approach, which could lead to automatic toxin classification. The neural network approach to sample classification, based on ^{1}H NMR spectra of urine, was in general predictive of the sample class. It appears to be reasonably robust and once the network is trained, the prediction of new samples is rapid and automatic. However, the principal disadvantage is common to all neural network studies in that it is difficult to ascertain from the network which of the original sample descriptors are responsible for the classification. Although recently it has been suggested that probabilistic

neural networks appear to be a useful and effective method for sample classification. Recently, comprehensive studies have been published using pattern recognition to predict and classify drug toxicity effects, including lesions in the liver and kidney, and using supervised methods as an approach to an expert system.

It appears that using ^{1}H NMR spectroscopy to follow the biochemical responses of animals or cells to foreign compounds may confer significant analytical advantages. Currently, in order to evaluate the toxicity of a drug candidate by conventional toxicological procedures an array of biochemical methods is required. This is necessarily a complex and time-consuming process and if an inappropriate range of biochemical methods or metabolic parameters is used important metabolic disturbances may be overlooked. The role of NMR spectroscopy in analytical toxicology is thus essentially one of biochemical exploration; that is, determining the range of biochemical perturbations caused by exposure to a toxin and whether these are biologically significant.

MAS NMR of Tissues

As described above, NMR spectroscopy of biofluids when coupled with pattern recognition analysis can be an efficient new method of investigating toxicity profiles of xenobiotics. In addition, while NMR spectroscopy *in vivo* might be used to investigate abnormalities in whole animals, such studies are hampered by the heterogeneity of the sample, low magnetic fields of whole body scanners (low sensitivity and poor spectral dispersion), and short NMR relaxation times, all leading to broad lines and loss of resolution. Within the last few years, with the development of high-resolution MAS technology, it has become possible to obtain very high quality ^{1}H NMR spectra on small ($\sim$10 mg) samples of whole tissue.

MAS involves spinning the sample about an axis at 54.7° to the magnetic field direction. This process removes the line broadening caused by dipolar couplings, chemical shift anisotropies, and anisotropic magnetic susceptibility artifacts. Tissue metabolites already enjoy a degree of molecular mobility such that the line broadenings are greatly reduced from their true solid values and this means that only modest rotation rates ($<$10 kHz) are required. This approach has now been applied to cells and tissues. The technique opens up many diagnostic possibilities since information on a variety of metabolites in different cellular environments can be rapidly obtained and specialized NMR experiments, such as those to measure molecular diffusion coefficients, can be used to probe compartmentation. Confirmation of biochemical composition can be obtained using standard high-resolution NMR of both aqueous (protein-free) and methanolic extracts. This produces a comprehensive set of metabolic information that can be used in integrated metabonomics studies. MAS NMR data, like biofluid NMR spectra, can also be subjected to computer pattern recognition methods in order to classify toxicity type (target organ and biochemical mechanism) and to map time-related biochemical trajectories associated with drug-induced biochemical changes. The ability to compare biofluid and tissue NMR spectra may provide further insight into mechanisms of toxicity or target organ identification.

Chemometric Analysis of Metabolic NMR Data

One general procedure, which has found wide application, is to first simplify ^{1}H NMR spectra of biofluids by means of data compression, by producing a segmentation of each NMR spectrum (usually with $\sim$250 intensity values per spectrum), integrating peak intensity in each segment. Each of these acts as a metabolic descriptor with which to classify the NMR spectra according to biochemical features. These data are then constructed in the form of a spreadsheet, which is used as the input into a pattern recognition/multivariate statistics software suite. Appropriate data reduction routines, such as principal components analysis (PCA) or partial least squares discriminant analysis (PLS-DA), can be used to classify the NMR-generated toxicity data in terms of toxin type and dose. Multidimensional metabolic trajectories can be constructed in order to visualize the biochemical time-course of the toxic episodes. More complex expert systems based on chemometric models in the multidimensional metabolic space can be constructed and used for class prediction.

Expert System Development

A variety of different supervised pattern recognition methods have been evaluated for detecting abnormalities in metabolite profiles caused by toxins. These are of two main types, those that relate to overall variance in the data sets, such as those based on latent variables, for example, SIMCA and PLS-DA, and those that examine relationships in the data in different ways, such as neural networks and genetic algorithms. The basic procedure is to train types of expert systems to produce classifications of biofluid samples based on known toxicity type according to standard methods of evaluation, such as histopathology. These systems are then tested against standard toxicological assessment

procedures using toxins unknown to the model in order to evaluate the robustness of each expert system approach for toxicity screening.

Recently, LC/MS, LC/MS/MS, and LC/TOFMS have also become very popular techniques due to their sensitivity, speed, and specificity. However, the use of these techniques requires an expertise in the instrumentation to ensure high-quality reproducible data. Using the LC/TOFMS approach, drug metabolites have successfully been identified following drug candidate administration against a backdrop of potentially endogenous interferences. These measurements using any of the above-mentioned techniques, coupled with multivariate statistical chemometric methods for the purpose of latent information extraction and sample classification, offer a powerful new approach to the whole system of diagnostics and metabolic function to identify the metabolic phenotypes that result from a combination of genetic and environmental factors.

Applications of Metabonomics

Significant opportunities exist for the application of metabonomics to the field of environmental health sciences, particularly in the area of biomarkers of exposure and disease. When toxins interact with cells and tissues they disturb the ratios, concentrations, and fluxes of endogenous biochemicals in key intermediary cellular metabolic pathways. Under mild toxic stress, cells attempt to maintain homeostasis and metabolic control by varying the composition of the body fluids that either perfuse them or are secreted by them. In more severe toxicity states, cell death leads to loss of organ function and more marked biochemical changes occur in biofluids due to loss of whole body homeostasis and metabolite leakage from damaged cells. Consequently, following either scenario there are characteristic organ-specific and mechanism-specific alterations in biofluid composition. Clearly, the detection of toxic lesions via biochemical effects is most difficult close to the toxic threshold, yet these are often the most important effects to define.

^{1}H NMR spectroscopy has been used to study the composition of biofluids before and after administering a wide range of toxicants. Predictive statistical models have been constructed to deal with toxicological profiling on three levels. The first and most basic level is determining if the sample is normal, for example, whether it belongs to a control population. The second level involves fitting abnormal samples to known cases of tissue or mechanism-specific toxicity to predict the toxicity of novel pharmacological agents. The final level is to identify the spectral regions that are responsible for deviations from normal profiles and to determine toxicity biomarkers within those regions that may help elucidate mechanisms of toxicity.

Using pattern recognition methods, NMR spectra can be used to:

- Classify the sample as being normal or abnormal (example: patients suffering from coronary artery occlusion have been identified on the basis of ^{1}H NMR spectra of their blood serum).
- Establish normal physiological variance in a population of human urine samples.
- Classify target-organ toxicity and site and mechanism of action within the organ.
- Evaluate the time-course of the effect; for example, the onset, evolution, and regression of toxicity.
- Differentiate between tissue extract spectra obtained from normal tissues and to classify tumors by type such as pituitary tumor, fibrosarcoma, hepatoma.
- Classify several inborn errors of metabolism using urine spectra.
- Use in functional genomics: Metabonomics can be used to separate classes of experimental animals, such as mice and rats, according to their strain on the basis of the endogenous metabolite patterns in their biofluids. This is possible because differences in 'silent-gene' function between strains can influence the fluxes of metabolites through many key intermediary pathways resulting in distinct animal 'metabotypes'. There is also a strong indication for the use of metabonomics in the phenotype of mutant or transgenic animals and the investigation of the consequences of transgenesis.
- Identification of novel biomarkers of toxicity: Previously, the detection of novel biomarkers of toxic effect has mainly been serendipitous. However, it is now possible to use a combined NMR–expert systems approach to systematically explore the relationships between biofluid composition and toxicity and to generate novel combination biomarkers of toxicity. Pattern recognition maps can be examined for evidence of clustering of data according to site and type of toxic lesion.

Limitations of Metabonomics

As with all 'omics' platforms, metabonomics has certain limitations in terms of the recovery of biological information. In the case of toxicity assessment, it is possible to generate false-positive data in situations in which the compound of interest causes significant metabolic changes without associated toxicity, because of marked physiological

or pharmacological effects. For example, acetazolamide is a renal carbonic anhydrase inhibitor that massively reduces the excretion of intermediates in the citric-acid cycle. Misinterpretation can, however, be minimized by using supervised methods that include models of such effects.

Conversely, certain pathologies, such as liver fibrosis, are associated with negligible effects on biofluids, as metabolic derangement does not occur until there is significant tissue damage. In the case of low-potency compounds, there might be particular difficulties in separating toxicological from physiological effects. However, in previous dose–response studies, NMR-based metabonomic methods were at least as sensitive as conventional methods for detecting lesions at the 'threshold-dose' level, and even minor physiological changes were detected in normal animals.

There are obvious limitations in terms of choice of biofluid; for instance, urine might not be as appropriate as cerebrospinal fluid for studying neuropathology. There is also the potential for confusion with mixed-toxicity drugs that, for example, affect both liver and kidney, as the biomarkers of toxicity will be a complex combination that relates to both sites and possibly to the multiple mechanisms. However, this offset by the fact that mixed toxicities often have different timescales and such effects can therefore be deconvoluted by making repeated sequential measurements in individual animals.

Conclusion

Metabonomics is now recognized as an independent and widely used technique for evaluating the toxicity of drug-candidate compounds, and has been adopted by several pharmaceutical companies into their drug development protocols. It is possible to identify the target organ toxicity, derive the biochemical mechanism of toxicity, and determine the combination of biochemical markers for the onset, progression, and regression of the lesion. Furthermore, this technique can provide a metabolic fingerprint of an organism (metabotyping) – a key to functional genomics – and hence has applications in the design of drug clinical trials and evaluation of genetically modified animals as disease models.

A particular strength of spectroscopy-based metabonomic methods is that they are rapid, economic, and not labor intensive. In biological terms, the most important advantage of metabonomics is that individual animals and subjects can be followed noninvasively (urine collection) through a disease-related metabolic trajectory, yielding a holistic picture of integrated biological function over time. Finally, metabonomics can be a very effective tool for biomarker discovery following disease states or toxicant exposure.

A large challenge in the future will be to create a database of metabolic profiles with linkages to protein and gene expression databases. It is envisioned that a new and fundamental understanding of organism's responses to environmental insult will emerge from the integration of metabonomic data with those obtained from the study of global patterns of gene and protein expression. Such integration of data types will also pave the way to understanding the relationships between gene function and metabolic control in health and disease.

See also: Biomarkers, Human Health; Biomonitoring; Genomics, Toxicogenomics; Proteomics.

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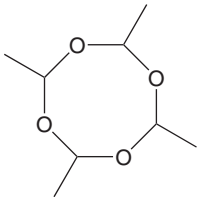
Metaldehyde

Guangping Chen

- Chemical Name: *r*-2,*c*-4,*c*-6,*c*-8-Tetramethyl-1,3,5,7-tetroxocane or 2,4,6,8-Tetramethyl-1,3,5,7-tetraoxacyclooctane
- Chemical Abstracts Service Registry Number: CAS 108-62-3
- Synonyms: Acetaldehyde tetramer; Metacetaldehyde; Acetaldehyde polymers; Acetaldehyde homopolymer; Antimilace; Antimitace; Ariotox; Cekumeta; Deadline; Halizan; Limatox; Meta; Metason; Namekil; Ortho Metaldehyde 4% Bait; Slug Death; Slug Pellets, Slug-Tox; Slugit; Limax; Limovet; Polyacetaldehyde; Schneckokorn; Schnex-schneckentod; Terrasan-schneckentod Gekoernt

- Chemical/Pharmaceutical/Other Class: Molluscicide
- Chemical Formula: $C_8H_{16}O_4$
- Chemical Structure:



Uses

Metaldehyde is the most common molluscicide for controlling slugs and snails. It is used in a variety of vegetable and ornamental crops. Slug and snail baits generally contain 3% metaldehyde. It is also used as a fuel for lamps and stoves in Europe.

Exposure Routes and Pathways

Metaldehyde poisoning typically results from the ingestion of products containing the active ingredient. The use of molluscicides increases the risk of exposure for pets with access to treated areas.

Toxicokinetics

Metaldehyde is readily absorbed from the gastrointestinal tract. Metaldehyde's primary decomposition product in the body is acetaldehyde. Metabolites can cross the blood–brain barrier and enter the central nervous system.

Mechanism of Toxicity

Products containing metaldehyde are in the Environmental Protection Agency Toxicity Class II or III. The toxic dosage can range from 100 to 1000 mg kg^{-1} of body weight. Metaldehyde toxicity causes rapid onset of neurological signs/symptoms that can be fatal if untreated. Signs of poisoning begin within 1–4 h of exposure. Repeated seizures due to metaldehyde poisoning can cause very high body temperature, which can lead to complications similar to those observed from heatstroke.

Acute and Short-Term Toxicity (or Exposure)

Metaldehyde is moderately toxic by ingestion. Metaldehyde is also moderately toxic by inhalation. It can cause irritation to skin, eyes, and mucous membranes of the upper airways and gastrointestinal tract. The following symptoms appeared in humans hours following ingestion: abdominal pain, nausea, vomiting, diarrhea, fever, convulsions, coma, and persistent memory loss. High acute exposure can also lead to tachycardia, respiratory panting, acute asthmatic reaction, depression, drowsiness, high blood pressure, excessive urination and defecation, muscle tremors, sweating, excessive salivation, tearing, cyanosis, acidosis, stupor, and unconsciousness. Some signs and symptoms can persist for months following acute poisoning.

Chronic Toxicity (or Exposure)

Long-term exposure to metaldehyde may result in dermatitis and affect brain function in humans.

Clinical Management

An emetic (ipecac) should be administered to induce vomiting and prevent further absorption. Gastric lavage can be performed to remove metaldehyde from the gastrointestinal tract. If hyperthermia is noted, a cool-water bath can be given to lower body temperature. Sedatives, e.g., diazepam, can be given to control anxiety, seizures, and tremors. Intravenous fluids should be given to correct dehydration and acidosis.

Environmental Fate

Metaldehyde is of low persistence in the soil (days). It weakly adsorbs to soil. Metaldehyde is soluble in water. Metaldehyde undergoes rapid hydrolysis to acetaldehyde in an aquatic environment.

Ecotoxicology

Metaldehyde is toxic to birds, wildlife, pets, and poultry. Metaldehyde is reported to be virtually nontoxic to aquatic organisms. Bait agents with 6% active ingredient are nontoxic to bees. Many types of flowers lose their color following contact with metaldehyde.

See also: Acetaldehyde.

Further Reading

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Relevant Websites

http://ace.orst.edu – Extension Toxicology Network.
http://www.inchem.org – International Programme on Chemical Safety.
http://www.petplace.com – Webpage of Pet Place.com.

Metallothionein

Shayne C Gad

Metallothioneins (MTs) are a class of intracellular, low molecular weight (6000–7000 Da), cysteine-rich proteins. They are ubiquitous in eukaryotes, and have unique structural characteristics giving them potent metal-binding and redox capabilities. Although MTs were discovered many years ago, their functional significance remains obscure, that is, the primary role for MTs has not been identified and new functions are being discovered. MTs have been shown to be involved in many pathophysiological processes such as metal ion homeostasis and detoxification, protection against oxidative damage, cell proliferation and apoptosis, chemoresistance and radiotherapy resistance. MTs have been implicated as a transient response to any form of stress or injury providing cytoprotective action. Further, MT isoforms have been shown to be involved in the carcinogenic process, cancer development and progression; however, the use of MT as a potential marker of tumor differentiation or cell proliferation, or as a predictor of poor prognosism, remains unclear.

Four major MT isoforms, MT-1, MT-2, MT-3, and MT-4, have been identified in mammals. The most widely expressed isoforms in mammals, MT-1 and MT-2, are rapidly induced in the liver by a wide range of metals, drugs, and inflammatory mediators. In the gut and pancreas, MT responds mainly to Zn status. A brain isoform, MT-3, has a specific neuronal growth inhibitory activity, while MT-1 and MT-2 have more diverse functions related to their thiolate cluster structure. These include involvement in Zn homeostasis, protection against heavy metal (especially Cd) and oxidant damage, and metabolic regulation via Zn donation, sequestration, and/or redox control. A possible role for MT-4 is related to copper requirements in epithelial differentiating tissues.

MTs are key in protecting organs and tissues against the toxic effects of heavy metals. Although found throughout the body, metallothionein is most prevalent in the kidneys and liver. MTs bind with metals, rendering them biologically inactive as they enter the kidneys and liver. Once within these organs, the metal–MT complex breaks down, releasing the metal, which may cause toxic effects.

Outside the hepatic system, MTs facilitate absorption of metals such as cadmium into the kidneys and liver. Also, the induction of MTs via reactive metals protects hepatic enzymes against cellular damage. Without this intervention, zinc may stimulate glutathione concentration and reduce catalase activity. Cadmium reduces the amount of cytochrome P450 and its activity toward testosterone oxidation. The liver usually can produce sufficient amounts of the protein to bind to any free metals.

The kidneys are at greater risk for toxicity because they must manage free metals that enter without MTs binding as well as those freed through biotransformation. In the kidney, the cadmium–MT compound is filtered by the glomerulus and then reabsorbed by the proximal tubule. Lysomal degradation in the tubular cells may break the molecular binding and release free cadmium into the kidney, which prompts further MT production. If the production within the kidney is insufficient to absorb the concentration of a free metal, it may produce kidney damage or other pathologies. Similar effects are seen with aluminum as well, which may create tubular damage in free concentrations.

Wilson's disease is an autosomal recessive inherited disorder of copper metabolism resulting in accumulation of copper in various tissues. Rats raised to have a large accumulation of copper had ~ 80 times greater concentration of MTs in their liver compared to controls ($5016\,\mu g\,g^{-1}$ vs. $65\,\mu g\,g^{-1}$).

MTs appear to respond to other reactive substances such as trihalomethanes. A single dose of chloroform significantly increased metallothionein levels for up to 6–12 h following exposure. Although this indicates a response, it is not clear whether metallothionein protects against the harmful effects of this substance as well.

Toxicity due to insufficient MTs depends on factors such as age, gender, and health status. Young mice were found to have a fourfold greater accumulation of cadmium in their brains than adult mice, and significantly less metallothionein. Because cadmium interacts with calcium, women may lose cellular calcium. Diabetics experience glomeral damage and greater sensitivity.

In protecting the body against toxic metals, MTs interact with endogenous metabolic enzymes such as glutathione and mercapturic acid. This makes sense since cysteine is a substrate of glutathione. Supplements such as cysteine may elevate levels of MTs and zinc in the kidneys.

See also: Glutathione; Kidney; Liver; Metals.

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Metals

Shayne C Gad

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No general principles that govern the toxicity of all metals and their compounds exist; however, a few generalizations are possible. For the purposes of this discussion, the term metals will include both their ions and compounds.

Oxidation state and solubility are critical factors in toxic reactions. Metals can react with enzymes, cell membranes, and specific cell components. These reactions can inhibit or stimulate the actions of these substances and components.

Metals are generally circulated bound to some blood protein and can selectively bioaccumulate; thus, metals can affect either specific target organs or multiple anatomical sites. For example, lead can deposit in the bone, affect the central nervous system (CNS), and interfere with the metabolism of the heme in hemoglobin; cadmium appears to concentrate in the kidneys and the liver, mercury is a CNS toxin.

The metabolic product of the metal can determine the action in the organ in which the metal is deposited. Usually, metabolism of metals can lead to detoxification and often to excretion. Some metals, such as selenium metal and oxides, are converted to the volatile trimethyl derivative and are exhaled. On the other hand, mercury is converted to methyl mercury chloride, which is soluble in lipids and appears to be concentrated overtime in organs with high lipid content.

Some metals (cadmium, zinc, copper, and mercury) induce special protein complexes called metallothioneines. Iron forms a number of other protein complexes (ferritin, hemosidern, and transferrin).

For the general population, inhalation is a secondary exposure route for metals. Usually, metals are ingested with food or with drinking water.

Acute toxicity from metals can follow similar patterns; these can be essentially nonspecific like nausea and vomiting. A few metals and their compounds are carcinogenic to humans; the vast majority is not.

A few metals, such as lead and mercury, can cross the placental barrier. The very young population and the older population are most susceptible to metal toxicity.

Some metals are essential for good health (e.g., copper, chromium, selenium). Others are suspected not to be essential (e.g., beryllium, lead, tin). Still others are under investigation (e.g., arsenic).

See also: Aluminum; Antimony; Arsenic; Barium; Beryllium; Bismuth; Boron; Cadmium; Cesium; Chromium; Cobalt; Copper; Gallium; Iron; Lead; Lithium; Manganese; Mercury; Metallothionein; Molybdenum; Nickel and Nickel Compounds; Platinum; Potassium; Selenium; Silver; Sodium; Tellurium; Thallium; Tin; Titanium; Tungsten; Uranium; Vanadium; Zinc.

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Methadone

Michael Hiotis

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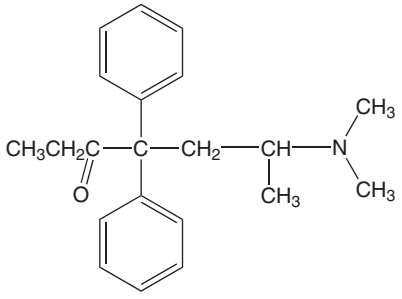
- Chemical Abstracts Service Registry Numbers: CAS 297-88-1; CAS 76-99-3
- Synonyms: 4,4-Diphenyl-6, dimethylaminoheptan-3-one
- Chemical/Pharmaceutical/Other Class: Synthetic opioid
- Chemical Formula: $C_{21}H_{27}NO$
- Chemical Structure:



Uses

Methadone is a synthetic opioid used primarily for detoxification and maintenance in patients who are dependent on opiates, mainly heroin, and in the treatment of chronic severe pain.

Methadone is a Schedule II controlled substance under the Federal Controlled Substances Act.

Exposure Routes and Pathways

Oral and parenteral administrations are the most common routes of exposure.

Toxicokinetics

Methadone is rapidly absorbed after all routes of exposure. When administered orally, methadone is approximately one-half as potent as when given parenterally. Oral administration results in a delay of the onset, a lowering of the peak, and an increase in the duration of analgesic effect. It is metabolized primarily in the liver where it undergoes *N*-demethylation. Protein binding is 85%. Urinary excretion of methadone and its metabolites is dose dependent and comprises the major route of excretion only in doses exceeding 55 mg day^{-1}. It is excreted by glomerular filtration and undergoes renal reabsorption that decreases as urinary pH increases. Half-life ranges between 13 and 47 h with an average of 25 h.

Mechanism of Toxicity

Its mechanism of action is similar to morphine and it stimulates a number of specific opioid receptors in the brain, causing central nervous system (CNS) and respiratory depression.

Acute and Short-Term Toxicity (or Exposure)

Animal

Methadone, like other opiates and their derivatives, cause miosis and CNS and respiratory depression, but has an excitatory effect on the CNS in cats and horses. Naloxone can be used at 0.02 mg kg^{-1}, if needed.

Human

Intentional or accidental overdose of methadone can lead to lethargy, miosis in mild intoxication, and stupor, coma, bradycardia, hypotension, hypothermia, respiratory depression, pulmonary edema, cardiovascular collapse, and death with higher doses. After abrupt discontinuation or administration of an antagonist such as naloxone, withdrawal syndrome can develop, consisting of lacrimation, rhinorrhea, sneezing, nausea, vomiting, fever, chills, tremor, tachycardia, and agitation. Accidental ingestions of methadone by children can lead to significant toxicity and even death.

Chronic Toxicity (or Exposure)

Animal

Pregnant rabbits and rats dosed up to 40 mg day^{-1} of methadone showed no fetal abnormalities. In B6C3F1 mice, doses of 1–30 mg kg^{-1} day^{-1} resulted in overall weight loss in the animals.

Human

Methadone has Food and Drug Administration indications for the management of pain in adults and for adult narcotic addiction. Adverse events are those expected from opiate exposures: gastrointestinal symptoms, CNS depression, and respiratory depression in larger doses, bradycardia, and constipation. Methadone appears to be fairly well tolerated by

most patients. Cases of edema have been described. Methadone appears to increase prolactin levels and can lead to gynecomastia.

In Vitro Toxicity Data

In *Drosophila* studies, methadone has not been shown to be mutagenic.

Clinical Management

Activated charcoal is the preferred method of decontamination. The use of syrup of ipecac is contraindicated because of the potential for serious CNS and respiratory depression. Naloxone, a specific opioid antagonist, should be used to reverse significant respiratory depression. Nalmefene and naltroxone are other opioid antagonists, with limited usefulness, similar to naloxone but with longer half-life and may be considered as an alternative to naloxone. The duration of naloxone effect is much shorter than that of methadone and relapse of opioid symptoms are common. For this reason naloxone infusion may be necessary and symptomatic patients should be monitored for 2 days.

See also: Heroin; Morphine.

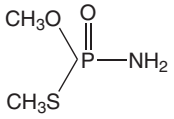
Further Reading

Wolff K (2002) Characterization of methadone overdose: Clinical considerations and the scientific evidence. *Therapeutic Drug Monitoring* 24(4): 457–470.

Methamidophos

Kevin N Baer

- Chemical Abstracts Service Registry Number: CAS 10265-92-6
- Synonyms: *O*,*S*-Dimethyl phosphoramidothioate; Tamaron; Tamanox; Monitor; Acephate-met
- Chemical/Pharmaceutical/Other Class: Organophosphorus insecticide
- Chemical Structure:



Uses

Methamidophos is used as an insecticide and acaricide. In the United States, cotton, potatoes and tomatoes are the principal crops for methamidophos use.

Exposure Routes and Pathways

Poisonings can occur from inhalation, skin absorption, or ingestion.

Toxicokinetics

Methamidophos can be readily absorbed through the skin, lung, and gastrointestinal tract. The bioactivation of methamidophos (replacement of covalent sulfur with oxygen) is accomplished primarily by liver microsomal enzymes. A variety of hydrolysis reactions to alkyl phosphates and various leaving groups can occur. Methamidophos is fairly well distributed throughout the tissues, with marked accumulation in the liver, kidney, and adipose tissue. Excretion in the urine is the major elimination pathway. Methamidophos is formed from biotransformation of another organophosphorus insecticide, acetphate.

Mechanism of Toxicity

Methamidophos is a potent, direct acetylcholinesterase inhibitor that acts by interfering with the metabolism of acetylcholine. As a result, acetylcholine accumulates at neuroreceptor transmission sites. Some evidence suggests that biotransformation of methamidophos may produce a more potent anticholinesterase.

Acute and Short-Term Toxicity (or Exposure)

Animal

Methamidophos has high mammalian toxicity. The oral LD_{50} value in rats is $\sim 20\,mg\,kg^{-1}$. Dermal LD_{50} values in rats and rabbits are about $100\,mg\,kg^{-1}$. The no-observed-adverse-effect level (NOAEL) from an acute neurotoxicity study based

on inhibition of plasma, erythrocyte, and brain cholinesterase in rats was 0.3 mg kg^{-1} day^{-1}.

Human

Classic signs of acute toxicity include pinpoint pupils, muscular fasciculations, slow pulse, excessive salivation and lacrimation, and gastrointestinal symptoms (nausea, abdominal cramps, diarrhea, and loss of sphincter control). In severe cases, convulsions, coma, and heart block are common. Death is generally attributed to respiratory insufficiency caused by the combination of respiratory center depression, paralysis, and increased bronchial secretions. In children, the classic signs described previously may be infrequent, with the major symptoms being central nervous system depression, stupor, flaccidity, dyspnea, seizures, and coma.

Chronic Toxicity (or Exposure)

Animal

Methamidophos can cause delayed neurotoxicity in hens. The NOAEL from a long-term dosing study in rats was 0.03 mg kg^{-1} day^{-1} based on brain cholinesterase inhibition.

Human

Cholinesterase inhibition may persist for 2–6 weeks. Signs of delayed neuropathy in humans have been reported following dermal and/or inhalation exposures. Progressive distal weakness, ataxia, flaccid paralysis, or quadriplegia may ensure. Additional chronic toxicities, such as memory impairment, language defects (slowed speech and slurring), and behavior disorders have been reported.

Clinical Management

For exposure to eyes, eyelids should be held open and the eyes flushed with copious amounts of water for 15 min. For exposure to skin, affected areas should be washed immediately with soap and water. Medical attention is necessary if irritation develops and persists.

For exposure through inhalation, the victim should be removed to fresh air and, if not breathing, given artificial ventilation. The victim should receive medical attention as soon as possible.

For ingestion victims, vomiting should be induced, keeping in mind the possibility of aspiration of solvents. Gastric decontamination should be performed within 30 min of ingestion to be most effective. Initial management of acute toxicity is establishment and maintenance of adequate airway and ventilation. Atropine sulfate in conjunction with 2-PAM (PAM, Pralidoxime) can be administered as an antidote. Atropine by intravenous injection is the primary antidote in severe cases. Test injections of atropine (1 mg in adults and 0.15 mg kg^{-1} in children) are initially administered, followed by 2–4 mg (in adults) or 0.015–0.05 mg kg^{-1} (in children) every 10–15 min until cholinergic signs (e.g., diarrhea, salivation, and bronchial secretions) decrease. High doses of atropine over several injections may be necessary for effective control of cholinergic signs. If lavage is performed, endotracheal and/or esophageal control is suggested. At first signs of pulmonary edema, the patient should be placed in an oxygen tent and treated symptomatically.

Ecotoxicology

Methamidophos may cause harm to nontarget species with approved applications. Field studies indicate bird mortality can occur with methamidophos use. Methamidophos residues on food that birds may eat (e.g., leaves, insects, invertebrates) show high acute and persistent exposure. In addition, residue data on food that wild mammals may eat indicate that there would be sufficient persistent residues to cause adverse chronic effects. Methamidophos is highly toxic to bees and some beneficial insects. Freshwater and estuarine invertebrate aquatic species may be affected with normal use of methamidophos but acute risks to fish are minimal.

Exposure Standards and Guidelines

The acute reference dose is 0.001 mg kg^{-1} and the chronic reference dose is 0.0001 mg kg^{-1} day^{-1}.

See also: Cholinesterase Inhibition; Organophosphates; Pesticides.

Further Reading

Gallo MA and Lawryk NJ (1991) Organic phosphorus pesticides. In: Hayes WJ Jr. and Laws ER Jr. (eds.) *Handbook of Pesticide Toxicology*, vol. 3, pp. 917–972, 1090. San Diego: Academic Press.

Relevant Website

http://www.epa.gov – United States Environmental Protection Agency.

Methane

Stephen R Clough

- Chemical Abstracts Service Registry Number: CAS 74-82-8
- Synonyms: Natural gas; Fire damp; Marsh gas; Methane (compressed, UN1971, DOT); Methane (refrigerated liquid, UN1972, DOT); Methyl hydride
- Chemical Formula: CH_4

Uses

In industry, methane is used to make methanol, halogenated methanes, ethylene, carbon tetrachloride, chloroform, acetylene, hydrogen cyanide, and methyl chloride. In the form of natural gas, methane is used as a fuel, as a source of carbon black, and as a starting material for the manufacture of synthetic proteins. It is also used in gas-fired utilities and in the home (home heating, gas dryers, and gas cooking).

Exposure Routes and Pathways

Because methane exists as a gas at normal temperatures and pressure, exposure generally occurs by inhalation. It is possible to spill liquid methane from a refrigerated tank, causing frostbite upon contact with the skin due to rapid evaporation and loss of heat.

Mechanism of Toxicity

Methane acts as an asphyxiant at concentrations that are high enough to displace oxygen (87–90%).

Acute and Short-Term Toxicity (or Exposure)

Animal

The toxicity of methane is similar to that in humans; that is, no direct toxicity but can cause asphyxiation at concentrations high enough to displace oxygen required for normal respiration.

Human

Methane is not toxic to humans but acts as an asphyxiant at high enough concentrations. A threshold concentration of 1000 ppm is commonly assumed. The American Conference of Governmental Industrial Hygienists suggests that methane be treated as a simple asphyxiant.

Clinical Management

Persons who are exposed to high concentrations should vacate or be removed from the source of the gas and seek fresh air.

Environmental Fate

Methane is likely to be in the vapor phase in the atmosphere where it may react with hydroxyl radicals. Volatilization is the most important fate process in soil and water. It is not expected to hydrolyze, adsorb to soil or sediment, or bioaccumulate to any great extent.

Other Hazards

Methane is highly flammable and is therefore an explosion and fire hazard; the lower explosive limit is 5–15% by volume. Extreme care must be taken to keep areas of high concentration free from ignition sources, such as sparks from static electricity. Explosion-proof equipment should be used in these areas. Many people believe that methane is an important greenhouse gas, and that the apparent threefold increase in atmospheric concentrations over the last 200 years affects the stratospheric ozone layer and the oxidizing capacity of the atmosphere.

Miscellaneous

Methane is a colorless, odorless, highly flammable gas that is lighter than air. It occurs in natural gas at a concentration of 60–80%. It evolves naturally in marshes and highly reducing sediments as a result of microbiological decay of vegetation and organic matter. Ruminants also produce large concentrations as a by-product of their digestive process (anaerobic fermentation). It is also found in coal deposits and is produced as a by-product of some industrial processes, including some types of fermentation and sludge digestion. Methane is also found in tobacco smoke and in emissions from the incomplete combustion of coal, wood, and petroleum fuels.

See also: Respiratory Tract; Veterinary Toxicology.

Further Reading

Patty F (ed.) (1963) *Industrial Hygiene and Toxicology, vol. II: Toxicology*, 2nd edn., p. 1196. New York: Interscience Publishers.

Methanol

Greene Shepherd

This article is a revision of the previous print edition article by Bradford Strohm, volume 2, pp. 295–297, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 67-56-1
- Synonyms: Methyl alcohol; Carbinol; Wood spirits; Wood alcohol
- Chemical/Pharmaceutical/Other Class: Alcohols
- Chemical Formula: CH_4O

Uses

Methanol is used primarily as an intermediate in the production of formaldehyde, acetic acid, and methyl *t*-butyl ether. Methanol itself has been used as an oxygenated fuel additive, as well as an alternate transportation fuel in addition to its use as a solvent.

Background Information

Large epidemics of methanol poisoning have resulted from it being substituted for ethanol in Moonshine (illegally produced drinking alcohol). Recently, a new antidote, fomepizole, has been approved for use in the United States.

Exposure Routes and Pathways

Exposure to methanol can occur via inhalation, ingestion, and skin absorption.

Toxicokinetics

Irrespective of route of exposure, methanol distributes readily and uniformly to all organs and tissues as a function of their water content. Absorption via inhalation has been reported to be $\sim$60% of the inhaled dose. Methanol is absorbed through intact human skin. Upon absorption, methanol is excreted unchanged in urine, exhaled in breath, or metabolized in the liver and eventually excreted as carbon dioxide. Greater than 90% of the administered dose is metabolized with $\sim$2% excreted unchanged in expired air and 1% unchanged in urine. Depending on dose, half-lives for methanol elimination range from $\sim$1 day or more for doses of $1\,g\,kg^{-1}$ or greater to $\sim$3 h for doses of $<0.1\,g\,kg^{-1}$.

The oxidation of methanol to CO_2 proceeds through several enzymatic steps. Methanol is first converted to formaldehyde via either a catalase/peroxidase reaction (prevalent in rats) or an alcohol dehydrogenase oxidation (predominant in humans and monkeys). Methanol-derived formaldehyde is then conjugated with glutathione to form *S*-formylglutathione via a nicotinamide adenine nucleotide-dependent formaldehyde dehydrogenase. The *S*-formylglutathione conjugate is subsequently hydrolyzed by thiolase to form formic acid and glutathione. Formic acid, in the form of formate, then enters the body's carbon pool in the liver through complexation with tetrahydrofolate, where upon subsequent oxidation it is released as CO_2.

Mechanism of Toxicity

The toxic properties of methanol are the result of accumulation of the formate intermediate in the blood and tissues of exposed individuals. Formate accumulation produces metabolic acidosis leading to the characteristic ocular toxicity (blindness) observed in human methanol poisonings.

Humans and primates appear particularly sensitive to methanol toxicity when compared to rats. This is attributed to the slower rate of conversion in humans of the formate metabolite via tetrahydrofolate. This step in methanol metabolism occurs in rats at a rate $\sim$2.5 times that observed in humans.

Formate appears to directly affect the optic nerve. It is believed that formate acts as a metabolic poison inhibiting cytochrome oxidase. The optic nerve is composed of cells that normally have low reserves of cytochrome oxidase due to their low metabolic requirements and thus may be particularly sensitive to formate-induced metabolic inhibition.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute lethality of methanol is low based on animal testing via oral, dermal, and inhalation routes of exposure. The acute oral lethal dose (LD) observed in rats, rabbits, and monkeys range from $\sim 7\,mg\,kg^{-1}$ (monkey) to $14.4\,mg\,kg^{-1}$ (rabbits). Acute dermal LD in rabbits have been reported as $\sim 20\,mg\,kg^{-1}$ and inhalation lethal concentrations ranged from $\sim$31 000 ppm (18 h exposure, rats) to 72 000 ppm (54 h exposure, mice).

Sublethal doses have been shown to elicit central nervous system (CNS) effects, metabolic acidosis, ocular toxicity, and liver effects. Rats receiving oral doses of 1.0, 100, or 500 mg kg^{-1} day^{-1} methanol for 1 month showed liver changes characterized by enlarged hepatic cells and changes in some microsomal enzymes. Rabbits exposed via inhalation to 46.6 ppm methanol for 6 months exhibited changes in the photoreceptor cells of the retina which were observed upon electron microscopic examination. Monkeys exposed to 0, 10, 100, or 1000 ppm methanol, 22 h day^{-1} for up to 2 years exhibited slight changes in the liver and kidney at the 1000 ppm dose level. Pathologic changes in the nervous system of all animals at 1000 ppm were observed but were considered transient and probably reversible.

The developmental toxicity of methanol has been examined in rats and mice via inhalation and oral exposure. Rats exposed by inhalation to 10 000 and 20 000 ppm methanol 7 h day^{-1} during gestation have produced offspring with reduced body weights and a high incidence of malformations. Similarly, mice exposed to 4000 and 5000 ppm methanol 7 h day^{-1} during gestational days 6–15 experienced a high incidence of embryotoxicity and encephalopathy in surviving offspring. Single oral dosing of rats on gestational day 10 produced an increase in malformations at 1.3, 2.6, and 5.2 ppm methanol. These malformations are likely due to toxic mechanisms discussed above and folate depletion that can occur as the body metabolized formic acid.

Human

Historically, injuries and fatalities have been reported from acute methanol overexposure via ingestion, inhalation, as well as prolonged or repeated skin contact. Inhalation toxicity can occur in occupational settings or as a result of inhalant abuse (huffing). Clinical studies of individuals acutely poisoned by methanol ingestion have identified visual disturbances and possibly blindness as the most notable toxic effects in humans. Methanol is also a CNS depressant, although less potent than ethanol, and has also been shown to produce liver damage upon overexposure.

At high doses, methanol can cause reversible or permanent blindness, and in severe cases, death. Visual problems include eye pain, blurred vision, constriction of visual field, and possibly permanent blindness, which can develop in as little as 48 h. The lethal dose of methanol in untreated individuals is estimated to be in the range of 0.8–1.5 mg kg^{-1}, which translates into ~56–100 g, or 70–130 ml of 100% methanol, for the average individual (70 kg). Typically, the effects noted in methanol poisoning can be divided into three stages: (1) narcosis or CNS depression similar to that observed in ethanol intoxication; (2) a latent period, generally 10–15 h but can be prolonged if ethanol is coingested; and (3) visual disturbances, metabolic acidosis and possibly multiorgan failure leading to death.

Chronic Toxicity (or Exposure)

Animal

Rats exposed during pregnancy to doses up to 20 000 ppm in air for 7 h day^{-1} developed fetal skeletal, cardiac, and urologic abnormalities.

Human

Methanol is a normal part of the human diet (via fresh fruits and vegetables) and is produced in the body by metabolic breakdown of other products.

In Vitro Toxicity Data

Mutagenicity studies in Syrian hamster embryos and *Neurospora crassa* have been negative.

Clinical Management

Gastric decontamination by gastric lavage may be beneficial for patients that present less than an hour after a large ingestion of methanol. Administration of ethanol or preferably fomepizole, to competitively block the metabolism of methanol, is the first line of therapy. Fomepizole has significantly higher binding affinity for ADH than ethanol with lesser side effects and consequently is the preferred therapy. Blocking therapy is preventative in nature and most effective when started soon after an overdose. Leucovorin (folininic acid) may be beneficial for patients that present in stage three as it provides a folate source to help detoxify circulating formate/formic acid. Hemodialysis to remove methanol, toxic metabolites and to correct acid-base disorders has been found to be the most effective therapy in methanol poisonings.

Environmental Fate

Methanol can be released from natural sources (e.g., emissions from certain plants, as a by-product of degradation of organic material) as well as from human use of methanol as a solvent. Methanol has a low vapor pressure and will volatilize into air. With

volatilization into the air, methanol degrades via reaction with airborne hydroxyl radicals and has a half-life of ~18 days. Methanol can be removed from air via rainfall. If released into water, methanol decomposition is expected to occur via biodegradation. If released to soil, methanol is expected to degrade and be susceptible to leaching. Because of the low vapor pressure of methanol, rapid evaporation from dry surfaces occurs.

See also: Ethanol; Ethylene Glycol; Formaldehyde.

Further Reading

Bennett IL Jr. (1953) Acute methyl alcohol poisoning: A review based on experience in an outbreak of 323 cases. *Medicine* 32: 431–463.

Brent J, McMartin K, and Phillips SP (2001) Fomepizole for the treatment of methanol poisoning. *New England Journal of Medicine* 344: 424–429.

Frenia ML and Schauben JL (1993) Methanol inhalation toxicity. *Annals of Emergency Medicine* 22: 1919–1923.

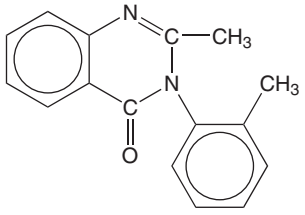
Kulig K, Duffy JP, and Linden CH (1984) Toxic effects of methanol, ethylene glycol, and isopropyl alcohol. *Emergency Medicine* 14–29.

Methaqualone

Christopher P Holstege

This article is a revision of the previous print edition article by Linda Hart, volume 2, pp. 297–298, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Numbers: CAS 72-44-6 (base); CAS 340-56-7 (hydrochloride)
- Synonyms: Ludes; Mequin; Parest; Quaalude; Sopes
- Chemical/Pharmaceutical/Other Class: Sedative–hypnotic
- Chemical Structure:



Uses

Historically, methaqualone was used as a hypnotic for the treatment of insomnia. However, it is less effective than the benzodiazepines for this indication. It also has anticonvulsant, antitussive, and weak antihistaminic properties. It no longer has clinical therapeutic value and is not manufactured in the United States for legitimate pharmaceutical purposes. It is manufactured by clandestine laboratories for drug abuse purposes.

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposures.

Toxicokinetics

Methaqualone is completely absorbed from the gastrointestinal tract within 2 h. The rate of absorption of the HCl salt is faster than that of the freebase form because of faster dissolution in the stomach. Methaqualone is nearly completely metabolized by the liver by hydroxylation. It is highly lipophilic with a volume of distribution of 2–6 l kg^{-1}. The elimination half-life is ~40 h for therapeutic doses and may be prolonged after overdose. Metabolites are excreted in the urine. These metabolites may be found in the urine up to 7 days postingestion.

Mechanism of Toxicity

Methaqualone is a central nervous system depressant similar to other sedative–hypnotics that cause enhanced gamma-aminobutyric acid activity.

Acute and Short-Term Toxicity (or Exposure)

Human

Signs and symptoms reported in overdose have included gastrointestinal distress, drowsiness, ataxia, slurred speech, paresthesias, agitation, convulsions, and coma. Unique among sedative–hypnotic drugs, hypertonicity, myoclonia, positive Babinsky responses, clonus, and hyperreflexia may develop following severe methaqualone poisoning. The cough reflex is decreased with this drug. Methaqualone inhibits platelet aggregation, prolongs prothrombin time, and partial thromboplastin time, and decreases factors V and VII, all of which may lead to conjunctival, retinal, and gastrointestinal hemorrhage.

Chronic Toxicity (or Exposure)

Animal

In chronic dosing studies in rats and dogs, 30 mg $kg^{-1} day^{-1}$ of methaqualone produced no toxic effects. Similar studies using doses of 130 mg kg^{-1} day^{-1} resulted in mortality of 60%.

Human

Tolerance and physical dependence may develop in persons who chronically use methaqualone. Abrupt discontinuation of chronic methaqualone abuse may result in a withdrawal syndrome consisting of anxiety, agitation, insomnia, tremors, headache, myalgias, nausea, vomiting, diaphoresis, and hyperpyrexia.

In Vitro Toxicity Data

Methaqualone use has been associated with reports of bleeding in humans. *In vitro* studies have demonstrated methaqualone induced blood platelet dysfunction in human platelets.

Clinical Management

Basic and advanced life-support measures should be implemented as necessary. Gastrointestinal decontamination procedures should be used as appropriate based on the patient's level of consciousness and history of ingestion. Activated charcoal can be used to adsorb methaqualone. The patient's level of consciousness and vital signs should be monitored closely. Obtunded patients with reduced gag reflex should be intubated to prevent pulmonary aspiration. Respiratory support, including oxygen and ventilation, should be provided as needed. If hypotension occurs it should be treated with standard measures including intravenous fluids, Trendelenburg positioning, and dopamine by intravenous infusion. Forced diuresis, hemoperfusion, and hemodialysis are of no value in methaqualone toxicity. Seizures should be treated with benzodiazepines. Coagulation studies and platelet counts should be obtained. If withdrawal signs and symptoms develop, treatment should consist of benzodiazepine therapy with a gradual dose reduction.

See also: Benzodiazepines; Drugs of Abuse.

Further Reading

Majelyne W, De Clerck F, and Demeter J (1979) Treatment evaluation of a severe methaqualone intoxication in man. *Veterinary and Human Toxicology* 21(suppl.): 201–204.

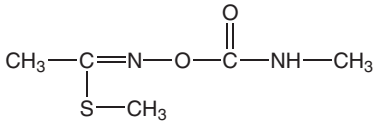
Matthew H, Proudfoot AT, and Brown SS (1968) Mandrax poisoning: Conservative management of 116 patients. *British Medical Journal* 2: 101–102.

Methomyl

Carey N Pope

This article is a revision of the previous print edition article by Janice Reeves and Carey N Pope, volume 3, pp. 298–299, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 16752-77-5
- Synonyms: Nudrin; Lannate; Methyl-*N*-((methylcarbomoyl)oxy)thioacetimidate
- Chemical/Pharmaceutical/Other Class: Carbamate insecticide
- Chemical Structure:



Uses

Methomyl is a broad-spectrum insecticide registered for use on several agricultural crops and commercially grown ornamental plants.

Exposure Routes and Pathways

Oral exposures are most common. Inhalation and dermal exposures are also possible, particularly in the workplace.

Toxicokinetics

Methomyl is rapidly absorbed from the oral route. Dermal exposure is less hazardous. It is biotransformed to acetonitrile and carbon dioxide. Methomyl is well distributed to the tissues. Elimination is rapid: less than 10% remains 24 h after an oral exposure. Approximately 75% of an absorbed oral

dose of methomyl is eliminated via exhalation either as CO_2 or as acetonitrile. The remainder is excreted in urine as polar metabolites.

Mechanism of Toxicity

Methomyl exerts toxicity by inhibiting acetylcholinesterase. As with other carbamate insecticides, acetylcholinesterase inhibition is much less persistent than with organophosphate intoxication.

Acute and Short-Term Toxicity (or Exposure)

Animal

Methomyl is extremely toxic to animals; the range of toxicity depends on the route and rate of exposure. Oral LD_{50} values in laboratory rats, mice, and guinea pigs range from about 10 to 24 mg kg^{-1}. The 4 h inhalation LC_{50} in rats was 0.3 mg l^{-1}. It is only slightly toxic by dermal application (LD_{50} in rabbits >5 g kg^{-1}).

Human

Methomyl is highly toxic by the oral route, moderately toxic by inhalation, and has low dermal toxicity. The primary symptom in acute methomyl poisoning is severe headaches, which may be accompanied by less severe symptoms such as nausea, vomiting, salivation, and abdominal pain. Other general symptoms are cramps, diarrhea, sweating, lassitude, weakness, runny nose, chest tightness, and blurring or dimness of vision. Sometimes the effects of acute exposure to carbamates can be long lasting. The probable lethal oral dose in humans is 5–50 mg kg^{-1}. The actual lethal dose of methomyl depends on the route and rate of exposure and the aggressiveness of the treatment used.

Chronic Toxicity (or Exposure)

Animal

In a 2 year feeding study in dogs, the no-observed-adverse-effect level was 5 mg kg^{-1} day^{-1}. Methomyl is not teratogenic or a reproductive toxicant.

Human

Because of its rapid biotransformation, methomyl does not tend to cause cumulative toxicity. However, repeated, frequent exposures could lead to cumulative inhibition of cholinesterase, resulting in flu-like symptoms including weakness, loss of appetite, and myalgia.

In Vitro Toxicity Data

Methomyl was negative in a variety of mutagenesis, DNA damage, and cytogenesis studies.

Clinical Management

Basic life-support measures must be maintained. The patient should be moved to fresh air, and exposed eyes and skin should be irrigated with large amounts of water. Atropine is used to counteract muscarinic side effects. Pralidoxime is contraindicated. Charcoal may be used to absorb methomyl.

Environmental Fate

Methomyl has moderate persistence in soil (half-life of 14 days). It is highly water soluble and can translocate to the groundwater. Methomyl readily undergoes microbial degradation in soil under anaerobic conditions. Dissipation in soil was influenced by differences in soil moisture content, which likely affect microbial activity, irrigation, and thus leaching. It is degraded in water with aeration, in sunlight, and under alkaline conditions (half-life in surface water of 6 days) but is more stable in neutral or acidic conditions.

Ecotoxicology

Methomyl is highly toxic to birds (oral LD_{50} in quail was 24–34 mg kg^{-1}, 28 mg kg^{-1} in chickens, and 10–45 mg kg^{-1} in other species). Methomyl is moderately to highly toxic to fish and aquatic invertebrates. In rainbow trout, the 96 h LC_{50} was 3.4 and 0.8 mg l^{-1} in bluegill. The 48 h LC_{50} for *Daphnia* was 0.0287 mg l^{-1}. Methomyl does not bioaccumulate. It is highly toxic in bees.

Exposure Standards and Guidelines

The chronic reference dose is 0.008 and the acceptable daily intake is 0.03 mg kg^{-1} day^{-1}.

See also: Carbamate Pesticides; Cholinesterase Inhibition; Pesticides.

Further Reading

Ecobichon DJ (2001) Carbamate insecticides. In: Krieger R (ed.) *Handbook of Pesticide Toxicology*, 2nd edn. San Diego, CA: Academic Press.

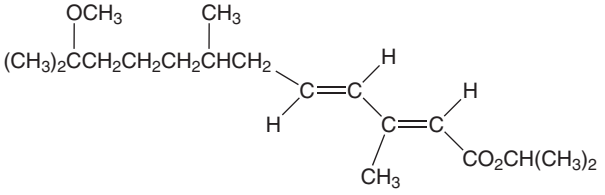
Relevant Websites

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.
http://www.epa.gov – US Environmental Protection Agency.

Methoprene

Eric M Silberhorn

- Chemical Abstracts Service Registry Number: CAS: 40596-69-8
- Synonyms: Altosid; Altosand; Apex; Manta; Minex; Diacon; Dianex; Kabat; Pharorid; Precor; Isopropyl(2*E*,4*E*,7*R*,*S*)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate
- Chemical/Pharmaceutical/Other Class: Synthetic terpenoid
- Chemical Formula: $C_{19}H_{34}O_3$
- Chemical Structure:



Uses

Methoprene is a broad-spectrum synthetic juvenile hormone mimic, which acts as an insect growth regulator (insecticide). It prevents larval insect stages from undergoing metamorphosis to viable adults and thus acts as a larvicide. It is useful for control of a variety of insect pests including ants, mosquitoes, flies, fleas, beetles, lice, and moths, but is only effective against larvae, not adults or pupae. Many different products (e.g., pesticides, veterinary drugs) and formulations containing methoprene are commercially available. Methoprene products used for protecting pets such as cats and dogs include capsules administered orally and flea collars used externally. Production animals (e.g., cattle) typically receive methoprene in the diet as a food additive. Other formulations of methoprene include emulsifiable concentrates, pellets and tablets, granules, and aerosols. Some of these are applied to water for mosquito control whereas others are sprayed in areas where foods are stored to prevent insect infestations.

Background Information

Methoprene is the common name for a racemic mixture of two enantiomers (*R* and *S* in a ratio of 1:1). The activity of the compound as a juvenile hormone mimic is restricted to the *S* enantiomer.

Exposure Routes and Pathways

Dermal contact and eye contamination are the most common routes of exposure for humans.

Toxicokinetics

Methoprene may be absorbed from the gastrointestinal tract, through the intact skin, and by inhalation of spray mist. The metabolism of methoprene has been studied in rat, mouse, guinea pig, cow, and chicken given single doses. No studies of metabolism after repeated exposures have been conducted. Available studies indicate that methoprene is metabolized rapidly and extensively when given in low doses and excreted in urine, feces, and expired air. Metabolism is primarily via hepatic microsomal esterases, which initially produce methoprene acid. This compound undergoes both alpha- and beta-oxidation to form acetate, of which the majority is transformed via the Kreb's cycle into carbon dioxide and respired as such. Some carbon dioxide was also incorporated into natural products such as triglycerides, bile acids, and cholesterol. When administered at high dose levels, large quantities of methoprene are excreted unchanged in the feces, but are not found in the urine or blood, suggesting that intestinal absorption is slowed greatly but that metabolism is not saturated.

Mechanism of Toxicity

Juvenile hormones are one of three types of internal regulators of insect growth and metamorphosis. These hormones are synthesized and released in a regulated way into the hemolymph. Immature larvae require these hormones to progress through the regular larval stages. Methoprene disrupts these hormonal processes and prevents metamorphosis.

Acute and Short-Term Toxicity (or Exposure)

Animal

Methoprene has extremely low acute toxicity in laboratory animals. The oral LD_{50} value for rats is $>30\,000\,mg\,kg^{-1}$ and the value for dogs is between 5000 and $10\,000\,mg\,kg^{-1}$. The acute dermal LD_{50} for rabbits is $>2000\,mg\,kg^{-1}$. The acute (4 h) inhalation LC_{50} in the rat and guinea pig is $>210\,mg\,l^{-1}$. Methoprene is not irritating to the eye or skin when tested on rabbits and did not act as a skin sensitizer when tested on guinea pigs.

Human

No clinical effects or overt signs of toxicity are known to have occurred in humans due to methoprene exposure. Because of its mechanism of action, methoprene has high selectivity for insects and no acute toxicity is expected in humans even after ingestion of large doses.

Chronic Toxicity (or Exposure)

Animal

Several chronic and subchronic toxicity studies have been conducted with methoprene and show that the compound has little toxic potential. The main effect at high dose levels is an increase in the liver weight compared to body weight. Rats receiving up to 5000 ppm in the diet for 2 years did not exhibit any increases in tumor incidences; however, increased numbers of hepatic lesions were found at the highest dose level. The no-observed-adverse-effect level (NOAEL) was 1000 ppm, equivalent to 44 mg kg^{-1} body weight per day. Similarly, in mice fed up to 2500 ppm in the diet for 18 months, no toxicologically relevant effects were observed except for focal accumulations of macrophages with brownish foamy cytoplasm in livers and an increased frequency of amyloidosis of the intestine at 2500 ppm. The no-observed-effect level (NOEL) for systemic toxicity in this study was established at 1000 ppm, equivalent to 130 mg kg^{-1} body weight per day. In separate 90 day feeding studies, the NOEL in both rats and dogs was 500 ppm in the diet. Increased liver weights in rats and dogs, and renal tubular degeneration effects in some rats were observed at higher dose levels; however, the significance of these effects is questionable because they were not observed in longer feeding studies. In a 30 day dermal toxicity study on rabbits, the NOEL values for systemic effects and local effects were 300 and 100 mg kg^{-1}, respectively. In a 21 day inhalation toxicity study in rats, the NOEL for methoprene was 20 mg l^{-1}.

Methoprene is not teratogenic or strongly fetotoxic based on the results of several developmental and reproductive toxicity studies conducted in rabbits, rats, and mice. In a study in which mice were treated on days 7–14 of gestation, no toxicologically relevant effects were observed in dams or fetuses at any dose tested; the NOAEL was 570 mg kg^{-1} body weight per day, the highest dose tested. This study was extended and pups in some litters were observed for an additional 7 weeks. Effects on organ weights were observed at the highest dose; therefore, the NOAEL for toxicity to offspring was 190 mg kg^{-1} body weight per day. In a similar study with rabbits that were treated on days 7–18 of gestation, the NOAEL for maternal, embryo and fetotoxicity was 190 mg kg^{-1} body weight per day. There was an increase in fetal deaths and abortions at the highest dose tested, 1900 mg kg^{-1} body weight per day. The NOAEL in a three-generation reproduction study conducted in rats was 500 ppm in the feed (equivalent to 29 mg kg^{-1} body weight per day), based on reduced growth of pups and a slight increase in the number of pups of the F_3 litters that were born dead.

Human

No clinical effects or symptoms of toxicity are known to have occurred in humans due to methoprene exposure alone; however, some commercial formulations may potentially contain additional ingredients that cause skin or eye irritation, or allergic reactions after repeated exposures. Based on negative results in several genotoxicity studies and the results of the studies of carcinogenicity with methoprene, it is very unlikely that methoprene poses a carcinogenic risk to humans.

In Vitro Toxicity Data

Methoprene is not genotoxic based on negative results in the Ames *Salmonella* test, several other genotoxicity assays, and a dominant lethal study in rats.

Clinical Management

In cases of skin exposure, the exposed area should be thoroughly washed with soap and water. Eyes should be washed with copious amounts of room-temperature water in cases of eye contamination. If small amounts are ingested, no treatment is generally needed. Emesis is seldom necessary due to low toxicity; it is contraindicated when methoprene is in a hydrocarbon base. Activated charcoal is preferred. It can be administered as aqueous slurry or as a mixture of charcoal with saline cathartics or sorbitol. Symptomatic treatment is recommended.

Environmental Fate

Methoprene degrades rapidly in sunlight, both in water and on inert surfaces. It is metabolized rapidly in soil under both aerobic and anaerobic conditions (half-life = 10–14 days). The major microbial degradation product is carbon dioxide. Degradation in both freshwater and saltwater is also quite rapid with a half-life of 10–35 days at 20°C. Methoprene is not very soluble in water (< 2 ppm) and as a result is not

highly mobile in soil. Because of this and its rapid biodegradation, methoprene does not persist for long periods in soil and is unlikely to contaminate groundwater. Based on studies with bluegill sunfish, significant bioconcentration of methoprene is not expected in fish tissues as a result of aquatic exposures.

Ecotoxicology

Methoprene is practically nontoxic to birds with an acute oral LD_{50} of $>2000\,mg\,kg^{-1}$ in mallard ducks and an 8 day dietary $LC_{50} > 10\,000$ ppm in bobwhite quail. In avian reproduction studies, methoprene had no effects on reproductive parameters at dietary concentrations of 30 and 3 ppm in quail and mallards, respectively.

Methoprene toxicity has been studied extensively in aquatic life as a result of concern over possible effects on nontarget organisms. Toxicity to fish is slight to moderate with a 96 h LC_{50} of $1.5\,mg\,l^{-1}$ (ppm) for bluegill sunfish and $>50\,mg\,l^{-1}$ for rainbow trout. Toxicity to aquatic invertebrates is generally moderate to high, although there is considerable species-to-species variation in sensitivity. Acute LC_{50} values for most invertebrates are $>900\,\mu g\,l^{-1}$ (ppb). Effects on reproductive parameters have been observed at lower concentrations in studies with invertebrates. For example, exposures of *Daphnia pulex* to nominal concentrations of 10 and $100\,\mu g\,l^{-1}$ caused changes in the incidences of all-male and all-female broods compared to controls. Methoprene has also been shown to produce developmental toxicity (malformations) in exposed amphibians, but only at concentrations that produce other toxic effects and those that are greater than those typically resulting from field applications of mosquito control products.

Exposure Standards and Guidelines

The acceptable daily intake (ADI) for racemic methoprene is up to $0.09\,mg\,kg^{-1}$ body weight. For *S*-methoprene, the ADI is up to $0.05\,mg\,kg^{-1}$ body weight.

See also: Pesticides.

Further Reading

Degitz SJ, Duhan EJ, Tietge JE, *et al.* (2003) Developmental toxicity of methoprene and several degradation products in *Xenopus laevis*. *Aquatic Toxicology* 64: 97–105.

Dodson SI, Kashian DR, and Peterson JK (2001) Methoprene and 20-OH-ecdysone affect male production of *Daphnia pulex*. *Environmental Toxicology and Chemistry* 20: 582–588.

Food and Agriculture Organization (FAO) and World Health Organization (WHO) (2001) Toxicological evaluations for methoprene and *S*-methoprene. In: *Pesticide Residues in Food: 2001*. Joint Meeting of the FAO Panel of Experts on Pesticides Residues in Food and the Environment and the WHO Core Assessment Group. Geneva, Switzerland.

US Environmental Protection Agency (1991) *Reregistration Eligibility Document Methoprene*. Washington, DC: Office of Pesticides Programs.

US Environmental Protection Agency (2001) *Methoprene Pesticide Fact Sheet. Update of 1991 R.E.D. Facts for Methoprene*. Washington, DC: Office of Pesticides and Toxic Substances.

Methoxychlor

Guangping Chen

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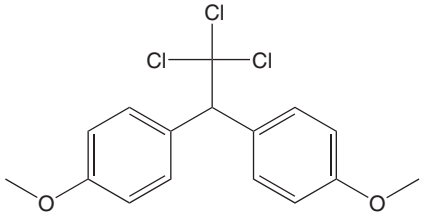
- Chemical Abstracts Service Registry Number: CAS 72-43-5
- Synonyms: 1,1,1-Trichloro-2,2-bis(*p*-methoxyphenyl)-ethane; 1,1′-(2,2,2-Trichloroethylidene)bis(4-methoxy-benzene); Dimethoxy-DT; DMDT; ENT 1716; Higalmetox; Methoxychlore; Marlate; Methoxy-DDT; OMS 466; Prentox; Marlate®; Metox®; Chemform
- Chemical/Pharmaceutical/Other Class: Organochlorine insecticide
- Chemical Formula: $C_{16}H_{15}Cl_3O_2$
- Chemical Structure:



Uses

Methoxychlor is a manufactured chemical. Pure methoxychlor is a pale-yellow powder. Methoxychlor is used as an insecticide against flies and a wide variety

of other insects. It is also used on agricultural crops and livestock, home garden, and on pets. Methoxychlor is used against the beetle vectors of Dutch elm disease.

Exposure Routes and Pathways

Ingestion and dermal contact are possible routes of exposure. Individuals may be exposed by ingestion of food or drinking water contaminated with methoxychlor.

Toxicokinetics

Chlorinated hydrocarbon insecticides, when dissolved in oil or other lipid, are readily absorbed by the skin and alimentary canal. Although methoxychlor is slowly metabolized to a small extent by pathways similar to those of DDT, the major pathway is by *O*-demethylation and subsequent conjugation. Methoxychlor has been detected in the blood of agricultural workers. All organochlorines are likely to be excreted in the milk of lactating women. Methoxychlor is excreted mainly in feces, and to a lesser extent in urine.

Mechanism of Toxicity

Methoxychlor undergoes hepatic microsomal monooxygenase(s)-mediated activation and the resultant reactive metabolites (possibly free radicals) bind covalently to microsomal components.

Animal Toxicity

Ocular toxicity has been reported from systemic exposure to methoxychlor. Chronic intoxication of dogs at dosages of $2000\,mg\,kg^{-1}\,day^{-1}$ in the diet led to convulsions in 6 weeks and death in 9 weeks; weight loss, high alkaline phosphatase and serum transaminase, and intestinal congestion were seen; and swine showed kidney injury and uterine and mammary enlargement. Rabbits given $200\,mg\,kg^{-1}\,day^{-1}$ orally died after four or five doses; autopsy findings included mild liver damage and nephrosis. Atrophy of the testes was observed in rats given 1% methoxychlor in the diet. In rats given 100 or $200\,mg\,kg^{-1}\,day^{-1}$, arrested spermatogenesis was noted after 70 consecutive days of treatment; corpora lutea failed to develop in female rats treated with similar dosages for 14 days before and after mating. Administration of $1000\,mg\,kg^{-1}$ in the diet to pregnant rats caused early vaginal openings in their offspring; both male and female offspring had reduced fertility when they attained maturity. No abortions were observed in pregnant cows given methoxychlor at $10\,mg\,kg^{-1}\,day^{-1}$. In a 27-month study in rats, 1000 ppm methoxychlor was administered in the diet; there were no differences in deaths or the incidence and distribution of benign or malignant tumors in treated animals versus controls. There is no evidence of carcinogenicity in animals. Results were negative in the *Escherichia coli* WP2, UVRA reverse mutation assay. Injection of a 0.1% solution of methoxychlor did not induce sex-linked recessive lethals in male *Drosophila melanogaster*.

Human Toxicity

In extreme overdoses, central nervous system depression may occur. In general, for chlorinated hydrocarbon insecticides, aspiration of insecticide-containing petroleum distillate may result in pneumonitis. In addition, nausea, vomiting, and diarrhea may follow ingestion; blood dyscrasias, anemia, and leukemia have been associated with organochlorine exposure, and extensive contact results in dermal irritation. The approximate fatal dose is $6\,g\,kg^{-1}$. Chronic exposure may cause kidney damage. There is no evidence of carcinogenicity in humans.

Clinical Management

In general, following acute exposure to chlorinated hydrocarbon insecticides, blood chlorinated hydrocarbon levels are not clinically useful; for most compounds it reflects cumulative exposure over a period of months rather than recent exposure. Emesis may be indicated and is most effective if initiated within 30 min postingestion. In addition, an activated charcoal/cathartic may be given. For seizures, diazepam should be administered as an intravenous bolus. Oils should not be given by mouth. Adrenergic amines should not be administered because they may further increase myocardial irritability and produce refractory ventricular arrhythmias. If clothing is contaminated, it should be removed.

Environmental Fate

Methoxychlor is very persistent in soil. Its half-life is $\sim$120 days. Methoxychlor degrades much more rapidly in aerobic conditions than in anaerobic conditions. Methoxychlor is tightly bound to soil and is insoluble in water. The risk to groundwater should be low. Movement of the pesticide is likely via adsorption to suspended soil particles.

Ecotoxicology

Methoxychlor is slightly toxic to bird species. Methoxychlor is highly toxic to fish and aquatic invertebrates. Methoxychlor accumulates in aquatic organisms because these organisms metabolize methoxychlor very slowly. The compound is relatively nontoxic to bees.

See also: Cholinesterase Inhibition; Cyclodienes; Pesticides.

Further Reading

Cummings AM (1997) Methoxychlor as a model for environmental estrogens. *Critical Reviews in Toxicology* 27(4): 367–379.

Gore AC (2001) Environmental toxicant effects on neuroendocrine function. *Endocrine* 14(2): 235–246.

Kanno J, Onyon L, Peddada S, *et al.* (2003) The OECD program to validate the rat uterotrophic bioassay. Phase 2: Coded single-dose studies. *Environmental Health Perspectives* 111(12): 1550–1558.

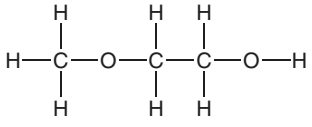
Relevant Websites

http://ace.orst.edu – Extension Toxicology Network.
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Methoxychlor.
http://www.epa.gov – United States Environmental Protection Agency.
http://www.oehha.ca.gov – California Environmental Protection Agency.

Methoxyethanol

Michael J Brabec

- Chemical Name: Ethylene glycol monomethyl ether
- Chemical Abstracts Service Registry Number: CAS 109-86-4
- Synonyms: 2-Methoxyethanol; Methyl cellosolve; Methyl glycol; Amsco-Solv EE; Dowanol EM; Ektasolve EM; Glycol methyl ether; Monoethylene glycol methyl ether; Methyl oxitol; Monomethyl glycol ether
- Chemical/Pharmaceutical/Other Class: Glycol ether
- Chemical Formula: $C_3H_8O_2$
- Chemical Structure:



Uses

Methoxyethanol is an industrial solvent with high water miscibility. It is widely used in paints, lacquers, stains, inks, surface coatings, and food-contact plastics. Products have applications in printing, leather processing, photography and photolithography processes, such as in the semiconductor industry, and in textile finishing. It is used as an anti-icing ingredient in jet fuels and lubricants.

Exposure Routes and Pathways

As a solvent with a relatively high vapor pressure, exposures to both liquid and vapor states can simultaneously occur. About equal amounts can be absorbed via inhalation and dermal exposure to methoxyethanol vapors. Oral exposure would be unlikely except by deliberate or accidental ingestion of methoxyethanol-containing liquids. Unfortunately, since methoxyethanol has a mild ethereal odor, is not a surface irritant, and lacks an unpleasant flavor, it has weak warning properties.

Exposure to methoxyethanol can also occur after exposure to the acetic acid ester, methoxyethyl acetate, since the ester is readily hydrolyzed to release methoxyethanol by esterases in tissues lining the respiratory system, by blood cells, and in deep body organs.

Toxicokinetics

Methoxyethanol is rapidly absorbed through the skin and lungs into the blood. Its water solubility favors distribution to all body tissues except adipose tissue. Metabolism occurs via two pathways. Methoxyethanol is a substrate for alcohol dehydrogenase, and the resultant methoxyacetaldehyde is metabolized to methoxyacetic acid by aldehyde dehydrogenase. In rats, pretreatment with phenobarbitol decreased formation of methoxyacetaldehyde but accelerated formation of methoxyacetate in liver cytosolic fractions. A minor pathway involves demethylation by undefined enzymes to ethylene glycol and CO_2.

In Sprague–Dawley rats, males and females quickly eliminate methoxyacetate at similar rates in the

urine, but the male excretes the unmetabolized compound more slowly ($t_{1/2}$ of 50 min vs. 30 min). This may be because the activity of alcohol dehydrogenase in the liver is higher in female rats than in the male rats. Methoxyacetate and methoxyacetylglycine are the principal metabolites in rat urine, with minor amounts of sulfate and glucuronide derivatives also excreted. Human volunteers exposed to low levels (5 ppm) of methoxyethanol vapors for 4 h excreted ~90% of the absorbed dose as methoxyacetate in the urine. The half-life of methoxyethanol is estimated to be ~77 h in humans. The compound does not bioaccumulate.

Mechanism of Toxicity

High acute doses of methoxyethanol have a narcotic effect. Kidney and lung damage, accompanied by hemoglobinuria, follow exposures to high doses. Toxicity is attributed to metabolites, including the putative intermediate methoxyacetaldehyde and methoxyacetate. *In vitro* studies with radiolabeled methoxyethanol indicate that formation of methoxyacetyl-coenzyme A may lead to the formation of methoxyacetyl derivatives of Krebs cycle intermediates. Methoxyacetate produces the same testicular lesions in rodents as does the parent compound, although the immunosuppression elicited by methoxyethanol exposure may depend on the putative metabolite, methoxyacetaldehyde. In both the testicular lesion and the immune suppression, some data suggest that the pattern of cell death termed apoptosis may be stimulated. Methoxyacetate stimulates synthesis of progesterone by luteal cells in culture. This disturbance of luteal function may be related to the prolongation of gestation in rodents. Teratogenicity appears to be related to interference by methoxyethanol, or its metabolites, with one carbon metabolism in the synthesis of nucleotide precursors, and can be relieved by administration of other substrates, such as serine and glycine, which also provide substrates for nucleotide synthesis.

Acute and Short-Term Toxicity (or Exposure)

Animal

Oral LD_{50} values range from 1 to 3 g kg^{-1} in rodents. A dermal LD_{50} of 1.3 g kg^{-1} was determined in rabbits. An inhalation LC_{50} of 4600 ppm was determined in mice. In animals, systemic effects from sublethal, or short-term exposures, are kidney damage, thymic involution, depression of blood cell counts, and depression of bone marrow cellularity. Depression of the primary antibody plaque-forming cell response in rats suggests that methoxyethanol could inhibit the humoral immune system. Testicular atrophy and depression of sperm counts are noted in animal studies. Methoxyethanol, along with several other known testicular toxins, causes an increase in the urinary creatine/creatinine ratio as one of the earliest and most sensitive markers of testicular damage after acute exposure. Methoxyethanol is considered to be a mild skin and eye irritant. Methoxyethanol is not a contact sensitizer.

Human

Deliberate or accidental human exposures have defined the toxicity expected from acute and short exposures to methoxyethanol by all routes. High doses can kill by severe central nervous system (CNS) depression. A human death resulted from hemorrhagic gastritis and degeneration of liver, kidney, and pancreas following ingestion of ~225 ml of the neat compound. Two individuals who drank ~100 ml each of methoxyethanol survived, but were severely ill with symptoms of severe intoxication, metabolic acidosis, hyperventilation, tachycardia, nausea, weakness, and cyanosis.

Chronic Toxicity (or Exposure)

Animal

Reproductive toxicity in both sexes is the most significant effect of chronic exposures to low doses of methoxyethanol in humans and animals. Both chronic and subchronic studies indicate that rabbits are more sensitive to methoxyethanol than rats and mice, and primates, including human, are more sensitive to the teratogenic effects of methoxyethanol than any rodent species. A dose of 12 mg kg^{-1} day^{-1} during the second trimester of pregnancy in monkeys produced 29% dead or resorbed fetuses, while 36 mg kg^{-1} day^{-1} caused 100% fetal death. Doses of 25 mg kg^{-1} day^{-1} are the lowest reported to cause fetal abnormalities in rodents, with skeletal abnormalities of the extremities as one remarkable feature. Dose levels of 25 mg kg^{-1} day^{-1} during the sensitive period of gestation in rodents are also associated with reduced litter size, reduced litter weight, and prolongation of pregnancy. Examination of rodent fetuses at different periods during development indicate that repair of early damage occurs when the exposure ceases, which may contribute to the greater tolerance rodents display toward methoxyethanol.

Methoxyethanol is a male reproductive toxin. Males display testicular atrophy, tubular degeneration, and reduced sperm counts at doses as low as

25 mg kg^{-1} day^{-1} (rabbits) with symptoms increasing in severity as dose levels increase. Loss of pachytene spermatocytes in a stage-specific manner is the earliest noted lesion. Effects are reversible after low doses.

As the dose level of chronic exposures increases, methoxyethanol becomes immunosuppressive. Thymic involution, decrease of spleen weight, and suppression of various lymphocyte and antibody responses are noted at doses as low as 50 mg kg^{-1} in rats and mice, although comparison of several studies indicate that rats may be more susceptible than mice. Effects became more severe as the dose level of methoxyethanol increased in the studies.

Human

Evidence gathered from pregnant women exposed to methoxyethanol in the semiconductor industry would strongly suggest that methoxyethanol is a human teratogen as well as a reproductive toxin. Decreased fertility, increased incidence (roughly twofold) of spontaneous abortion, and prolongation of the menstrual cycle were found in women exposed to a variety of solvents that included methoxyethanol. In one report, six children delivered by five women heavily exposed to methoxyethanol in the workplace displayed characteristic dysmorphic features. Cleft palate, CNS malformations, mental retardation, and musculoskeletal malformations were noted in the offspring born to mothers with exposure to methoxyethanol. Thirty-five children delivered by a matched population of 23 nonexposed women in the same workforce did not present a similar pattern of lesions. Although it is difficult to ascertain the exposure history associated with the human subjects, effects were noted at exposure levels below 1 ppm, with more dramatic effects as exposures increased.

Reduced sperm counts, decreased testes size, decreased circulating testosterone and follicle stimulating hormone (FSH) levels, and an increase in luteinizing hormone (LH) were reported in men occupationally exposed to methoxyethanol at levels in the range 5.4–8.5 ppm. A tendency toward reduced fertility in the spouses of men exposed to methoxyethanol was also observed in this study. Increases in the incidence of oligospermia and azoospermia were found in painters exposed to methoxyethanol (mean level of exposure less than 1 ppm). However, no reduction of fertility was noted. The study was complicated by the simultaneous exposure of the workers to ethoxyethanol. No paternal effects on offspring have been associated with human methoxyethanol exposures.

Examinations of male and female workers exposed to methoxyethanol reveal hematopoietic effects manifested as anemia and alterations in numbers of white blood cells. Exposures were estimated as at, or below, 1 ppm.

In Vitro Toxicity Data

Methoxyacetate inhibits lactate production by testicular cells in culture.

Short-term tests for mutagenicity, such as the Ames assay, and various mammalian cell culture assays, indicate that methoxyaldehyde may be mutagenic at high doses but methoxyethanol does not produce any positive response. Thirty-two millimole concentrations of methoxyethanol (but not equimolar concentrations of methoxyacetate) inhibited gap junction communication in cultures of rat myometrial myocytes. The failure of the end metabolite, methoxyacetate, and the relatively high concentrations required to produce a positive result led the authors to conclude that inhibition of gap junction communication was not likely as a mechanism of female reproductive toxicity.

Clinical Management

Symptoms of patients acutely exposed to methoxyethanol depend on route of exposure. Inhalation exposure can lead to irritation of the respiratory tract and delayed presentation of pulmonary edema. Oral consumption will produce systemic toxicity, so prompt induction of vomiting following several glasses of water can reduce exposure. Contaminated clothing should be removed and methoxyethanol washed away with soapy water as quickly as possible after dermal exposure. Prompt flushing with copious amounts of water is recommended after eye contact. Observation of the patient for 24 h after exposure is important because presentation of pulmonary, liver, and kidney damage may not be evident for 12 h or longer after exposure. Animal studies indicate that attempts to alter the pharmacokinetic behavior of methoxyethanol by simultaneous, or subsequent, consumption of ethyl alcohol were of limited success in alleviating toxicity.

Environmental Fate

Since methoxymethanol is highly miscible with water and readily breaks down by several enzymatic routes, bioconcentration is unlikely and has not been shown to occur.

Ecotoxicology

The acute and chronic effects of methoxyethanol and its acetate ester on aquatic ecosystems have been examined. The effects of methoxyethanol are judged to

be negligible at the range of concentrations expected in the environment.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists exposure limit is 5 ppm, with a warning about dermal exposure routes. The Occupational Safety and Health Administration recommends a permissible exposure limit, 8 h time-weighted average, of 25 ppm. Exposure controls include the use of personal protective equipment such as respirators, chemical-resistant gloves, and chemical safety goggles. Contaminated clothing should be washed before reuse. Spills on skin, or eye splash should be thoroughly removed by flushing with copious amounts of water. Methoxyethanol is subject to SARA section 313 reporting requirements.

See also: Jet Fuels; Reproductive System, Female; Reproductive System, Male.

Further Reading

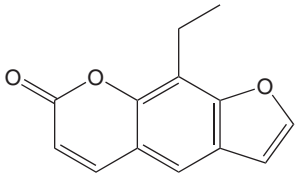
Johanson G (2000) Toxicity review of ethylene glycol monomethyl ether and its acetate ester. *Critical Reviews in Toxicology* 30(3): 307–345. Material Safety Data Sheets may be obtained from principal suppliers of methoxyethanol.

Vincoli JW (ed.) (1997) Ethylene glycol monomethyl ether. *Risk Management for Hazardous Chemicals*, vol. 1, pp. 1317–1320. Boca Raton, FL: CRC Press, Lewis Publishers.

Methoxypsoralen, 8-

Samantha E Gad

- Chemical Abstracts Service Registry Number: CAS 298-81-7
- Synonyms: Xanthotoxin; 9-Methoxypsoralin; Ammoidin; Oxsoralen; Methoxsalen; 8-MOP
- Chemical/Pharmaceutical/Other Class: Coumarin
- Chemical Formula: $C_{12}H_8O_4$
- Chemical Structure:



Uses

8-Methoxypsoralen and other psoralens are naturally found in plants, including common fruit and vegetable crops. Synthetic forms of 8-methoxypsoralen and 5-methoxypsoralen (bergapten) are widely used as drugs in skin photochemotherapy, for example, with long-wave ultraviolet (UV) light in the treatment of psoriasis, vitiligo, and mycosis fungoides. The combination of UV light and psoralens is called PUVA therapy. They have also been used as tanning activators in many sunscreen preparations. Unfortunately, the use of psoralens in skin photochemotherapy has been shown to have major side effects. Concomitant therapy with 8-methoxypsoralen and other systemic or topical photosensitizing agents (e.g., anthralin, coal tar or coal tar derivatives, griseofulvin, phenothiazines, nalidixic acid, halogenated salicylanilides, sulfonamides, tetracyclines, thiazides, or certain organic staining dyes such as methylene blue, toluidine blue, rose bengal, and methyl orange) may produce additive photosensitizing effects. Particular caution is necessary if 8-methoxypsoralen is administered concomitantly with any topical or systemic photosensitizing agent.

Exposure Routes and Pathways

Dermal and oral exposures are the most likely to occur.

Toxicokinetics

8-Methoxypsoralen is extensively metabolized, and is demethylated to 8-hydroxypsoralen (8-HOP). 8-Methoxypsoralen and 8-HOP are conjugated with glucuronic acid and sulfate; other unidentified metabolites have also been detected. 8-Methoxypsoralen and 8-HOP and their conjugates are excreted in urine. Following oral administration of 8-methoxypsoralen, 80–90% of the drug is excreted in urine within 8 h as hydroxylated, glucuronide, and sulfate metabolites; less than 0.1% of a dose is excreted in urine as unchanged drug. About 95% of the drug is excreted in urine within 24 h as metabolites. When oral 8-methoxypsoralen is administered with food, the extent of absorption and the peak serum concentration appear to be increased. The mechanism of this interaction is not known but may involve the effect of food on dissolution or hepatic metabolism of 8-methoxypsoralen.

Mechanism of Toxicity

The toxic effects of psoralens almost never occur without exposure to UV light. These are photosensitizing

materials that exert their primary effect on the skin. 8-Methoxypsoralen, when activated by long-wavelength UV light in the range of 320–400 nm is strongly erythemogenic, melanogenic, and cytotoxic in the epidermis. The mechanisms of action of 8-methoxypsoralen in inducing repigmentation of vitiliginous skin have not been established. Repigmentation depends on the presence of functioning melanocytes and UV light. 8-Methoxypsoralen may activate the functional and dihydroxyphenylalanine-positive melanocytes present in vitiliginous skin. An increase in the activity of tyrosinase, the enzyme that catalyzes the conversion of tyrosine to dihydroxyphenylalanine (a precursor of melanin), has been shown in melanin-producing cells exposed *in vitro* to trioxsalen and UVA light. In addition, binding of photoactivated psoralens (in triplet states) to pyrimidine bases of nucleic acids, with subsequent inhibitions of DNA synthesis, cell division, and epidermal turnover, has been demonstrated. Following photoactivation, 8-methoxypsoralen forms covalent bonds with DNA to produce monofunctional (addition to a single strand of DNA) and bifunctional adducts (cross-linking to both strands of DNA). Reactions with other proteins also occur. Psoralens may also increase melanin formation by producing an inflammatory reaction in the skin. Other mechanisms of increased pigmentation may include an increase in the number of functional melanocytes (and possibly activation of dormant melanocytes); enhancement of melanin granule synthesis; stimulation of the movement of melanocytes up hair follicles resulting in melanocytic repopulation of the epidermis; and/or hypertrophy of melanocytes and increased arborization of their dendrites. Since psoriasis is a hyperproliferative disorder and other agents effective in the treatment of psoriasis are known to inhibit DNA synthesis, the therapeutic effect of 8-methoxypsoralen in the treatment of psoriasis probably involves binding to DNA and inhibition of DNA synthesis resulting in decreased cell proliferation; other vascular, leukocyte, or cell regulatory mechanisms may be involved. It has been suggested that at low drug load, 8-methoxypsoralen binds to DNA as an intercalator, whereas at higher ratios of 8-methoxypsoralen to DNA, it binds to the outside of DNA, probably in the minor groove and causes some compaction in DNA. Protective eyewear is used to prevent irreversible binding of 8-methoxypsoralen to proteins and DNA components of the lens. The central hypothesis for the reproductive toxicity of 8-methoxypsoralen is that it produces reproductive effects by disrupting the hypothalamus–pituitary axis, and the alternate hypothesis is that this compound targets gonadal function, resulting in alteration of pregnancy outcome.

Acute and Short-Term Toxicity (or Exposure)

Animal

The rat LD_{50} is $>51\,mg\,kg^{-1}$. Female rats had reduced birth weights, a reduced number of implantation sites, pups, and corpora lutea when given 8-methoxypsoralen.

Human

Since 8-methoxypsoralen is a strong photosensitizer capable of producing severe burns if used improperly, it should be used only under the supervision of a physician with special training and experience in photochemotherapy. 8-Methoxypsoralen lotion should be applied only by a physician under controlled conditions for light exposure and subsequent light shielding. The 8-methoxypsoralen lotion should be applied only to small, well-defined vitiliginous lesions, preferably those lesions that can be protected by clothing or a sunscreen from subsequent exposure to UVA light. Because of the potential for serious adverse effects (e.g., ocular damage, aging of the skin, and skin cancer (including malignant melanoma)) resulting from PUVA therapy, the patient should be fully informed by the physician of the risks associated with the treatment. To prevent serious adverse effects, the physician should carefully instruct the patient to adhere to the prescribed 8-methoxypsoralen dosage regimen and schedules for UVA exposure.

Side effects after oral 8-methoxypsoralen therapy are usually mild and include gastric discomfort, nausea, nervousness, insomnia, and depression. Mild, transient erythema occurring 24–48 h after PUVA therapy is an expected cutaneous reaction, and indicates that a therapeutic interaction between 8-methoxypsoralen and UVA has occurred. Areas of skin showing fiery erythema with edema should be shielded during subsequent UVA exposures until the erythema has resolved. Fiery erythema with edema that occurs within 24 h following UVA exposure may indicate a potentially severe burn, since the peak erythemal reaction usually occurs 48–72 h following PUVA therapy. Following 8-methoxypsoralen ingestion and controlled exposure to UVA or sunlight, patients must avoid additional, direct, or indirect exposure to sunlight for at least 8 h; following topical treatment with 8-methoxypsoralen, additional exposure to UV light should be avoided for at least 12–48 h. If exposure to sunlight cannot be avoided, the patient should wear protective clothing (e.g., hat, gloves) and/or apply sunscreens to all areas of the body that may be exposed to the sun (including lips).

Exposure of animals to large doses of PUVA without eye protection has produced cataracts and 8-methoxypsoralen enhances this effect; however, in patients receiving PUVA therapy who use appropriate eye protection, there is no evidence for an increased risk of cataract formation. Prior to the initiation of PUVA therapy and yearly thereafter, patients should have an ophthalmologic examination because of the cataractogenic potential of psoralens.

Because psoralens have caused photoallergic contact dermatitis and may precipitate sunlight allergy, 8-methoxypsoralen should be used with caution in patients with a family history of sunlight allergy. The drug should also be used with caution in patients with gastrointestinal diseases or chronic infection. Oral or topical 8-methoxypsoralen should be used with particular caution in patients receiving topical or systemic therapy with known photosensitizing agents. Oral or topical 8-methoxypsoralen is contraindicated in patients exhibiting idiosyncratic reactions to psoralens or with a history of a sensitivity reaction to the drugs; in patients with diseases associated with photosensitivity (e.g., lupus erythematosus, porphyria cutanea tarda, erythropoietic protoprophyria, variegate porphyria, xeroderma pigmentosum, albinism, hydroa vacciniforme, leukoderma of infectious origin, polymorphous light eruptions), except under special circumstances; in patients with melanoma or history of melanoma; and in patients with invasive squamous cell carcinoma. Oral 8-methoxypsoralen is also contraindicated in patients with aphakia (absence of lenses) because of the increased risk of retinal damage.

Damage to the nail beds can be induced by 8-methoxypsoralen and sunlight. Histological examination of the nail beds showed that the photosensitizing effect of the drug induced the generation of many multinucleated epithelial cells and fibroblasts in the dermis.

The 8-methoxypsoralen concentrations and UV irradiation conditions to which human lymphocytes are exposed therapeutically *in vivo* have been shown to be too low to induce observable numbers of sister chromatid exchanges. However, more point mutations, as indicated by the increased incidence of 6-thioguanine-resistant lymphocytes, were observed in patients treated with psoralen drugs and UV irradiation than in healthy controls. Because oral 8-methoxypsoralen and UVA radiation therapy is mutagenic, concern exists about the potential for teratogenic effects resulting from the use of this therapy at the time of conception and during pregnancy. The pregnancy outcomes among over 1000 patients who were documented and who received UVA radiation treatments have been examined in a prospective study. Although the power of this study to detect an increase in the risk of specific defects is limited, the results found no evidence to suggest that UVA radiation is a potent teratogen.

Chronic Toxicity (or Exposure)

Animal

8-Methoxypsoralen is a reproductive and developmental toxicant in rats; however, in rabbits, doses that caused minor maternal toxicity did not affect fetal growth, viability, or morphological development. The International Agency for Research on Cancer (IARC) has deemed 8-methoxypsoralen to be carcinogenic to animals. For example, rats exposed by gavage for 2 years had clear evidence of carcinogenic activity of 8-methoxypsoralen without UV radiation for male rats, as shown by increased incidences of tubular cell hyperplasia, adenomas, and adenocarcinomas of the kidney and carcinomas of the Zymbal gland. Subcutaneous tissue fibromas and alveolar/bronchiolar adenomas of the lung in male rats may have been related to 8-methoxypsoralen administration. Dose-related nonneoplastic lesions in male rats included increased severity of nephropathy and mineralization of the kidney and forestomach lesions. There was no evidence of carcinogenic activity of 8-methoxypsoralen for female rats in the same study.

Human

8-Methoxypsoralen can cause hyperpigmentation and abnormal nail pigmentation. The IARC has classified 8-methoxypsoralen to be in group 1 (i.e., the agent is carcinogenic to humans). Studies have shown the patients with a history of methotrexate, ionizing radiation, or skin types I or II have an increased chance of developing cutaneous carcinomas when psoralen–UV light therapy is used.

In Vitro Toxicity Data

8-Methoxypsoralen is mutagenic in the Ames *Salmonella* assay, and in yeast, human lymphocyte, sister chromatid exchange, unscheduled DNA synthesis (UDS), and Chinese hamster ovary chromosome aberration studies. In a micronucleus test using V79 cells adapted to photogenotoxicity testing, 8-methoxypsoralen was found to cause micronuclei toxicity upon photochemical activation. Induction of UDS by 8-methoxypsoralen plus UVA was investigated in the epidermis of female hairless mice by means of an *in vivo–in vitro* assay using a liquid scintillation counting method. The results showed that PUVA causes a small induction of UDS, which might be due to slow DNA excision repair over a long period.

Clinical Management

Many of the acute symptoms of psoralen toxicity can be avoided by simply avoiding UV light. Severely exposed patients should be kept in a darkened room for 8–48 h depending on which psoralen is involved. Treatment of burns after PUVA therapy is symptomatic and supportive. UV absorbing wrap should be used around sunglasses for 24 h after 8-methoxypsoralen ingestions to decrease the potential for cataract formation. The body should be shielded from sunlight for at least 48 h after 8-methoxypsoralen exposure.

See also: Photoallergens; Toxicity Testing, Mutagenicity.

Further Reading

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Chignell CF, Haseman JK, Sik RH, Tennant RW, and Trempus CS (2003) Photocarcinogenesis in the Tg.AC mouse: Lomefloxacin and 8-methoxypsoralen. *Photochemistry Photobiology* 77: 77–80.

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Kersten B, Zhang J, Brendler-Schwaab SY, Kasper P, and Móuller L (1999) The application of the micronucleus test in Chinese hamster V79 cells to detect drug-induced photogenotoxicity. *Mutation Research* 445: 55–71.

Mori M, Kobayashi H, Katsumura Y, and Furihata C (2001) Induction of unscheduled DNA synthesis in hairless mouse epidermis by 8-methoxypsoralen plus ultraviolet A (PUVA). *Journal of Toxicolgical Sciences* 26: 1–8.

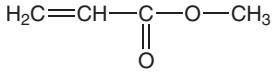
Relevant Website

http://ntp-server.niehs.nih.gov – (US) National Institute of Environmental Health Sciences, National Toxicology Program (NTP). 8-Methoxypsoralen (testing status from the NTP).

Methyl Acrylate

Ralph J Parod

- Chemical Abstracts Service Registry Number: CAS 96-33-3
- Synonyms: Methyl 2-propenoate; 2-Propenoic acid methyl ester; Acrylic acid methyl ester
- Chemical/Pharmaceutical/Other Class: Ester
- Chemical Formula: $C_4H_6O_2$
- Chemical Structure:



Uses

Methyl acrylate is combined with other monomers to produce copolymers used in the production of surface coatings (e.g., latex finishes, floor and fabric finishes), acrylic fibers (used in carpets, clothing, blankets, and curtains), plastics (e.g., medical and dental prostheses), as well as adhesives, detergents, and sealants.

Exposure Routes and Pathways

Exposures to methyl acrylate monomer are most likely to occur in an occupational environment via skin contact and inhalation. However, these exposures are limited since methyl acrylate is a chemical intermediate that is manufactured and processed within closed systems accompanied by the use of industrial hygiene controls and personal protective equipment. The acrid odor of methyl acrylate, which can be detected at 0.002–0.014 ppm, also serves to limit exposure. Studies in workers involved in the production of the monomer have indicated that mean exposures to methyl acrylate are typically less than 1 ppm. The general population is not expected to receive a significant exposure to methyl acrylate monomer due to low concentrations of residual monomer in consumer products. The public may be exposed to methyl acrylate via ingestion because it is found naturally in some foods (e.g., pineapple puree).

Toxicokinetics

Data from animal experiments indicate that methyl acrylate is readily absorbed from the respiratory and gastrointestinal tracts. Absorption of methyl acrylate through the skin occurs less readily and may be limited by evaporation of methyl acrylate if the applied dose is unoccluded.

The primary route of methyl acrylate metabolism is its rapid hydrolysis by tissue and circulating carboxylesterases to acrylic acid and methanol, which

undergo further metabolism. Another route of methyl acrylate metabolism is conjugation with the sulfhydryl group of glutathione. Methyl acrylate is rapidly distributed throughout the body. Methyl acrylate and/or its metabolites can be detected in all organ systems, with the highest concentrations being present in the urine, expired air, and organ of entry (i.e., stomach, upper respiratory tract, and skin). More than 90% of orally administered methyl acrylate is excreted within 72 h via the lungs (>50%) as CO_2 and via the kidneys (40–50%) as products of methyl acrylate–glutathione.

Mechanism of Toxicity

Pretreatment of rats with a carboxylesterase inhibitor enhances the respiratory irritation and lethality produced by the inhalation of methyl acrylate. This observation suggests that the toxicity of methyl acrylate becomes manifest when local detoxification/defense mechanisms become overwhelmed.

Acute and Short-Term Toxicity (or Toxicity)

Animal

The acute oral LD_{50} in rat is 765 mg kg^{-1}; the rabbit dermal LD_{50} is 1250 mg kg^{-1}. The 4 h LC_{50} for methyl acrylate vapor in rat is 1600 ppm. Methyl acrylate may produce an allergic contact dermatitis and may cross-react with other acrylic esters. Methyl acrylate was not clastogenic in two (oral and inhalation) *in vivo* mouse micronucleus assays.

Human

Methyl acrylate can be highly irritating to the skin, eyes, and the respiratory tract. The severity of the reaction will depend on the concentration of the applied dose as well as the duration and frequency of contact. Methyl acrylate may cause an allergic contact dermatitis that may cross-react with other acrylate esters.

Chronic Toxicity (or Exposure)

Animal

Repeated inhalation exposures to irritating concentrations of methyl acrylate can damage the respiratory tract and eyes, while systemic toxicity is relatively minor, being limited to some organ weight changes without accompanying histopathology. Toxicity associated with repeated oral exposures is generally limited to decrements in body weight and the exacerbation of spontaneously occurring disease states.

In a 3 month drinking water study, rats were exposed to methyl acrylate at doses of 0, 1, 5, and 20 mg kg^{-1}. Toxicity was limited to the high dose and consisted of slight decreases in body weight gain and water consumption as well as an increase in relative kidney weight and in the severity of chronic progressive nephropathy that occurs spontaneously in Fischer 344 rats. The histopathology of other organs, including the sex organs, was normal. The study no-observed-adverse-effect level (NOAEL) was 5 mg-kg^{-1}. In a lifetime inhalation study, rats were exposed to methyl acrylate concentrations of 0, 15, 45, and 135 ppm for 6 h day^{-1}, 5 days week^{-1}. At these irritating concentrations, the primary effect was damage to the nasal and respiratory mucosa and the eyes at all three concentrations; systemic toxicity was observed primarily in the high dose group and was limited to a slight but reversible body weight gain and changes in organ weights without associated histopathology. The histopathology of the sex organs was normal. Tumor incidences were not increased.

In a developmental study, pregnant rats were exposed to methyl acrylate at concentrations of 0, 25, 50, or 100 ppm for 6 h day^{-1} on days 6–20 of gestation. The two highest doses produced marked decrements in maternal body weight gain and food consumption. Decrements in fetal body weight were observed only at 100 ppm; decreases in embryonic survival and increases in fetal malformations were not observed at any concentration. The NOAELs for maternal toxicity, developmental toxicity, and teratogenicity were 25, 50, and 100 ppm, respectively.

The normal sex organ histopathology noted in animals above combined with the occurrence of rat fetotoxicity only in the presence of maternal toxicity suggests that methyl acrylate does not pose a significant reproductive and developmental hazard to humans.

Human

No data on the chronic toxicity of methyl acrylate in humans are available.

In Vitro Toxicity Data

Data from several *in vitro* mutagenicity studies were negative, both with and without metabolic activation. However, methyl acrylate was clastogenic in Chinese hamster cells in the absence of metabolic activation.

Clinical Management

Clinical management involves removal from exposure and treatment of symptoms.

Environmental Fate

Methyl acrylate is a volatile (89 hPa at 20°C) liquid under normal environmental conditions. At equilibrium in the environment, methyl acrylate will partition primarily to air (92%) with lesser amounts to water (7.2%), soil (<1%), and sediment (<0.1%). In air, methyl acrylate will be removed by reaction with photochemically produced hydroxyl radicals (13.6 h half-life). When released to water, methyl acrylate will be removed by volatilization to air (Henry's law constant of $1.5\,Pa\,m^3\,mol^{-1}$) or biodegradation (60% removal in 28 days). Based on its estimated organic-carbon partition coefficient (K_{oc} of 6.4), methyl acrylate will exhibit high mobility in soil where it may leach to ground water or volatilize to air from surface soils. Similarly, it is not expected to bind significantly to sediments or suspended particulate matter. Based on its relatively low octanol–water partition coefficient ($\log K_{ow}$ of 0.74), methyl acrylate does not pose a significant bioaccumulation hazard.

Ecotoxicology

Methyl acrylate is acutely toxic to aquatic organisms. In a series of studies with analytically measured concentrations, methyl acrylate exhibited a 96 h LC_{50} of $3.4\,mg\,l^{-1}$ in freshwater fish (rainbow trout) and $1.1\,mg\,l^{-1}$ in marine fish (*Cyprinodon variegatus*), a 48 h EC_{50} (immobilization) of $2.6\,mg\,l^{-1}$ in an aquatic invertebrate (*Daphnia magna*), and a 96 h growth rate EC_{50} (biomass) of $1.99\,mg\,l^{-1}$ in algae (*Selenastrum capricornutum*).

Other Hazards

Methyl acrylate is highly flammable with lower and upper explosive limits of 2.1 and 14.5%, respectively.

Exposure Standards and Guidelines

International occupational exposure limits (OEL) for methyl acrylate generally range from 2 to 10 ppm as an 8 h time-weighted average (TWA); 2 ppm is the TWA OEL established by the American Conference of Governmental Industrial Hygienists. International short-term excursion limits generally range from 5 to 20 ppm. The US Occupational Safety and Health Administration lists a permissible exposure limit of 10 ppm for methyl acrylate (TWA). The National Institute of Occupational Safety and Health has a recommended exposure limit of 10 ppm methyl acrylate (TWA) and lists 250 ppm methyl acrylate as immediately dangerous to life or health. The International Agency for Research on Cancer indicates that methyl acrylate is not classifiable as to its carcinogenicity to humans (group C).

See also: Carboxylesterases; Glutathione; Polymers; Respiratory Tract.

Further Reading

Murphy SR and Davies JH (1993) Methyl acrylate health effects overview. In: Tyler TR, Murphy SR, and Hunt EK (eds.) *Health Effect Assessments of the Basic Acrylates*, pp. 33–52. Boca Raton, FL: CRC Press.

Methyl Bromide

Danny Villalobos and Marilyn Weber

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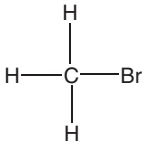
- Chemical Abstracts Service Registry Number: CAS 74-83-9
- Synonyms: Bromomethane; Monobromomethane; Halon 1001; Haltox; Zytox
- Chemical/Pharmaceutical/Other Class: Halogenated hydrocarbon/fumigant
- Chemical Formula: CH_3Br
- Chemical Structure:



Uses

The uses of methyl bromide include preplant soil fumigant treatment for production of flowers, nursery crops, tomatoes, strawberries as well as other produce including carrots, legumes, and other assorted vegetables. The largest worldwide end use of methyl bromide is for soil fumigation; however, it is also used to fumigate durable and perishable commodities. Methyl bromide is also used to fumigate structures, dwellings, office buildings, warehouses, silos, mills, vaults, shipping, and freight cars for the control of fungi, insects, and rodents. As an outside fumigant, methyl bromide is typically used under approved gas-proof sheeting or tenting to control pests in soils and orchards. The largest usage of methyl bromide in developed countries occurs in the United States, Japan, Italy, France, Belgium, and

South Africa. It is also used in chemical synthesis. The use of methyl bromide in the United States as a fumigant is scheduled to cease in 2005.

Exposure Routes and Pathways

Exposures may occur via inhalation of the vapor or by dermal contact with the liquid. Ingestions are unlikely due to its gaseous form at room temperature. Childhood exposures may result in higher doses due to greater lung (surface area)/(body weight) ratios and increased minute volumes/weight ratios. Additionally, due to methyl bromide's density, children's shorter stature may also lead to relatively higher levels of exposure.

Toxicokinetics

Methyl bromide is rapidly absorbed by inhalation. Rats are more efficient than humans at absorbing methyl bromide following inhalation exposure, with absorption being directly proportional to air concentration up to ~about 300 ppm. Absorption is also rapid and extensive (97%) in rats after oral administration. Methyl bromide is distributed in fat, lung, liver, adrenals, and kidney, with less distribution into brain. Methyl bromide is rapidly and extensively metabolized to methanol, bromide, and finally to CO_2. After oral or inhalation exposure in rats, 85% was eliminated within 65–72 h. Most (30–50%) was recovered as expired CO_2 and 4–20% as expired parent compound. About 16–40% was recovered in the urine and a small percentage was eliminated in the feces. Extensive enterohepatic circulation is possible. Tissue half-lives of methyl bromide range from 0.5 to 8 h.

Mechanism of Toxicity

Methyl bromide methylates sulfhydryl groups of enzymes, causing cellular disruption and reduced glutathione levels. Cellular disruption, primarily in the central nervous system, results in progressive dysfunction. In sublethal poisoning, a latency period of 2–48 h can occur between exposure and onset of symptoms. Methanol, a metabolite of methyl bromide, may also contribute to the neurologic and visual effects, but this is only likely to be significant at high levels of exposure.

Acute and Short-Term Toxicity (or Exposure)

Animal

Methyl bromide is moderately toxic by the oral and inhalation routes. The oral LD_{50} in rats was 104–214 mg kg^{-1}. Toxicity by the inhalation route is both time and concentration dependent. In mice, LC_{50} values were 1700 ppm (~6.6 mg m^{-3}) for a 30 min exposure to 405 ppm (~1.6 mg m^{-3}) for a 4 h exposure. In rats, the LC_{50} for a 30 min exposure was reported as 2833 ppm (~11 mg m^{-3}) while for an 8 h exposure the value was 302 ppm (1.2 mg m^{-3}).

Human

Primary effects of methyl bromide are on the nervous system, lungs, nasal mucosa, kidneys, eyes, and skin. Neurologic symptoms include blurred vision, mental confusion, paresthesias, tremors, and speech defects. Severe exposure may result in narcosis, seizures, coma followed by respiratory paralysis, and circulatory failure. Contact with the skin and eyes can lead to irritation and burns. After an acute single, small with prompt recovery, no delayed or long-term effects are likely to occur. In larger exposures inhalation can cause injury to the nervous system, lungs, and throat. High doses can also injure the kidneys and liver.

Chronic Toxicity (or Exposure)

Animal

Rats, rabbits, or female rhesus monkeys exposed to 0, 17, 33, 66, 100, or 220 ppm methyl bromide 7–8 h per day, 5 days per week for 6 months exhibited mortality in rats and monkeys at 100 ppm. Rabbits showed mortality at 33 ppm. Severe effects, including paralysis, were seen after exposure to 66 ppm in rabbits and monkeys. No signs of overt toxicity were noted at 17 ppm. Other studies have shown focal lesions in the brain and heart in rats after inhalation of 150 ppm methyl bromide (4 h per day, 5 days per week for 11 weeks). Rats exposed to 0, 200, 300, or 400 ppm methyl bromide (4 h per day, 5 days per week for 6 weeks) exhibited coronary lesions and exposures of 300 ppm or greater resulted in neurologic dysfunction including ataxia and paralysis. Testicular atrophy was noted at 400 ppm.

Rabbits appear more sensitive than rats to neurotoxicity of methyl bromide. Rats and rabbits were exposed to 0 or 65 ppm methyl bromide (7.5 h per day, 4 days per week for 4 weeks) but nerve conduction velocity and eyeblink reflex were impaired only in the rabbits.

Rats were exposed to 0, 3, 30, or 90 ppm methyl bromide (6 h per day, 5 days per week) and observed at 14, 53, and 105 weeks of exposure. Exposures of males and females to 90 ppm resulted in reduced body weight and significant lesions in the heart (cartilaginous metaplasia and thrombus in males; myocardial

degeneration and thrombus in females). Exposure at 30 or 90 ppm led to decreased kidney weights. Histological changes in the nose, heart, esophagus, and forestomach were noted. At the lowest concentration (3 ppm), slight degenerative changes in the nasal epithelium and olfactory basal cell hyperplasia were noted in both sexes at termination. In a 13 week study, mice were exposed to 0, 30, 60, or 120 ppm methyl bromide (6 h per day, 5 days per week). Serious effects, including 58% body weight loss, 17% mortality, and severe curling and crossing of the hindlimbs were observed with the high dose. Exposure of males to 40 ppm or higher led to decreased hemoglobin and increased red blood cell count.

No teratogenic effects were noted in rats or rabbits exposed to 20 ppm methyl bromide (7 h per day, 5 days per week for 3 weeks) during gestation days 1–19 (rats) or 1–24 (rabbits).

A two-generation reproductive and developmental toxicity study in rats was conducted using 0, 3, 30, or 90 ppm methyl bromide (6 h per day, 5 days per week during premating, gestation, and lactation through two generations). Significant decreases in body weight were observed in males exposed to 90 ppm. Neonatal body weights were decreased by exposure to 30 ppm. There was a decreased cerebral cortex width in the 90 ppm F1 group, reduced brain weight in 30 ppm F1 females, and reduced fertility in the 30 and 90 ppm F2b groups.

Methyl bromide induced sister chromatid exchanges and micronuclei in bone marrow cells in both male and female rats and mice following inhalation exposures.

There were no significant increases in tumors in rats exposed to concentrations up to 90 ppm methyl bromide for 29 months or mice exposed to concentrations up to 100 ppm for 2 years. Degeneration and hyperplasia of the nasal olfactory epithelium were noted in both species. No increased tumor incidence was seen in a 2 year dietary study in rats.

Human

Repeated exposures have been associated with peripheral neuropathies (especially sensory neuropathy), impaired gait, behavioral changes, and mild liver and kidney dysfunction. Visual impairment secondary to atrophy of the optic nerve has been reported. Chronic exposure may be more serious for children because of their potential longer latency period. Workers exposed to methyl bromide had a higher rate of neurological symptoms and performed less well on several behavioral tests. Several confounding factors (age, alcohol consumption, prescription medication, illegal drug use, education, ethnicity) may have influenced these findings, however.

In Vitro Toxicity Data

Methyl bromide was positive in *Salmonella typhimurium* strain TA100, with or without exogenous metabolic activation; negative results were obtained with TA98 in this assay.

Clinical Management

There is no antidote for methyl bromide and patients are treated symptomatically. Although there is no specific laboratory data to detect the presence of or diagnose an exposure to methyl bromide, serum bromide levels may be used to document that an exposure occurred. Bromide levels do not aid in the acute treatment of an exposure and do not accurately predict clinical course.

Environmental Fate

As a soil fumigant methyl bromide leaves no toxic residue in soils. The volatile gas rises into the atmosphere. Methyl bromide is an ozone-depleting substance. Although methyl bromide is very soluble in water, its high vapor pressure in various soil types indicates a low tendency to adsorb to soils and rapid evaporation. Methyl bromide has a half-life in air estimated from 0.3 to 1.6 years. Degradation is primarily due to photolysis. In soils, the half-life is 0.2–0.5 days. In water, a half-life of 3 h was calculated.

Ecotoxicology

Methyl bromide does not accumulate in aquatic species. Methyl bromide causes acute lethality in a number of aquatic species at concentrations of 0.7–20 $mg\,l^{-1}$. A 96 h LC_{50} in trout was 3.9 $mg\,l^{-1}$, with NOECs of 1.9 and 2.9 $mg\,l^{-1}$ for clinical signs and mortality, respectively. In *Daphnia*, a 48 h LC_{50} was 2.6 $mg\,l^{-1}$.

Exposure Standards and Guidelines

- Occupational Safety and Health Administration ceiling limit is 20 ppm (skin).
- Reference exposure level is 5 $\mu g\,m^{-3}$. The reference concentration is also 5 $\mu g\,m^{-3}$.
- National Institute for Occupational Safety and Health IDLH (immediately dangerous to life or health) value is 250 ppm.
- American Industrial Hygiene Association ERPG-2 (the maximum airborne concentration below which it is believed that nearly all individuals could be exposed for up to 1 h without experiencing or developing irreversible or other serious health effects or symptoms which could impair an individual's ability to take protective action) value is 50 ppm.

Miscellaneous

Methyl bromide is a colorless, odorless gas or liquid at low concentrations. At very high concentrations, it has a sweet, fruity odor. Additives such as chlorpicrin are often mixed with methyl bromide to warn of its presence.

See also: Bromine; Carbon Tetrabromide; Ethyl Bromide; Methanol.

Further Reading

Yang RS, Witt KL, Alden CJ, and Cockerham LG (1995) Toxicology of methyl bromide. *Reviews of Environmental Contamination and Toxicology* 142: 65–85.

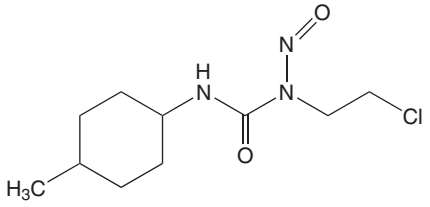
Relevant Websites

http://www.chem.unep.ch – United Nations Environment Programme.
http://www.epa.gov – United States Environmental Protection Agency.

Methyl CCNU

Jaya Chilakapati and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 13909-09-6
- Synonyms: 1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea; Semustine; Methyl-CCNU
- Chemical/Pharmaceutical/Other Class: Alkylating agent
- Chemical Formula: $C_{10}H_{18}ClN_3O_2$
- Chemical Structure:



Uses

MeCCNU is an investigational drug used in chemotherapy to treat various types of cancers like Hodgkin's disease, malignant gliomas, gastrointestinal tract adenocarcinomas, breast carcinomas, and squamous-cell carcinomas, malignant melanoma and epidermoid carcinoma of the lung.

Exposure Routes and Pathways

The most common exposure pathway is ingestion.

Toxicokinetics

MeCCNU easily crosses the blood–brain barrier as it is lipid soluble. It has a short plasma half-life. This allows good therapeutic utility in various CNS neoplasms.

Mechanism of Toxicity

MeCCNU exerts its toxicity by cross-linking, that is, DNA alkylation, carbamoylates proteins and causes DNA strand breakage. It is cytotoxic in all stages of the cell cycle.

Acute and Short-Term Toxicity (or Exposure)

Animal

MeCCNU causes toxicity to kidney in male Fischer 344 rats. Even a single acute dose may lead to chronic and irreversible effects on the kidney. But lethal doses of MeCCNU (100–180 $mg\,kg^{-1}$) produced minimal proximal tubule injury. A 250 $mg\,kg^{-1}$ (1 $mmol\,l^{-1}$) dose of MeCCNU resulted in massive papillary necrosis within 7 days, with only limited necrosis to the proximal tubules. Sublethal doses resulted in a similar, chronic, progressive nephropathy, which was delayed in onset and was characterized by polyuria, enzymuria, a decrease in urine concentrating ability, and in renal slice organic ion accumulation. Studies suggest that hepatic metabolism contributes significantly to the alkylating activity of MeCCNU in the liver and the kidney, and indicate that a liver-derived metabolite may be responsible for the renal toxicity of MeCCNU.

Human

Protracted myelosuppression, a condition in which bone marrow activity is decreased, resulting in fewer red blood cells, white blood cells, and platelets, is the dose-limiting toxicity of MeCCNU. Renal and hepatic toxicities and pulmonary fibrosis are seen infrequently after exposure to MeCCNU. It exhibits dose-dependent nephrotoxicity. Proximal tubular cells are attacked, with renal failure occurring when

high doses are administered. It also produces acute nausea and vomiting.

Chronic Toxicity (or Exposure)

Animal

Data on methyl-CCNU were included in a report in which a large number of cancer chemotherapeutic agents were tested for carcinogenicity by intraperitoneal injection in Sprague–Dawley rats and Swiss-Webster mice. In male rats injected with methyl-CCNU thrice weekly for 6 months, total tumor incidence was reported to be increased 1.5–2-fold over that in controls at 18 months. A slight increase in tumor incidence was reported in mice. Intravenous administration of methyl-CCNU to rats induced lung tumors.

In another study, a single subcutaneous injection of MeCCNU (20–140 mg kg^{-1}) resulted in rapid decrease in renal function leading to a chronic progressive nephropathy in male Fischer 344 rats.

Human

Adjuvant treatment with methyl-CCNU has been evaluated in 3633 patients with gastrointestinal cancer treated in nine randomized trials. Among 2067 patients treated with methyl-CCNU, 14 cases of acute nonlymphocytic leukemia (ANLL) occurred, whereas one occurred among 1566 patients treated with other therapies. Cumulative (actuarial) risk was 4% at 6 years and was not affected by concomitant radiotherapy or immunotherapy. A subsequent report described a strong dose–response relationship, adjusted for survival time, giving a relative risk of almost 40-fold among patients who had received the highest dose.

Clinical Management

A patent airway should be established. Suction can be used if necessary. Signs of respiratory insufficiency should be watched for and ventilations should be assisted if needed. Oxygen should be administered by nonrebreather mask at 10–15 l min^{-1}. Pulmonary edema and shock should be monitored and treated if necessary. Seizures should be anticipated and treated if necessary. For eye contamination, eyes should be flushed immediately with water. Emetics should not be used.

See also: Alkyl Halides; Methylnitrosourea.

Further Reading

National Toxicology Program (2002) 1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (MeCCnU). *Report on Carcinogens: Carcinogen Profiles* 10: 53–54.

Methyl Disulfide

Sara J Risch

- Chemical Abstracts Service Registry Number: CAS 624-92-0
- Synonym: Dimethyl disulfide
- Chemical/Pharmaceutical/Other Class: Alkyl sulfide
- Chemical Formula: $C_2H_6S_2$
- Chemical Structure: $CH_3–S–S–CH_3$

Uses

Methyl disulfide is used as a component of flavoring materials. It has an intense onion odor by itself. It is used in combination with other flavor compounds in food products including baked goods, frozen dairy products, meat products, soft candy, gelatin, puddings, and both alcoholic and nonalcoholic beverages. This compound has been found in nature.

Exposure Routes and Pathways

Dimethyl sulfide can be either a liquid or vapor by itself or in combination with other materials that can come into contact with the skin or be inhaled. It can be irritating to the skin and eyes. Based on single exposure animal tests, dimethyl sulfide is considered moderately toxic if swallowed, slightly toxic if absorbed through the skin, and slightly toxic if inhaled. The compound has a strong, objectionable odor that can cause nausea, dizziness, or headache.

Occupational exposure may occur through inhalation or dermal contact where the compound is produced, in the manufacture of flavoring materials where it is a component of that flavor, or when the final flavor is being added to a food product. Most exposure for the general population is through various food products.

Toxicokinetics

Methyl disulfide has been found as a normal component of mouse urine vapor, indicating that it

is not metabolized but is excreted. It has also been found in the expired air of both diabetics and nondiabetics as well as some samples of human milk.

Acute and Short-Term Toxicity (or Exposure)

Prolonged contact can result in the removal of oil from the skin and may dry the skin and cause irritation, redness, and a rash. High vapor concentration may be irritating to the eyes and respiratory tract. High vapor concentrations may also result in headache, dizziness, drowsiness, nausea, vomiting, and loss of muscle coordination. In severe exposure, loss of consciousness is possible.

Animal

Oral exposure showed methyl disulfide to be moderately toxic to rats (LD_{50} 190 mg kg^{-1}). Dermal testing with rabbits showed it to be no more than slightly toxic (LD_{50} >2000 mg kg^{-1}). Inhalation exposure for rats showed the material to be slightly toxic (4 h LC_{50} 805 ppm). Methyl disulfide is slightly irritating to the eyes and skin of rabbits.

A single application to the skin of rabbits produced no mortality but did result in eye irritation and an effect on the central nervous system and respiratory system. All of the effects disappeared within a day after exposure.

In rats, 5–40 µl was found to inhibit the thyroid function. Toxic levels were between 50 and 400 µl. In pigs, there was no effect on thyroid function at 100 nmol ml^{-1}.

Human

The principal hazard of methyl disulfide is as an irritant to the skin and eyes.

Chronic Toxicity (or Exposure)

Animal

Toxicity testing using rats showed that exposure to levels of 250 ppm by inhalation, up to 6 h day^{-1}, 5 days $week^{-1}$ for up to 4 weeks resulted in lethargy, respiratory difficulties, low weight gain. On autopsy, the organs were found to be congested. Exposure under the same conditions to levels of 100 ppm showed no toxic signs and the organs were normal.

Oral administration for 5 months found a no-effect level of 0.007 mg kg^{-1}. At 0.5 mg kg^{-1}, there were cardiovascular and kidney effects with dystrophic disease of the kidneys and cardiac muscles as well as impairment of the oxidation process.

Human

No specific exposure levels have been reported and there are no airborne exposure guidelines. The effects in humans are not known; however, excessive exposure may result in similar effects to what was observed in rats.

In Vitro Toxicity Data

Methyl disulfide was classified as a nonirritant in human *in vitro* studies. In a study of the inhibition of soy lipoxygenase, the IC_{50} was determined to be 1090 µmol l^{-1}.

Clinical Management

Eyes should be flushed with a large amount of water for at least 15 min. Skin should be washed with soap and water. If irritation continues, medical attention should be sought.

If inhalation exposure occurs, the person should be removed to fresh air immediately and monitored for respiratory distress. If breathing has stopped, artificial respiration should be initiated and oxygen administered if necessary.

For a person who is conscious, two glasses of water or milk should be given if the material is ingested. Vomiting should be induced and medical attention sought. If the person is unconscious or drowsy, liquids should not be given and medical attention should be sought immediately.

Environmental Fate

Methyl disulfide is not readily biodegradable and thus will be present in the environment. It is sometimes used as a marker of pollution.

Ecotoxicology

Methyl disulfide has been found to be moderately toxic to *Daphnia* with a 48 h LC_{50} of 4 mg l^{-1} and to trout with a 120 h LC_{50} of 1.75 mg l^{-1}. It is slightly toxic to algae with a 72 h LC_{50} of 11–35 mg l^{-1} and to guppies with a 96 h LC_{50} of 50 mg l^{-1}.

Exposure Standards and Guidelines

There are no established airborne exposure guidelines.

Miscellaneous

Methyl disulfide is a widely used flavoring ingredient. It is Food and Drug Administration approved as a

flavor material (21 CFR 172.515) and is FEMA GRAS number 3536. The applications for use as a flavor material include alcoholic and nonalcoholic beverages, baked goods, relishes, frozen dairy, gelatin and puddings, meat products, soft candy, and sweet sauces. It has been noted that the powerful and penetrating odor of this material may limit voluntary exposure.

See also: Food Additives.

Further Reading

Mikhailov VM (1970) Toxic effect of dimethyl sulfide and dimethyl disulfide under prolonged oral administration. *Gigiena truda i professional'nye zabolevaniia* 86–87.

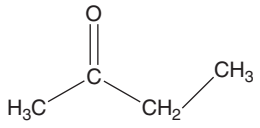
Munro IC and Kennepohl E (2001) Comparison of estimated daily capita intakes of flavoring substances with no-observed-effect levels from animal studies. *Food and Chemical Toxicology* 39(4): 331–354.

Transy MF, Kendall FM, Fantasia J, *et al.* (1981) Acute and subchronic toxicity studies of rats exposed to vapors or methyl mercaptan and other reduced sulfur compounds. *Journal of Toxicology and Environmental Health* 8: 71–88.

Methyl Ethyl Ketone

Samantha E Gad

- Chemical Abstracts Services Registry Number: CAS 78-93-3
- Synonyms: MEK; 2-Butanone; 2-Oxobutane; Ethyl methyl ketone
- Chemical/Pharmaceutical/Other Class: Ketone
- Chemical Formula: $CH_3COCH_2CH_3$
- Chemical Structure:



Uses

Methyl ethyl ketone (MEK) is used as a solvent for various coating systems, for example, vinyl, adhesives, nitrocellulose, and acrylic coatings. It is used in paint removers, lacquers, varnishes, spray paints, sealers, glues, magnetic tapes, printing inks, resins, rosins, cleaning solutions, and for polymerization. It is found in other consumer products, for example, household and hobby cements, and wood-filling products. MEK is used in dewaxing lubricating oils, the degreasing of metals, in the production of synthetic leathers, transparent paper and aluminum foil, and as a chemical intermediate and catalyst. It is an extraction solvent in the processing of foodstuffs and food ingredients. MEK can also be used to sterilize surgical and dental equipment.

In addition to its manufacture, environmental sources of MEK include exhaust from jet and internal combustion engines, and industrial activities such as gasification of coal. It is found in substantial amounts in tobacco smoke. MEK is produced biologically and has been identified as a product of microbial metabolism. It has also been found in plants, insect pheromones, and animal tissues, and MEK is probably a minor product of normal mammalian metabolism. It is stable under ordinary conditions but can form peroxides on prolonged storage; these may be explosive.

Exposure Routes and Pathways

Inhalation, ingestion, and dermal contact are all possible routes of exposure. MEK is a natural component in some foods. Indoor air pollution (and inhalation) can occur from volatilization of MEK from consumer and building products.

Toxicokinetics

MEK is rapidly absorbed by inhalation, skin contact and ingestion and transferred into the blood and other tissues. MEK is metabolized in the liver, mainly to 3-hydroxy-2-butanone and 2,3-butanediol that are eliminated in urine. Most MEK probably enters the general metabolism in the body and is converted to acetate that is eventually broken down to carbon dioxide and water that are then eliminated in exhaled air and urine. Small amounts of MEK itself are also eliminated in exhaled air and urine. MEK and its metabolites are mostly cleared from the body within 24 h, and MEK does not accumulate in the body.

Mechanism of Toxicity

There is very limited information on the mechanisms of toxicity of MEK. Relatively high inhaled concentrations of 1475–29 500 mg m^{-3} (500–10 000 ppm) caused pulmonary vasoconstriction and hypertension

in cats and dogs. There are several human case reports of neurological effects resulting from high exposure to MEK in combination with other solvents, and animal studies have confirmed synergism between MEK and ethyl *n*-butyl ketone, methyl *n*-butyl ketone, *n*-hexane, carbon tetrachloride, 2,5-hexanedione, and chloroform. The main target organs involved in toxicological interactions are the nervous system and liver, and the lung has also been mentioned.

Acute and Short-Term Toxicity (or Exposure)

Animal

MEK causes central nervous system depression in animals. The oral LD_{50} in rats is $6.86\,ml\,kg^{-1}$ ($2737\,mg\,kg^{-1}$) and $4050\,mg\,kg^{-1}$ in mice. Inhalation LC_{50} is $23\,500\,mg\,m^{-3}$ in rats and $32\,mg\,m^{-3}$ in mice. The intraperitoneal LD_{50} is $607\,mg\,kg^{-1}$ in rats, $616\,mg\,kg^{-1}$ in mice, and $2\,g\,kg^{-1}$ in guinea pigs. Animal studies indicate that MEK is a mild-to-moderate skin irritant, and a moderate to severe eye irritant. MEK was not mutagenic in mouse and hamster micronucleus cytogenetic assays. Pregnant rats were exposed by inhalation to up to 3000 ppm on days 6–15 of gestation; at 3000 ppm, there was a low but statistically significant increase in malformations. Sternebral and soft tissue anomalies were also increased. There was also a statistically significant increase in total skeletal anomalies at 1000 ppm. Maternal toxicity was not observed. In subsequent studies, pregnant rats and mice were exposed to up to 3000 ppm by inhalation during days 6–15 of gestation. There were no embryotoxic or teratogenic effects at any exposure level. There were fetotoxic effects (increased incidence of minor skeletal variations; delayed bone formation; reduced fetal weight) with very slight maternal toxicity at 3000 ppm.

Human

The major acute toxicity of MEK is mucosal irritation, and MEK can irritate the eyes, nose, and the respiratory system. No irritation was produced when 20% MEK in petrolatum was applied to volunteers for 48 h in a closed patch test; however, it can be irritating to the skin by defatting. It can cause dizziness, fatigue, memory alteration, dermatitis, headaches, nausea, and paresthesia of extremities, diminished vision acidosis, and vomiting. Acute inhalation can cause central nervous system depression. MEK vapor is irritating to mucous membranes and conjunctivae at 200 ppm after 15 min, but the odor is noticeable at 25 ppm. The results from animal evidence suggest that MEK can be aspirated during ingestion or vomiting, and could result in severe lung damage (edema), respiratory failure, cardiac arrest, and death.

Chronic Toxicity (or Exposure)

Animal

Exposure to 5000 ppm for 13 weeks produced an exposure-related effect on body and liver weights in rats, as well as a depression in brain weight in females. Guinea pigs and rats were exposed to 235 ppm for 12 weeks ($5\,day\,week^{-1}$, $7\,h\,day^{-1}$). There were no deaths and no signs of toxicity. Extensive neurological studies with high exposures have shown no effects. In one study, rats were initially exposed to 10 000 ppm that was reduced to 6000 ppm due to severe irritation of the upper respiratory tract. Temporary signs of muscle incoordination and gait disturbances were observed. Exposures continued for only 7 of the planned 15 weeks because some animals died of bronchopneumonia; there were no neurological symptoms. In the other study, rats were exposed to 1125 ppm continuously for up to 55 days with no signs of neurotoxicity.

Human

A mortality study of hundreds of workers who had worked at MEK dewaxing plants concluded that there was no evidence of a cancer hazard. The average follow-up was 14 years. This study is limited by the small size of the cohort and the relatively short follow-up period. The International Agency for Research on Cancer has not evaluated the carcinogenicity of MEK, the American Conference of Governmental Industrial Hygienists has not assigned a carcinogenicity designation for MEK, and the US National Toxicology Program has not listed MEK in its report on carcinogens. MEK is classified as a Group D chemical by the US Environmental Protection Agency, that is, it is not classifiable as to human carcinogenicity.

In Vitro Toxicity Data

MEK was not mutagenic in Ames (*Salmonella*) and *Escherichia coli* tests, but induced aneuploidy in *Saccharomyces cerevisiae*. MEK was not found to be genotoxic in the mouse lymphoma assay, in Chinese hamster ovary cell, unscheduled DNA synthesis assay, and micronucleus assays.

Clinical Management

Vomiting should not be induced.

Environmental Fate

MEK evaporates readily into the atmosphere and is subject to rapid photochemical decomposition. It reacts to form a haloform that is more toxic than the original compound in water containing free halogens or hypohalites. MEK does not accumulate in any environmental compartment, and it is rapidly metabolized by microbes and mammals. There is no evidence of bioaccumulation.

Ecotoxicology

MEK is not acutely toxic to fish or aquatic invertebrates. The LC_{50} values range from 1382 to 8890 mg l^{-1}. MEK is produced by fungi to concentrations that affect the germination of some plants.

Exposure Standards and Guidelines

The (US) Occupational Safety and Health Administration permissible exposure limit, 8 h time-weighted average (TWA) is 200 ppm, and the (US) National Institute for Occupational Safety and Health (NIOSH) recommended exposure level, averaged over a 10 h work day is 200 ppm. The NIOSH short-term exposure limit, for a 15 min exposure, is 300 ppm.

See also: Neurotoxicity.

Further Reading

Noraberg J and Arlien-Soborg P (2000) Neurotoxic interactions of industrially used ketones. *Neurotoxicology* 21: 409–418.

Yang RS (1986) The toxicology of methyl ethyl ketone. *Residue Reviews* 97: 121–143.

Relevant Websites

http://www.inchem.org – International Programme on Chemical Safety (IPCS). Methyl Ethyl Ketone (Environmental Health Criteria 143).

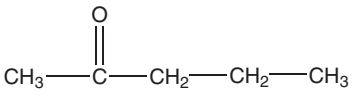
http://www.intox.org – International Programme on Chemical Safety. Methyl Ethyl Ketone.

Methyl Isobutyl Ketone

Samantha E Gad

This article is a revision of the previous print edition article by Shayne C Gad, volume 2, pp. 312–314, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 108-10-1
- Synonyms: MIK; Hexone; 4-Methyl-2-pentanone; Isopropyl acetone; MIBK; Isobutyl methylketone
- Chemical/Pharmaceutical/Other Class: Ketone
- Chemical Formula: $C_6H_{12}O$
- Chemical Structure:



Uses

Methyl isobutyl ketone (MIK) is used as a solvent for vinyl, epoxy, acrylic, natural resins, nitrocellulose, paints, varnishes, lacquers, protective coatings, rare metal extraction, and dyes. In addition, it is used as a denaturant for rubbing alcohol, as a synthetic flavoring adjuvant, and as a fruit flavoring agent. Other uses include in extracting uranium from fission products, dewaxing mineral oils, manufacturing antibiotics, dry-cleaning preparations, limited reported usage in cosmetics, and in the synthesis of methyl isobutyl carbinol.

Exposure Routes and Pathways

The general population can have inhalation and dermal contact during use of consumer products that contain MIK, together with oral exposures to MIK via its natural occurrence in oranges, grapes, and vinegar. Some segments of the general population may also be exposed to MIK via inhalation of contaminated air near industrial users, or areas near landfills and by ingestion of contaminated drinking water. In the workplace, inhalation of vapors and skin and eye contact are the most likely routes of exposure.

Toxicokinetics

MIK is absorbed by ingestion, inhalation, and dermal exposure. The metabolism of MIK involves oxidative hydroxylation, followed by reduction to the secondary alcohol. A single intraperitoneal administration of 450 mg kg^{-1} MIK to male guinea

pigs yielded two serum metabolites: 4-hydroxy-4-methyl-2-pentanone and 4-methyl-2-pentanol. The biological half-life for MIK elimination from the serum of guinea pigs was 66 min. The elimination times for MIK and 4-hydroxy-4-methyl-2-pentanone from these animals were 6 and 16 h, respectively.

The maximum percutaneous absorption rate in guinea pigs is 1.1 $\mu mol\,min^{-1}\,cm^{-2}$ at 10–45 min. The toxicokinetics of MIK were studied in human volunteers during inhalation exposure. The relative pulmonary uptake was ~60% and the total uptake increased linearly with increasing exposure concentration. The concentration of MIK in blood rose rapidly after the onset of exposure and no plateau level was reached during exposure. The concentration of unchanged MIK in the urine after exposure was proportional with the total uptake. Only 0.04% of the total MIK dose was eliminated unchanged via the kidneys within 3 h postexposure.

Mechanism of Toxicity

Organic solvents in general have the potential at acute high-level vapor pressure to cause narcosis and death, likely as a result of physical interaction of the solvent with cells of the central nervous system (CNS). MIK has been observed to enhance the neurotoxicity of *n*-hexane. This may be related to its ability to induce liver microsomal cytochrome P450, resulting in increased metabolic activation of *n*-hexane to more potent neurotoxic metabolites. MIK has also been observed to enhance the CNS effects of ethanol by reducing the action of alcohol dehydrogenase, thereby reducing the rate of ethanol metabolism and elimination.

Acute and Short-Term Toxicity (or Exposure)

Animal

MIK has a low acute toxicity; for example, the oral lethal doses for mice and rats were 2.85 and 4.6 $g\,kg^{-1}$, respectively. MIK causes eye and skin irritations and causes narcosis at high concentrations.

Human

MIK vapors may cause headaches and dizziness, are anesthetic, and may have other CNS effects. Small amounts of the liquid aspirated into the respiratory system during ingestion, or from vomiting, may cause bronchiopneumonia or pulmonary edema. An odor threshold for MIK has been reported at 15 ppm. The exposure to 200 ppm causes irritation of the eyes, nose, and throat.

Chronic Toxicity (or Exposure)

Animal

Effects from chronic exposure to MIK have included kidney damage and behavioral effects. Increased kidney/body weight ratios in rats occurred following exposure via inhalation to 100 ppm MIK for 2 weeks. Kidney and liver weights and the organ body weight ratios were also increased after exposure to 200 ppm for 2 weeks, and to 100 ppm for 90 days; however, dogs and monkeys did not demonstrate these effects after the 2 week exposures. Rats exposed to 100 ppm for 2 weeks experienced kidney damage in the form of hyaline droplet degeneration of the proximal renal tubules with occasional focal tubular necrosis; the tubular damage was considered to be transient and reversible. Chronic inhalation studies in rats have found TC_{Lo} levels of 410 $mg\,m^{-3}$ for 90 days and 1002 ppm for 6 $h\,day^{-1}$ for 14 weeks. In baboons, discriminatory behavior and memory were not affected by exposure to 20–40 ppm MIK; however, there was impairment on the accuracy of performance of tasks in a delayed match-to-sample discrimination test at an exposure of 50 ppm for 7 days. Inhalation exposure of rats to 86–127 $mg\,m^{-3}$ MIK for 4 $h\,day^{-1}$ for 4.5 months caused disturbances in the conditioned reflexes, and in the detoxifying function of the liver; a decrease of the eosinophil count in the blood was also observed.

MIK did not induce any treatment-related increases in embryotoxicity or fetal malformations in inhalation studies of pregnant rats or mice at concentrations of 300, 1000, or 3000 ppm. There was evidence of treatment-related maternal toxicity only at the highest concentration tested. MIK applied to the tail of rats daily at 300 or 600 $mg\,kg^{-1}$ for 4 months produced changes in the testes, including a reduction in the number of spermatocytes, spermatids, and spermatozoa. A carcinogenicity study of MIK is being conducted by the US National Toxicology Program.

Human

Repeated or prolonged inhalation may cause mucous irritation. Repeated or prolonged contact may cause defatting and drying of the skin. MIK is known to enhance the neurotoxicity of linear six-carbon solvents. Chronic exposure may cause axonal neuropathy, paresthesia, muscle weakness, and kidney damage.

In Vitro Toxicity Data

MIK was not mutagenic in the Ames test or in a mitotic gene-conversion assay in bacteria. MIK was also nonmutagenic in the mouse lymphoma,

unscheduled DNA synthesis, micronucleus, cell transformation, and chromosomal aberration test systems.

Clinical Management

Affected eyes should be flushed immediately with large amounts of water for at least 15 min. Affected skin should be flushed immediately with large amounts of water; soap should be used if available. Contaminated clothing, including shoes, should be removed after flushing has begun. In cases of inhalation, the victim should be removed from exposure, be kept at rest, and receive prompt medical attention. If breathing has stopped, artificial respiration should be administered. In cases of ingestion, vomiting should not be induced. Instead, 4–8 oz of milk or water (not to exceed 15 mg kg^{-1} in a child) should be given to dilute. The victim should get medical attention.

Environmental Fate

MIBK has a short half-life in the atmosphere and is also biodegraded in water. It is not expected to bioaccumulate. Based on an experimental vapor pressure of 19.9 mmHg at 25°C, MIBK is expected to exist solely as a vapor in the ambient atmosphere. Vapor-phase MIBK is degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals with an estimated atmospheric half-life of ~27 h. Methyl isobutyl ketone is expected to have high mobility in soils based upon an estimated K_{oc} value of 123. Volatilization from dry soil surfaces is expected based upon the vapor pressure of this compound.

Ecotoxicology

Numerous ecotoxicology studies are available for MIK; for example, the oral LD_{50} for *Angelaius phoeniceus* (Redwinged blackbird) is 100 mg kg^{-1}, the LC_{50} for *Carassius auratus* (goldfish) is 460 mg l^{-1} for 24 h of exposure, and the LC_{50} for *Pimephales promelas* (fathead minnow) is 505 mg l^{-1} in a 96 h flowthrough bioassay. MIBK can contribute to the formation of photochemical smog when it reacts with other volatile organic carbon substances in air.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value, 8 h time-weighted average (TWA), is 50 ppm, and the ACGIH short-term exposure limit (STEL) is 75 ppm. The US Occupational Safety and Health Administration permissible exposure limit, 8 h TWA, is 100 ppm (410 mg m^{-3}). The US National Institute for Occupational Safety and Health (NIOSH) recommended exposure level, averaged over a 10 h work day, is 50 ppm (205 mg m^{-3}), the NIOSH STEL, for a 15 min exposure, is 75 ppm (300 mg m^{-3}), and the NIOSH Immediately Dangerous to Life or Health level is 500 ppm. MIK is listed by the US Environmental Protection Agency as a Clean Air Act hazardous air pollutant generally known or suspected to cause serious health problems.

See also: Neurotoxicity.

Further Reading

Johnson W Jr. (2004) Safety assessment of MIBK (methyl isobutyl ketone). *International Journal of Toxicology* 23(Suppl. 1): 29–57.

Noraberg J and Arlien-Soborg P (2000) Neurotoxic interactions of industrially used ketones. *Neurotoxicology* 21: 409–418.

Phillips RD, Moran EJ, Dodd DE, *et al.* (1987) A 14-week vapor inhalation toxicity study of methyl isobutyl ketone. *Fundamental and Applied Toxicology* 9: 380–388.

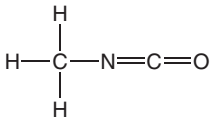
Relevant Website

http://cira.ornl.gov – US Oak Ridge National Laboratory, Center for Integrated Risk Assessment. Toxicity summary for Methyl Isobutyl Ketone (May 1994).

Methyl Isocyanate

Pallavi B Limaye and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 624-83-9
- Synonyms: Isocyanomethane; Isocyanatomethane; Methylcarbylamine, MIC
- Chemical Formula: C_2H_3NO
- Chemical Structure:



Uses

Methyl isocyanate (MIC) is used as an intermediate in organic synthesis, especially in the production of carbamate based pesticides. It is also used to produce polyurethane foams and plastics. Occasionally, it is present in cigarette smoke in very minute quantities.

Background Information

MIC is produced industrially by reacting methylamine with phosgene. At temperatures below 39°C (102°F), MIC is a highly flammable, colorless liquid. When exposed to air it readily evaporates. MIC in gaseous form is ~1.4 times heavier than air. Therefore, it tends to settle near the ground.

Exposure Routes and Pathways

Inhalation is the major route of exposure of MIC. MIC in the gaseous form is readily absorbed through the lungs. The odor threshold is ~2–5 ppm. However, odors of MIC may not provide adequate warning of hazardous concentrations since the immediately dangerous to life or health (IDLH) limit is only 3 ppm. Acute exposure to MIC vapors below the odor threshold can be irritating to the eye and respiratory epithelium. Acute exposure to higher vapor concentrations may cause severe pulmonary edema and injury to the alveolar walls of the lung leading to death due to respiratory failure. Significant exposures to MIC occur primarily in occupational settings or due to accidental release as occurred in Bhopal, India in 1984. The primary adverse effect was pulmonary edema with some alveolar wall destruction leading to respiratory failure that took several lives. Exposure in poorly ventilated, enclosed, or low-lying areas could result in asphyxiation. Children exposed to the same levels of MIC as adults may receive larger doses because they have relatively greater lung surface area:body weight ratios and higher minute volume:weight ratios. In addition, they may be exposed to higher levels than adults in the same location because of their short stature and the higher levels of MIC found nearer to the ground since it is heavier than the air.

Toxicokinetics

Studies conducted in Swiss Webster mice (males and pregnant females) indicate that the absorption of MIC is very rapid and it appears in the arterial and venous blood within few minutes after exposure to MIC vapors. Clearance of MIC is slower and may take ~3 days. The clearance is more rapid in urine than in bile. The highest concentrations of MIC in male mice 2 hours after exposure are found in the lung, sternum, gastrointestinal tract, spleen, and kidney. Twenty-four hours after exposure, highest MIC concentrations are found in the blood and lungs. In female mice, the highest concentrations at 2 h after exposure are found in the lungs, fetus, spleen, uterus, and kidney. After 24 h, the highest concentrations appear in the lung, spleen, and fetus.

Since MIC is highly reactive, it is not metabolized in the classical sense. Conjugation of MIC with glutathione (GSH) forming an adduct *S*-(*N*-methylcarbamoyl) glutathione, and corresponding cysteine adduct, *S*-(*N*-methylcarbamoyl) cysteine appears to represent an important pathway of biotransformation of MIC in the rats exposed to MIC intraperitoneally. The reaction of MIC with GSH and with cysteine is reversible, and can provide a source of free MIC in the tissues. It is speculated that these carbamate thioester conjugates of MIC may actually contribute to toxic effects of MIC. Similar studies in experimental animals exposed to MIC by the inhalation route have not been reported.

Mechanism of Toxicity

The exact mechanisms of MIC toxicity are not known, however, carbamylation of globin and other blood proteins have been speculated to contribute to MIC-induced toxicity. Acute exposure via inhalation of MIC vapors is known to cause irritation to the respiratory tract causing severe pulmonary edema and injury that can lead to death. It is also corrosive to the eyes causing severe corneal damage. Survivors of acute exposures may exhibit long-term respiratory and ocular effects. Direct skin contact of MIC in the liquid or gaseous form causes irritation of the skin.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute and subacute toxicity studies conducted in Charles Foster rats showed that MIC exposure significantly inhibits weight gain in a dose-dependent manner. These rats showed pathological lesions in the viscera, bronchial tree, lungs, liver, and kidneys. In another study, F344 rats exposed to 3 ppm MIC for 6 h day^{-1} for 4 days showed significant mortality within 28 days.

According to one report, in case of the Bhopal accident several thousand animals (cattle ~4000)

were reported dead due to MIC leakage. Information on fowl and other animals is not available.

Human

The respiratory system is the major target for MIC toxicity. In addition, MIC is also corrosive to skin and eyes. Upon ingestion, MIC shows corrosive action in the gastrointestinal tract. MIC exposure is rare in the general population except for the exposure occurring due to accidental release as occurred in Bhopal accident. The estimated immediate mortality resulting from this accidental exposure to MIC is believed to be between 2500 and 5000. Respiratory failure due to MIC inhalation was the principal cause of death. MIC caused bronchial necrosis and pulmonary edema. Within the first 24 h after the accident, around 90 000 patients were admitted in local hospitals and clinics with multiple symptoms of respiratory distress, breathlessness, choking, cough, chest pain, and hemoptysis. Acute ophthalmic effects were also reported with severe eye irritation and watering of the eyes.

Reproductive and gynecological effects were investigated by retrospective cohort studies. In an epidemiological survey conducted nine months after the accident, it was seen that 43% of 865 pregnancies amongst exposed women suffered fetal loss, as compared to 6–10% among the general Bhopal population. The spontaneous abortion rate was highest among those exposed during their first trimester. A study conducted by Shilotri *et al.* after 105–110 days of the accident showed a higher incidence of abnormal uterine bleeding and abnormal Pap smears amongst exposed women in the childbearing age.

Few immunological toxicity studies of MIC have been reported. A study of humoral and cell mediated immunity, in exposed subjects two months after exposure, found that cell-mediated immunity was suppressed, and that MIC-specific antibodies persisted for several months after the accident.

Chronic Toxicity (or Exposure)

Animal

No data were found.

Human

Around 50 000 survivors are estimated to be suffering from long-term health effects that are termed as 'Bhopal syndrome' due to a lack of information on the exact constituents of the gas cloud other than MIC. The Indian Council for Medical Research established a field office called Bhopal Gas Disaster Research Centre (BGDR) immediately after the accident. In addition, International Medical Commission on Bhopal (IMCB) was established in 1993 comprising 15 professionals from 12 different countries. BGDR and IMCB have reported that after 15 years of exposure, the affected population is still suffering from multisystemic toxicities. The major long-term health effects observed are shortness of breath, chest pain, muscle/bone pain, asthma, reproductive problems in the form of increased spontaneous abortions, and certain psychological problems. A randomized retrospective cohort study undertaken 10 years after exposure by Cullinan *et al.* indicates the presence of persistent small airways obstruction. The lung examination carried out in the survivors several months later exhibited presence of obliterative bronchiolitis and interstitial fibrosis. Thirty-nine percent of 783 patients examined showed ventilatory impairment.

A recent study published in the *Journal of the American Medical Association* in October of 2003 indicates that even the second generation of the exposed population is adversely affected. According to this study, a significant growth retardation was observed in boys who were either exposed to the gases as toddlers or born to exposed parents. Interestingly, no significant effects have been observed in girls.

In Vitro Toxicity Data

In vitro studies in Chinese hamster ovary cells indicate that MIC is capable of inducing chromosomal aberrations and sister chromatid exchanges in the 0.9–3.1 $\mu g\,ml^{-1}$ dose range, however it is not a mutagen.

Clinical Management

In acute exposure prompt medical attention is critical. Persons exposed only to MIC gas pose no risk of secondary contamination to rescuers. Persons whose skin or clothing is contaminated with liquid MIC can secondarily contaminate response personnel by direct contact or through off-gassing of vapor. There is no antidote for MIC poisoning. Treatment consists of removal of the victim from the contaminated area and support of respiratory and cardiovascular functions. In the event of ingestion of MIC, intragastric instillation of activated charcoal is useful; however, emesis is strictly avoided since this may cause additional corrosion of the gastrointestinal tract. Upon eye and skin exposures, washing the exposed area with ample amount of water is necessary. If the injury and pain are evident, the patient should be transferred to the Critical Care Unit to ensure continuous support therapy.

Environmental Fate

Terrestrial Fate

If MIC is released to soil, it is expected to rapidly hydrolyze if the soil is moist. Since it rapidly hydrolyzes, adsorption to and volatilization from moist soil are not expected to be significant processes, although no specific data regarding the fate of MIC in soil are available.

Aquatic Fate

If MIC is released to water, it is expected to rapidly hydrolyze with half-lives of 20 and 9 min at 15°C and 25°C, respectively. The products of hydrolysis may include *N*-carboxymethylamine, methylamine, carbon dioxide, and *N*,*N*′-dimethylurea. Since MIC rapidly hydrolyzes, bioconcentration, volatilization, and adsorption to sediment and suspended solids are not expected to be significant processes. However, some studies have shown that the alkylisocyanates like MIC are relatively resistant (compared to the arylisocyanates) to hydrolysis in water. Hence, despite the high water reactivity of MIC, this compound could possibly persist in the environment for many days after an initial release. No data were found concerning biodegradation of MIC.

Atmospheric Fate

If MIC is released to the atmosphere, it is expected to exist almost entirely in the vapor phase, based upon a reported vapor pressure of 348 mmHg at 20°C. It is also susceptible to photooxidation via vapor phase reaction with photochemically produced hydroxyl radicals, though this process is slow ($t_{1/2}$ 3 months).

Other Hazards

MIC reacts violently with water. MIC is incompatible with oxidizers, acids, alkalis, amines, iron, tin, and copper.

Exposure Standards and Guidelines:

Over an 8 h workshift, the Occupational Safety and Health Administration permissible exposure limit is 0.02 ppm. The IDLH established by the National Institute for Occupational Safety and Health is 3 ppm for MIC.

American Industrial Hygiene Association Emergency Response Planning Guidelines-2 (AIHA ERPG-2) defined as maximum airborne concentration below which nearly all persons could be exposed for up to 1 h without experiencing or developing irreversible or other serious health effects or symptoms that could impair their abilities to take protective action is 0.5 ppm. The current exposure standards and guidelines are summarized in **Table 1**.

Table 1 Summary of exposure criteria for MIC

Agency	*Criteria*		*Averaging time*
NIOSH	IDLH	3 ppm	NA
OSHA	PEL	0.02 ppm	8 h workshift per day
CalEPA	Chronic REL	0.001 mg m^{-3}	

EPA has not established a reference concentration or a reference dose for MIC. CalEPA stands for California Environmental Protection Agency.
Conversion: 1 ppm = 3.19 mg m^{-3}.

See also: Isocyanates; Bhopal.

Further Reading

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Relevant Website

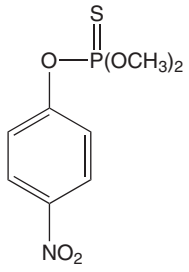
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Methyl Isocyanate.

Methyl Parathion

Kelly McCracken

This article is a revision of the previous print edition article by Carey N Pope, volume 2, pp. 317–318, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 298-00-0
- Synonyms: Penncap-M; Bladan M; Dalf; Folidol-M; Metacide; Nitrox
- Chemical/Pharmaceutical/Other Class: Synthetic organophosphorus insecticide in the phosphorothionate class
- Chemical Formula: $C_8H_{10}O_5NPS$
- Chemical Structure:



Uses

Methyl parathion is a contact insecticide for use on a variety of crop insects.

Background Information

In March 1997, there were reported misuses of methyl parathion leading to prosecution. Over 15 000 homes and businesses in Mississippi and Ohio were sprayed with methyl parathion by unlicensed operators. Methyl parathion is prohibited for use indoors. Authorities had to relocate over 1100 people to temporary accommodations with clean-up costs approaching $50 million dollars. With these reported misuses, local veterinarians reported deaths of household pets due to methyl parathion exposure. In July 1997, there was also an illegal application of methyl parathion by an illegal applicator to control cockroaches in the Chicago area.

Exposure Routes and Pathways

The dermal and inhalation routes are the most important means of occupational exposure. Accidental exposure through the oral route has also been reported. Methyl parathion is available as emulsifiable concentrates, wettable powders, and dusts of various concentrations.

Toxicokinetics

Methyl parathion is rapidly absorbed by all routes. Maximum tissue levels are achieved in 1 or 2 h following oral exposure. Methyl parathion is activated via the P450 mixed function oxidase system to the oxygen analog, methyl paraoxon. The oxon is metabolized in the liver to *p*-nitrophenol and dimethyl phosphate and these can be conjugated as glucuronides and glycosides. Glutathione-mediated demethylation also occurs. Methyl parathion is rapidly distributed to various tissues. The water-soluble metabolites are primarily excreted through the urine. A trace amount of the unmetabolized parent compound is also eliminated through the urine. Excretion of the major metabolite, *p*-nitrophenol, is essentially complete in 24 h following oral exposure. The excretion of dimethylphosphate is more protracted.

Mechanism of Toxicity

As with other organophosphorothioate agents, the toxicity of methyl parathion is due to inhibition of acetylcholinesterase by the active metabolite (i.e., methyl paraoxon), resulting in stimulation of the central nervous system, the parasympathetic nervous system, and the somatic motor nerves.

Acute and Short-Term Toxicity (or Exposure)

Animal

Clinical signs include hypersalivation, gastrointestinal hypermotility, abdominal cramping, vomiting, diarrhea, sweating, dyspnea, cyanosis, miosis, muscle fasciculations (in extreme cases, tetany followed by weakness and paralysis), and convulsions. The oral LD_{50} in adult rats and mice is $\sim 20\,mg\,kg^{-1}$. As with many other organophosphate insecticides, young animals appear to be more sensitive than adults to acute toxicity (lethality) from high doses. These age-related differences appear to be related in part to maturation of detoxification processes.

Human

Eye contact may cause pain, moderate eye irritation, and temporary corneal injury. Prolonged exposure may cause skin irritation. Ingestion of methyl parathion has caused typical symptoms of acute organophosphorus poisoning including headache, weakness, incoordination, fasciculations, tremor, nausea, cramps, diarrhea, and sweating. When inhaled the first adverse effects include bloody or

runny nose, coughing, chest discomfort, and difficulty breathing.

Chronic Toxicity (or Exposure)

Animal

Dietary administration of methyl parathion in dogs ($1.25\,mg\,kg^{-1}day^{-1}$) for 12 weeks caused red blood cell and plasma cholinesterase inhibition. A three-generation reproductive toxicity test in rats (0.5 and $1.5\,mg\,kg^{-1}\,day^{-1}$) led to reduced weanling survival, reduced birth weight at both dosages and increased stillbirths at the high dose only. In developmental studies, subacute exposures ($3\,mg\,kg^{-1}\,day^{-1}$ for 15 days) caused fetal toxicity but no teratogenic effects. In a 2 year study with mice, methyl parathion was not carcinogenic at dietary levels up to 50 ppm (9.2 mg per kg body weight per day).

Human

With repeated exposures, acetylcholinesterase inhibition can persist without indications of toxicity. In most cases, cholinesterase inhibition is without overt effects. Methyl parathion cannot cause delayed neurotoxicity.

In Vitro Toxicity Data

Methyl parathion is not mutagenic in standard *in vitro* assays.

Clinical Management

For exposure to eyes, eyelids should be held open and the eyes flushed with copious amounts of water for 15 min. For exposure to skin, affected areas should be washed immediately with soap and water. The victim should receive medical attention if irritation develops and persists.

For exposure through inhalation, the victim should be removed to fresh air and, if not breathing, given artificial ventilation. The victim should receive medical attention as soon as possible.

First aid for ingestion victims would be to induce vomiting, keeping in mind the possibility of aspiration of solvents. Gastric decontamination should be performed within 30 min of ingestion to be most effective. Initial management of acute toxicity is establishment and maintenance of adequate airway and ventilation. Atropine sulfate in conjunction with pralidoxime chloride can be administered as an antidote. Atropine by intravenous injection is the primary antidote in severe cases. Test injections of atropine (1 mg in adults and $0.15\,mg\,kg^{-1}$ in children) are initially administered, followed by 2–4 mg (in adults) or $0.015–0.05\,mg\,kg^{-1}$ (in children) every 10–15 min until cholinergic signs (e.g., diarrhea, salivation, and bronchial secretions) decrease. High doses of atropine over several injections may be necessary for effective control of cholinergic signs. If lavage is performed, endotracheal and/or esophageal control is suggested. At first signs of pulmonary edema, the patient should be placed in an oxygen tent and treated symptomatically.

Exposure Standards and Guidelines

Methyl parathion is a restricted use pesticide. The chronic reference dose for methyl parathion is $0.000\,02\,mg\,kg^{-1}\,day^{-1}$.

See also: A-Esterases; Carboxylesterases; Cholinesterase Inhibition; Neurotoxicity; Organophosphates; Pesticides.

Further Reading

Garcia SJ, Abu-Qare AW, Meeker-O'Connell WA, Borton AJ, and Abou-Donia MB (2003) Methyl parathion: A review of health effects. *Journal of Toxicology and Environmental Health, Part B: Critical Review* 6(2): 185–210.

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Methyl Parathion.

http://www.epa.gov – US Environmental Protection Agency.

Methylamine

Dale J Marino

- Chemical Abstracts Service Registry Number: CAS 74-89-5
- Synonyms: Methanamine; Monomethylamine; Aminomethane; Carbinamine; MMA
- Chemical/Pharmaceutical/Other Class: Amine
- Chemical Formula: CH_5N
- Chemical Structure: CH_3NH_2

Uses

Methylamine is primarily used in the organic synthesis of pharmaceuticals, insecticides, herbicides, pesticides, fungicides, electrostatic automotive coatings,

as well as certain solvent and paint strippers, photographic developers, and water treatment chemicals. Methylamine has also found use in tanning and dyeing processes, in rocket propellants, and in the manufacture of rubber chemicals, surfactants, surface-active agents, and accelerators.

Background Information

Methylamine has been reported to occur in various fresh fruits and vegetables, coffee, tea, and cocoa, as well as cheeses, and fish.

Exposure Routes and Pathways

Exposure to methylamine primarily occurs in occupational settings. Because methylamine is a gas, such exposures typically occur via inhalation, although dermal contact (and to a lesser extent ocular contact) with liquefied methylamine or aqueous solutions of methylamine would also be possible. The general population is potentially exposed to low concentrations of methylamine by ingestion from food, and by inhalation from releases to air.

Toxicokinetics

The metabolism of methylamine is believed to occur in two stages. The amino group is initially dehydrogenated to an intermediate imine (methyl imine), which reacts spontaneously with water, forming the corresponding aldehyde (formaldehyde) and ammonia. The final metabolic products, reported to be formic acid and urea or methylurea, are excreted in the urine. To a lesser extent, methylamine is also metabolized to dimethylamine. Methylamine is a normal constituent of mammalian and human urine.

Mechanism of Toxicity

The toxic effects of methylamine are due primarily to its corrosive action on tissues.

Acute and Short-Term Toxicity (or Exposure)

Methylamine is corrosive to the eyes and skin, and is a severe respiratory tract irritant.

Animal

The acute rat oral LD_{50} of a 40% aqueous solution of methylamine is $100\,mg\,kg^{-1}$. Reported inhalation LC_{50} values in rats and mice are 448 ppm ($570\,mg\,m^{-3}$) (2.5 h) and $2400\,mg\,m^{-3}$ (2 h), respectively. In rabbits, a 40% aqueous solution of methylamine produced corneal damage and dermal necrosis, and a 5% aqueous solution caused conjunctival hemorrhages, superficial corneal opacities, and edema.

Human

Brief exposure to concentrations of 20–100 ppm produced transient irritation of the eyes, nose, and throat. A concentration of 2–60 ppm has been reported to cause bronchitis.

Exposure to elevated concentrations of various amines, and presumably methylamine, is known to cause transient visual disturbance called glaucopsia, which is often referred to as blue haze or halovision. Exposure to elevated levels of methylamine is expected to potentially cause severe ocular and respiratory tract irritation with eye burns and pulmonary edema possible. Dermal contact with aqueous methylamine is expected to cause severe skin irritation and skin burns, with cold burns/frost bite also possible following dermal contact with liquefied methylamine. Ingestion of aqueous methylamine is expected to cause burns of the mouth, throat, esophagus, and stomach with bleeding, vomiting, diarrhea, and possible perforation of the esophagus and stomach.

Chronic Toxicity (or Exposure)

Animal

Repeated inhalation exposure of rats to methylamine for $6\,h\,day^{-1}$, $5\,days\,week^{-1}$ for 2 weeks produced mild nasal irritation at 75 ppm; damage to the respiratory mucosa of the nasal turbinates at 250 ppm; and bodyweight loss, liver damage, and nasal degenerative effects at 750 ppm.

No adverse reproductive effects or fetal abnormalities were observed in CD-1 mice treated daily with 0.25, 1, 2.5, or 5 mmol methylamine per kg by intraperitoneal injection during gestation days 1–17.

Human

No symptoms of irritation were evident following repeated exposure to 10 ppm. Repeated exposures to higher concentrations are expected to produce irritation of the eyes, nose, and throat. Repeated exposures could potentially aggravate existing respiratory diseases.

In Vitro Toxicity Data

In vitro mutagenicity assays yielded negative (not mutagenic) results in the *Salmonella*/microsome

reverse mutation assay, and positive (mutagenic) results in the mouse lymphoma cell forward mutation assay.

Clinical Management

Exposed skin and eyes should be irrigated with copious amounts of water. After inhalation exposures, the victim should be moved to fresh air and monitored for respiratory distress. Humidified, supplemental oxygen (100%) with assisted ventilation should be administered as required. If coughing or breathing difficulties are noted, the patient should be evaluated for irritation, bronchitis, or pneumonitis, including chest X-rays and determination of blood gases. If pulmonary edema is present, positive-end expiratory pressure ventilation and steroids should be considered. For ingestion exposures, emesis or lavage should be avoided. Use of diluents is controversial. Delayed abdominal pain and tenderness or shock may indicate gastric or esophageal perforation.

Environmental Fate

Given its high vapor pressure of over 2 atm at 25°C, methylamine will remain in the vapor phase, if released to the atmosphere where it will react with photochemically produced hydroxyl radicals ($T_{1/2}$ of ~18 h). Dissolution into rain droplets is also an important removal process. Other atmospheric removal processes, for example, photolysis and hydrolysis are not significant.

The predominant form of methylamine under environmental conditions is the ionized (protonated) species, which is expected to bind to soil constituents, suspended sediments, and bed sediments to a greater degree than the neutral form. As such, migration from soil to groundwater is expected to be less than would be anticipated for the neutral form. Volatilization from moist soils or surface water is not expected to be an important fate process. Biodegradation is expected to be an important loss process in both soil and water. The potential for bioconcentration in aquatic biota is low.

Ecotoxicology

The reported nonlethal concentration in algae (*Scenedesmus*) is 4 mg l^{-1}. The reported no-observed-effect concentration in crustaceans (*Daphnia*) is 480 mg l^{-1}. The 24 h LC_{50} in fish (cheek chub) is 10–30 mg l^{-1}.

Other Hazards

High airborne concentrations of methylamine can form, given its vapor pressure, with the potential for severe eye, nose, and respiratory tract irritation; escape impairment; and possible death. The immediately dangerous to life or health concentration for methylamine is 100 ppm. Anhydrous methylamine is a flammable gas, and aqueous methylamine is a flammable liquid. Vapors can travel a considerable distance to an ignition source and flash back because methylamine vapor density is heavier than air.

Exposure Standards and Guidelines

Occupational exposure standards and guidelines for methylamine include the following:

- United States: Occupational Safety and Health Administration permissible exposure limit (Table Z-1) is 10 ppm (12 mg m^{-3}).
- United States: National Institute for Occupational Safety and Health recommended exposure limit is 10 ppm (12 mg m^{-3}).
- United States: American Conference of Governmental Industrial Hygienists threshold limit value is 5 ppm (6.4 mg m^{-3}), with a 15 min short-term exposure limit of 15 ppm (19 mg m^{-3}).
- Australia: 10 ppm.
- Germany: 10 ppm, with a short-term level of 20 ppm for 10 min (four times/shift).
- Sweden: 10 ppm, with a short-term value of 20 ppm for 15 min, (skin notation).
- United Kingdom: 10 ppm.

See also: Corrosives; Respiratory Tract.

Relevant Websites

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Methylamine.

http://www.state.nj.us/health/eoh/rtkweb/1225.pdf – Methylamine (Right-to-Know Hazardous Substance Fact Sheet from the state of New Jersey).

Methylcholanthrene, 3-

Richard A Parent

This article is a revision of the previous print edition article by R A Parent, T R Kline, and D E Sharp, volume 2, pp. 305–306, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 56-49-5
- Synonyms: 3-MC; 3-MCH; 3-Methyl-I,j-cyclopentabenz(*a*)anthracene; 3-Methylcyclopentabenzophenathrene
- Chemical/Pharmaceutical/Other Class: Polynuclear aromatic hydrocarbon (PAH); Polycyclic aromatic hydrocarbon
- Chemical Formula: $C_{21}H_{16}$

Uses

3-Methylcholanthrene (3-MC) is used experimentally as a positive control in cancer research and in biochemical research to induce specific forms of cytochrome P450. Other than this, there is no particular use for this chemical except as a possible chemical intermediate.

Exposure Routes and Pathways

3-MC may be absorbed via inhalation, ingestion, or dermal contact. It is contained in coal tar pitch volatiles which may result in dermal or ocular exposure.

Toxicokinetics

Animal studies with structurally related polynuclear aromatic hydrocarbons (PAHs), such as benzo(*a*)pyrene, benz(*a*)anthracene, and 3-MC, confirmed that intestinal transport readily occurs primarily by passive diffusion after oral dosing. From the partitioning parameters, the rate-limiting step involves solvation of transfer species in the interfacial water at the phospholipid surface.

Metabolic products vary with the type of enzyme inductions. In fetal rat livers, several compounds, such as 1- or 2-hydroxy-, *cis*- and *trans*-dihydroxy-, 11,12-dihydroxy-11,12-dihydro-, and 1- and 2-keto-3-cholanthrene have been identified. Most frequently, it is the liver that produces a variety of electrophilic reactants that covalently bind to macromolecules. Metabolism or bioactivation may also be extramicrosomal or be carried out by fetoplacental tissue or gut bacteria.

PAHs are highly soluble in adipose tissue and lipids. *In vivo* binding of 3-MC to liver and lung DNA was studied in A/J mice and demonstrated DNA binding in the liver and lung.

Mechanism of Toxicity

Metabolic activation of PAHs consists of an oxidation of the rings of unsubstituted PAHs. These oxidations are carried out by mixed function oxidases of the liver which contain cytochromes P450 and P448 and require reduced nicotine adenine dinucleotide and oxygen. In this oxidation, an epoxide intermediate is formed which has been shown to have the requisite chemical reactivity to bind covalently with DNA and histones and to serve as the ultimate carcinogenic form of PAH. Administration of 3-MC to rats increased hepatic nuclear proteins and caused a turnover of protein of the endoplasmic reticulum. Studies of ^{14}C amino acid incorporation showed that 3-MC causes increased protein synthesis and reduced degradation of protein.

Acute and Short-Term Toxicity (or Exposure)

Animal

3-MC has a low order of acute toxicity but at high concentrations it can produce irritation of mucous membranes.

Human

The minimum lethal human exposure and the maximum tolerated human exposure to this agent have not been delineated. PAHs are eye irritants and produce photosensitivity, respiratory irritation, cough, mild hepatotoxicity, and nephrotoxicity. The minimal lethal dose of 3-MC in humans has not been established.

Chronic Toxicity (or Exposure)

Animal

In mice, skin application leads rather quickly to carcinoma formation. Subcutaneous injection produces sarcomas in rats or mice. Oral administration in sesame oil to female Sprague–Dawley rats results in rapid induction of breast cancer, while oral administration to mice during the last week of pregnancy produced a threefold increase in incidence of tumors. The most common types were lymphoma and lung tumors. 3-MC has reportedly produced skin tumors in mice, injection site tumors in rats, large

intestinal tumors in Sprague–Dawley rats treated intrarectally, lung adenomas in mice treated via intraperitoneal injection, and lung tumors in neonatal mice treated transplacentally.

3-MC is a powerful irritant and is an experimental carcinogen producing neoplastic responses by various dosing routes including oral, dermal, intravenous, parenteral, subcutaneous, intrarenal, intrapleural, intratracheal, and implant. 3-MC is hepatotoxic, nephrotoxic, and immunotoxic and has been reported to produce agranulocytosis, anemia, leukopenia, and pancytopenia in exposed animals.

Exposure of mice to 3-MC produces a marked depression in serum antibody response to shee erythrocytes. Subsequent studies confirmed that 3-MC does indeed suppress the immune system resulting in long term immunsuppression.

Human

Chronic exposure to 3-MC can result in irritation, chronic cough, bronchitis, and bronchogenic cancer. Leukoplakia and cancers of the lip and oral cavity can develop. Dermal contact has been associated with precancerous lesions called 'coal tar warts' which are enhanced by exposure to UV light. Erythema, dermal burns, acneiform lesions, photosensitization and cancer may develop upon chronic exposure.

Workers routinely exposed to PAHs have been reported to show increased incidences of skin, bladder, lung, and gastrointestinal cancers. Other studies also demonstrated increased incidences of lung and scrotal cancer. 3-MC is a PAH and is considered to be carcinogenic in humans.

PAHs as a class of compounds are generally classified by the International Agency for Research on Cancer as being possibly carcinogenic to humans while mixtures of these compounds such as coal tar pitch and coke production are classified as being carcinogenic to humans. Increased incidences of skin, bladder, lung, and possibly gastrointestinal tract cancers have been reported in polynuclear aromatic hydrocarbon (PNA)-exposed workers, particularly associated with coal carbonization, coal gasification, and coke oven work.

Increased numbers of chromosomal aberrations have been reported to be a sensitive marker for exposure to PAHs.

In Vitro Toxicity Data

3-MC is mutagenic in a number of *in vitro* and *in vivo* assays and is used regularly as a positive control in these assays, and it has been shown to covalently bind to DNA and other macromolecules. Using TA100 strain of Salmonella in the Ames test, S-9 liver microsomal fractions from mice, rats, hamsters, pig, and humans produced a doubling of the reversion rate for all liver fractions except the pig. 3-MC has also produced positive findings in the Ames test using S9 rat liver microsomal fractions stimulated with PCB Aroclor 1254 in strains TA100, TA1535, TA1537, TA1538, and in TA1538, TA98 with rat liver S9 fractions stimulated with phenobarbital. Positive mutagenic findings were also noted in Chinese hamster V-79 thioquanine assay, in unscheduled DNA assays done in human fibroblasts, and in rodent primary cells and hepatocytes, in Chinese hamster ovary HGPRT assay but not in several *Escherichia coli* WP2 UVRA assays with S9 activation. Positive findings were also reported in human lymphocyte assays showing increased sister chromatid exchanges. Numerous other reports of both positive and negative findings may be found in the literature.

Clinical Management

Acute toxicity relating to ingestion of PAHs is highly unlikely. Inhalation exposure to PAHs may involve other materials capable of causing acute respiratory and systemic effects. Treatment should be according to symptomatology. For dermal contact, it is important to remove contaminated clothing and wash the exposed area with soap and water. Burns should be treated in the usual manner.

Environmental Fate

3-MC, when released to soil should adsorb strongly to the soil and not leach. It will not biodegrade or hydrolyze significantly but may evaporate from dry soil. If released to water, it is expected to adsorb strongly to sediment and to bioconcentrate in aquatic organisms. Again it will not biodegrade or hydrolyze significantly. If released into the atmosphere, it may be subject to direct photolysis since it absorbs strongly in the UV spectrum of light. It may also react with peroxyl radicals already present in the atmosphere. Its estimated half-life in the atmosphere is 2.81 h. Considering an octanol/water log concentration ratio of 6.42, a bioconcentration factor of 45 000 has been estimated.

Other Hazards

Treated pregnant rats produce offspring with major birth defects including open neural tubes, abnormal flexure rotation and proencephalic defects, among others. In experimental animals, PAHs such as 3-MC and metabolites have been noted to produce

decrements in fertility as a result of decreased numbers of oocytes. PAHs are strongly lipophilic and are excreted in breast milk thereby resulting in a secondary exposure to nursing infants. They also cross the placenta adding an additional burden to the fetus and neonate.

Exposure Standards and Guidelines

The US Environmental Protection Agency's Carcinogen Assessment Group has placed 3-MC on a list of compounds for which strong evidence exists for either causing cancer in humans or in multiple animal species.

No occupational exposure limits have been established for 3-MC. There may be no safe level of exposure.

Miscellaneous

3-MC can be isolated by recrystallization from benzene/ether producing pale yellow slender prisms with a melting point of 280°C. It is highly soluble in organic solvents including the BTEX solvents, chlorinated aliphatics, and various ketone and ester solvents. It strongly absorbs UV light with a maximum absorption at 327 nm. Its vapor pressure is low (3.8×10^{-6} mmHg) limiting is volatility. One part per million in the environment is equivalent to $10.98\,mg\,m^{-3}$.

See also: Polycyclic Aromatic Hydrocarbons (PAHs).

Relevant Websites

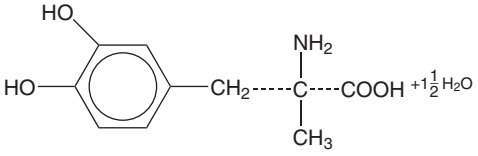
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Methylcholanthrene, 3-.

http://www.state.nj.us – New Jersey Department of Health and Senior Services, Hazardous Substance Fact Sheet, 3-Methylcholanthrene; August 1988.

Methyldopa

Elizabeth J Scharman

- Chemical Abstracts Service Registry Number: CAS 555-30-6 (anhydrous)
- Synonyms: 3-Hydroxy-α-methyl-L-tyrosine; α-Methyldopa; Methyldopate hydrochloride; Aldomet®
- Chemical/Pharmaceutical/Other Class: Hypotensive agent, centrally acting
- Chemical Formula: $C_{10}H_{13}NO_4$
- Chemical Structure:



Uses

Methyldopa is used in the management of moderate to severe hypertension and in the management of hypertension in pregnant women.

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposures to methyldopa. Methyldopa is available in an oral dosage form (alone or in combination with a thiazide diuretic, hydrochlorothiazide) and a parenteral dosage form (methyldopate hydrochloride).

Toxicokinetics

Approximately 50% of an oral dose is absorbed, individual variations occur. The peak effect occurs within 4–6 h; pharmacologic effects do not correlate with plasma concentrations. Metabolism is mainly by conjugation in the gastrointestinal tract and the liver. In patients with renal insufficiency, the rate of conjugation is decreased. Oral administration results in more sulfate conjugation than when methyldopa is given via intravenous administration. The sulfate conjugate may be active therapeutically. Urinary metabolites include: α-methyldopa-mono-*O*-sulfate; 3-*O*-methyl-α-methyldopa; 3,4-dihydroxyphenylacetone; α-methyldopamine; 3-*O*-methyl-α-methyldopamine, and their conjugates. Seventy percent of an absorbed dose is excreted in urine as methyldopa and the mono-*O*-sulfate conjugate. The volume of distribution is $0.371\,kg^{-1}$. Less than 15% is protein bound. Methyldopa crosses the placenta and is excreted into breast milk. Dialysis and peritoneal dialysis will remove methyldopa. Elimination is biphasic. The half-life during the first phase is 1.8 h. The half-life during the second phase is longer.

Mechanism of Toxicity

In the brain, methyldopa is enzymatically decarboxylated to α-methyldopamine, which undergoes subsequent enzymatic conversion, via hydroxylation, to α-methylnorepinephrine, an α-adrenergic agonist. Stimulation of central inhibitory α-adrenergic receptors causes a decrease in sympathetic outflow manifested as a decrease in blood pressure. A decrease in plasma renin activity may also play a role in methyldopa's hypotensive effects.

Acute and Short-Term Toxicity (or Exposure)

Animal

Specific information on the effects of methyldopa toxicity in domesticated animals is lacking.

Human

A minimum toxic dose is not well defined. Drug levels do not guide treatment. Hypotension, bradycardia, weakness, dizziness, sedation, and coma may occur. Gastrointestinal effects may include nausea, vomiting, and diarrhea. A withdrawal syndrome is not expected.

Chronic Toxicity (or Exposure)

Animal

Similar effects are seen in animals as those seen in humans (e.g., primarily central nervous system depression). Two year feeding studies of rats demonstrated no evidence of carcinogenicity despite doses up to 6300 ppm methyldopa in diet.

Human

Side effects may include drowsiness, headache, dry mouth, nasal congestion, and constipation. If orthostatic hypotension occurs, the dosage should be decreased. Sodium retention requiring concurrent therapy with a diuretic may occur. A positive Coombs' test has been reported in 10–20% of patients taking methyldopa; most commonly 6–12 months after starting therapy. However, not all patients with a positive test develop hemolytic anemia. A drug-induced fever may occur during the first 3 weeks after therapy is initiated. Methyldopa should be discontinued if fever, jaundice, or alterations in liver function tests occur.

In Vitro Toxicity Data

Ames *Salmonella* assays of mutagenicity have been negative.

Clinical Management

Activated charcoal will adsorb methyldopa and should be considered in patients with substantial recent ingestions. Standard supportive therapies, such as support of airway, breathing, and circulation, should be utilized as clinically necessary. Administration of vasopressors may be required for patients experiencing profound cardiovascular effects. Hemodialysis is of theoretical value if standard therapies fail.

Further Reading

Johnston GD and Smith AMJ (1990) Management of overdose due to antihypertensive agents. *Adverse Drug Reaction and Acute Poisoning Review* 9: 75–89.

Shnaps Y, Almog S, and Halkin H (1982) Methyldopa poisoning. *Journal of Toxicology Clinical Toxicology* 19: 501–503.

Methylene Chloride

Richard A Parent

This article is a revision of the previous print edition article by R A Parent, T R Kline, and D E Sharp, volume 2, pp. 308–310, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 75-09-2
- Synonyms: Dichloromethane; 1,1-Dichloromethane; DCM; Methane, dichloro
- Chemical/Pharmaceutical/Other Class: Chlorinated aliphatic hydrocarbon
- Chemical Formula: CH_2Cl_2

Uses

Methylene chloride has been used as a solvent in paint stripping formulations; as a propellant in aerosols; as a process solvent in the manufacture of drugs, pharmaceuticals, and film coatings; as a metal

cleaning and finishing solvent in electronics manufacturing; and as an agent in urethane foam blowing. Aerosol products in which methylene chloride may be found include paints, automotive products, and insect sprays. Methylene chloride has also been used as an extractant solvent for spice oleoresins, hops, and for the removal of caffeine from coffee.

Exposure Routes and Pathways

Methylene chloride is a widely used industrial chemical with reported atmospheric emissions of more than 126 million pounds annually in the United States. The principal route of exposure for the general population to methylene chloride is by inhalation. Occupational and consumer exposure to methylene chloride commonly occurs from spray painting and contact with consumer products such as paint strippers or aerosol cans, that contain methylene chloride. Exposures may occur as a result of breathing the vapors given off by the product or from direct dermal contact. Occupational exposure to methylene chloride by the inhalation route offers the most opportunity for exposure but it can also be absorbed through the skin.

Toxicokinetics

The principal route of human exposure to methylene chloride is inhalation, but dermal exposure has been noted albeit at a slower rate and oral ingestion has also been reported. During absorption through the lungs, the concentration of methylene chloride in alveolar air, in equilibrium with pulmonary venous blood content, approaches the concentration in inspired air until a steady state is achieved. After tissue and total body steady state is achieved through the lungs and other routes, uptake is balanced by metabolism and elimination. Elimination is mostly via the lungs in the exhaled air. Steady-state blood methylene chloride concentrations appear to be reached after 2–4 h of exposure. Evaluation of pulmonary uptake in humans indicated that 70–75% of inhaled methylene chloride vapor was absorbed. Uptake also increases with the percentage body fat since methylene chloride dissolves in fat to a greater extent than it dissolves in aqueous media. Once exposure ceased, methylene chloride was rapidly cleared from the blood. In animals, limited available data suggest that methylene chloride is easily absorbed from the gastrointestinal tract.

Methylene chloride is removed from the body primarily in expired air and urine. Methylene chloride excretion in the expired air was most evident in the first 30 min after exposure. In rats, methylene chloride was excreted in the expired air, urine, and feces following a single 6 h exposure to methylene chloride. Exhaled air accounted for 58–79% of the dose.

Mechanism of Toxicity

Available data suggest that there are two pathways by which methylene chloride is metabolized. One utilizes the mixed function oxidase enzymes and produces carbon monoxide, while the other pathway involves glutathione transferase and produces carbon dioxide. The mixed function oxidase pathway seems to be the preferred pathway for methylene chloride metabolism following inhalation exposures. In addition to carbon monoxide and carbon dioxide, methylene chloride is also metabolized to a lesser extent to formaldehyde and formic acid.

Human subjects exposed by inhalation to 500 ppm or greater for 1 or 2 h experienced elevated carboxyhemoglobin concentrations indicating that methylene chloride was metabolized to carbon monoxide by the mixed function oxidase pathway. Metabolism of methylene chloride in animals is similar to that in humans. Animal data on metabolism indicate that the process is similar for both inhalation and oral exposures.

When methylene chloride is absorbed through the lungs, it is thought that it will dissolve in the lipoprotein components of the blood and be distributed from the systemic circulation to the body organs.

Distribution studies in rats demonstrate that methylene chloride and its metabolites are present in the liver, kidney, brain, lungs, muscle, and adipose tissue after inhalation exposures. One hour after exposure, the highest concentration of radioactively labeled material was found in the adipose tissue followed by the liver. The concentrations in the kidney, adrenals, and brain were less than half of that in the liver. The affinity of methylene chloride for nucleophilic groups such as mercaptans present in proteins and DNA itself is thought to be a key factor in its mechanism of action.

Acute and Short-Term Toxicity (or Exposure)

Animal

Studies in animals confirm that methylene chloride may be lethal after inhalation exposure at high concentrations. Acute exposure to 16 000–19 000 ppm methylene chloride for 4–8 h caused death in rats and mice. Data suggest there is a narrow margin between concentrations causing anesthesia and those causing death. Repeated exposure in longer-term studies at levels from 1000 to 16 000 ppm has been reported to

cause increased deaths in rats, mice, guinea pigs, rabbits, dogs, and primates. Exposure to methylene chloride has reportedly resulted in fatty changes in the liver and elevated plasma enzymes. These effects were reversible after exposure ceased. A 28 day study in rats also showed elevated hepatic microsomal enzyme activities at 250 ppm in air. Nonspecific tubular degenerative and regenerative changes were observed after continuous exposure in rats at 25 and 100 ppm for 100 days, while others report splenic fibrosis and decreased cerebellar enzyme levels in rats and splenic atrophy in dogs. Methylene chloride has also been shown to cross the placental barrier but has not been shown to be teratogenic in rats and mice.

Human

Methylene chloride is a skin irritant on prolonged contact and eye irritant at high airborne concentrations and as a liquid. Case studies of methylene chloride poisoning during paint stripping operations have demonstrated that inhalation exposure can be fatal in humans. Quantitative estimates of exposure levels were not reported; however, methylene chloride was detected at autopsy in various tissues, including the liver ($14.4\,mg\,dl^{-1}$), blood ($50\,mg\,dl^{-1}$), serum ($29\,\mu g\,ml^{-1}$), and brain (24.8 mg per 100 g). The cause of death in these cases was uncertain; however, myocardial infarction was reported in one case.

Acute and prolonged exposures to methylene chloride have been reported to result in a number of signs and symptoms including headache, lightheadedness, nausea, vomiting, eye irritation, pulmonary irritation and cough, paresthesias, somnolence, altered sleep patterns, changing cardiac patterns, syncope, memory loss, intellectual impairment, cardiac sensitization, and gastrointestinal ulceration and bleeding, the latter resulting only from oral exposure. Less common symptoms include delerium, auditory and visual hallucinations, spontaneous abortions, hepatic effects, and renal effects including acute tubular necrosis. At very high levels of exposure, euphoria, central nervous system (CNS) depression with associated respiratory failure, seizures, and death have been reported. Single exposures to methylene chloride at 300 ppm caused decreased visual and auditory functions. These effects were reversible once exposure ceased. Similarly, psychomotor performance was impaired, but this occurred at higher exposure levels (800 ppm for 4 h). Alterations in visual evoked response have been observed in humans exposed to methylene chloride at 515–986 ppm for 1 or 2 h. Reversible CNS depression and carboxyhemoglobin formation are considered the major human toxicological effects of exposure to methylene chloride.

Chronic Toxicity (or Exposure)

Animal

Chronic oncogenicity studies in animal models have led to methylene chloride being classified as an animal carcinogen. In treated mice, hepatocellular carcinomas and broncho/alveolar neoplasm have been reported to be significantly elevated over controls, while salivary gland sarcomas have been noted in male rats and increased incidences of leukemia and mammary adenomas found in female rats. Other lesions found in oncogenicity studies include mesotheliomas at multiple sites, mononuclear cell leukemias and hemangiomas.

Human

Long-term low-level exposures have been known to result in neurophysiological and neurobehavioral disturbances.

Several epidemiological studies have detected no excess risk of death from malignant neoplasms in workers exposed to methylene chloride, while at least one study suggests an increased risk of pancreatic cancer.

Methylene chloride is considered to be a probable human carcinogen based on animal carcinogenicity studies and genotoxicity studies. Human studies are deemed inadequate in terms of establishing the human carcinogenic properties of methylene chloride.

In Vitro Toxicity Data

Methylene chloride has been shown to be genotoxic in some short-term assays including *Salmonella typhimurium* and *S. crevisiae*, but in other tests equivocal or negative results were reported. Methylene chloride has been reported to induce gene conversion, mitotic recombination, and gene mutations in *Saccharomyces cerevesia* D7 when cells were grown under conditions that lead to production of endogenous cytochrome P450 but did not induce DNA damage in a DNA repair assay in isolated rat hepatocytes. Other genotoxicity assays produced mixed findings. Attempted cell transformation using the BALB/3T3 mouse cell line was unsuccessful as was an attempt to cause chromosomal aberrations in the bone marrow cells of Sprague–Dawley rats.

Clinical Management

After inhalation exposures, the patient should be moved out of the exposure area and administered 100% oxygen. The carboxyhemoglobin level may be monitored as an indicator of state of intoxication.

Should seizures occur, intravenous benzodiazepam may be considered and if they continue, phenobarbital may be appropriate. For oral exposure, emesis is not recommended because of the potential for seizures and CNS depression. Gastric aspiration may be accomplished using a flexible nasogastric tube when ingestion is considerable.

Environmental Fate

When released into the environment as a vapor, methylene chloride does not usually undergo direct photolysis but does degrade by reaction with photochemically produced hydroxyl radicals. Its half-life in the atmosphere is estimated at 119 days. In soil and water, a major fate pathway involves volatilization to the atmosphere and subsequent degradation. Activated sludge studies have, however, demonstrated biodegradation of methylene chloride. Little bioconcentration in aquatic species can be expected.

Exposure Standards and Guidelines

- US Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) for an 8 h period is 25 ppm time-weighted average (TWA).
- US OSHA short-term exposure limit (STEL) for 15 min sampling is 125 ppm.
- American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV) for 8 h TWA is 50 ppm.
- ACGIH TLV – TWA for 30 min exposure during workday is 250 ppm.
- US National Institute of Safety and Health recommends that methylene chloride be handled as an occupational carcinogen.
- US Environmental Protection Agency classifies methylene chloride as a B2 carcinogen, a probable human carcinogen based on inadequate human data but sufficient evidence for carcinogenicity in animals.
- ACGIH classifies methylene chloride as an A3 carcinogen, a confirmed animal carcinogen with unknown relevance to man.
- International Agency for Research on Cancer has classified methylene chloride as a group 2B carcinogen, possibly carcinogenic to humans based on sufficient evidence in experimental animals.
- Methylene chloride has been designated as a hazardous air pollutant under Section 112 of the Clean Air Act and is subject to effluent limitations since it has been designated as a toxic pollutant pursuant to Section 307(a) (1) of the Federal Water Pollution Control Act.
- The maximum contaminant level promulgated in the National Revised Primary Drinking Water Regulations for methylene chloride in community and nontransient noncommunity water systems is $0.005\,\mathrm{mg\,l^{-1}}$.
- EPA's Federal Drinking Water Standard for methylene chloride is $5\,\mu\mathrm{g\,l^{-1}}$.

Miscellaneous

Methylene chloride is a clear volatile colorless liquid having a chloroform-like odor that is detectable at concentrations from 540 to $2160\,\mathrm{mg\,m^{-3}}$ in air. At atmospheric pressure it boils at $\sim 40^{\circ}\mathrm{C}$ and is soluble in organophilic solvents and oils. Its vapor has a density of 2.93 in air and a vapor pressure of 435 mmHg at 25°C. One part per million in air is equal to $2.48\,\mathrm{mg\,m^{-3}}$. Combustion of methylene chloride may result in the production of highly toxic gases including hydrogen chloride, phosgene, and carbon monoxide. Smoking in a methylene-contaminated atmosphere can result in toxic inhalation of these potentially lethal pyrolysis products.

See also: Aerosols; Caffeine; Pollution, Air.

Relevant Websites

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Methylene Chloride.

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Methylene Chloride.

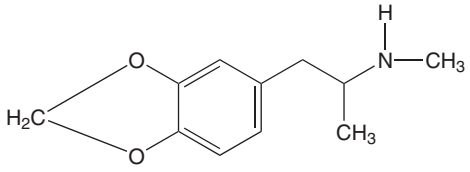
http://www.epa.gov – Methylene chloride (dichloromethane), US EPA Technology Transfer Network Air Toxic Website.

Methylenedioxymethamphetamine

Alexander B Baer and Christopher P Holstege

This article is a revision of the previous print edition article by Janet E Bauman, volume 2, pp. 311–312, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 69610-10-2
- Synonyms: 3,4-Methylenedioxymethamphetamine; Adam; Bean Doctor; E; Ecstasy; Essence; MDM; MDMA; M & Ms; Roll; The Substance; X; XTC
- Chemical/Pharmaceutical/Other Class: Synthetic phenylalkylamine derivative of amphetamine
- Chemical Structure:



Uses

Formerly a psychotherapeutic agent, methylenedioxymethamphetamine (MDMA) is now abused as a hallucinogenic amphetamine.

Exposure Routes and Pathways

MDMA is available illicitly in tablet, capsule, and powder forms. It is most commonly ingested, but insufflation and intravenous injection has also been reported.

Toxicokinetics

MDMA is rapidly absorbed with the onset of effects occurring in 20–60 min. Concentrations peak at 2–3 h and typically last 4–6 h. Prolonged effects lasting up to 48 h may be seen following large doses. MDMA is metabolized in the liver by cytochrome P450, chiefly CYP2D6, to form methylenedioxyamphetamine (MDA). The volume of distribution is considered large ($>5\,l\,kg^{-1}$). MDMA and its metabolites are excreted renally, with 75% as unchanged MDMA and 7% as MDA. Elimination is usually complete within 24 h.

Mechanism of Toxicity

MDMA induces norepinephrine release from presynaptic vesicles. MDMA also effects serotonin neurotransmission by causing release of serotonin (5-hydroxytryptamine (5-HT)) and inhibiting its uptake. In animal models, it has been demonstrated to cause long-term destruction of 5-HT axons. Studies demonstrate lowered concentrations of the 5-HT metabolite 5-hydroxyindoleacetic acid in the cerebrospinal fluid of regular MDMA users. This correlates with a similar decrease reported in primates with brain damage induced by MDMA.

Acute and Short-Term Toxicity (or Exposure)

Animal

In animals, a toxic dose is estimated to be $10–30\,mg\,kg^{-1}$. Reported effects in small animals include hyperthermia, rapid respirations, rapid heartbeat, dilated pupils, lactic acidosis, hypertension, arrhythmias, vomiting, and diarrhea. Renal failure, seizures, and coma are possible.

Human

Symptoms noted in acute toxicity include anxiety, mydriasis, hypertension, tachycardia, tachypnea, hallucinations, bruxism, and diaphoresis. Hyperthermia, arrhythmias, hyperreflexia, seizures, metabolic acidosis, ischemia, rhabdomyolysis, and renal failure may be seen in severe toxicity. Hyponatremia, hyperkalemia, coagulopathies, pulmonary edema, and adult respiratory distress syndrome have also been reported. MDMA may cause liver injury. While the vast majority of these cases have spontaneous recovery, an increasing number of fulminate hepatic failure reports are now appearing in the literature. Hyponatremia is a recognized complication that is thought to have several contributing factors including sodium loss through excessive sweating, hemodilution with large free water volume intake, and inappropriate secretion of antidiuretic hormone leading to water retention. Sudden death is likely due to cardiac arrhythmias, seizures, and central nervous system depression. Blood levels do not correlate with toxicity but can confirm exposure.

Chronic Toxicity (or Exposure)

Animal

Several models of MDMA exposure in animals have described long-term adverse effects on emotion. These effects have responded to treatment with serotonergic agents such as fluoxetine.

Human

Positron Emission Tomography (PET) scans of former abusers of MDMA have revealed a decrease

in the brain serotoninergic neurons. These changes are of unknown consequences but may include depression, anxiety, and memory impairment. Chronic paranoid psychosis, depression, flashbacks, panic disorders, and some impairment of cognitive function have been related to long-term use.

In Vitro Toxicity Data

Several studies have demonstrated that MDMA can suppress neutrophil phagocytosis as well as suppress the production of tumor necrosis factor-alpha and interleukin.

Clinical Management

There is no antidote for MDMA poisoning. General supportive care is the mainstay of therapy. Activated charcoal may be used to adsorb the MDMA within an hour of ingestion. Benzodiazepines may be a useful adjunct for the immediate management of an acutely agitated or psychotic patient that poses an immediate threat to healthcare staff or self.

Hypertensive emergencies with end-organ ischemia may be treated with antihypertensive agents such as nitroprusside. Beta-blocking agents should be used with caution due to concern of causing unopposed alpha agonism leading to worsening end-organ ischemia. Phentolamine may be useful in cases of hypertensive emergencies or end-organ ischemia refractory to nitrate infusions.

Hypothermic blankets, ice water baths, chilled intravenous fluids, gastric and bladder lavage with cooled fluids may be needed to reduce body temperature. Dantrolene has also been utilized for the treatment of MDMA-related hyperthermia. Measuring creatinine phosphokinase and urine myoglobin levels can be helpful in recognizing those at risk of developing acute renal failure due to rhabdomyolysis. Ensuring adequate urine output with intravenous fluids is the mainstay of treatment for preventing acute tubular necrosis.

See also: Drugs of Abuse.

Further Reading

Dowling GP, McDonough ET, and Bost RO (1987) 'Eve' and 'Ecstasy'. A report of five deaths associated with the use of MDEA and MDMA. *Journal of the American Medical Association* 257: 1615–1617.

Henry JA, Jeffreys KJ, and Dawling S (1992) Toxicity and deaths from 3,4-methylenedioxymethamphetamine ('ecstasy'). *Lancet* 340: 384–387.

McCann UD, Slate SO, and Ricaurte GA (1996) Adverse reactions with 3,4-methylenedioxymethamphetamine (MDMA; 'ecstasy'). *Drug Safety* 15: 107–115.

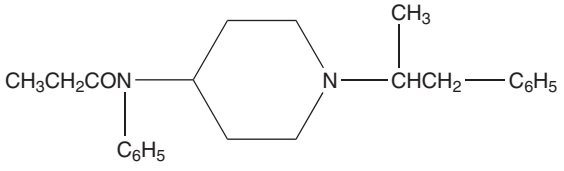
Milroy CM (1999) Ten years of 'ecstasy'. *Journal of the Royal Society of Medicine* 92: 68–72.

Shannon M (2000) Methylenedioxymethamphetamine (MDMA, "Ecstasy"). *Pediatric Emergency Care* 16: 377–380.

Methylfentanyl, α-

Abraham Dalu

- Chemical Abstracts Service Registry Number: CAS 79704-88-4
- Synonyms: *N*-[1-(1-Methyl-2-phenylethyl)-4-piperidinyl]-*N*-phenylpropanamide; China white
- Chemical/Pharmaceutical/Other Class: α-Methylfentanyl is a narcotic analgesic, a designer drug derived from fentanyl
- Chemical Formula: $C_{23}H_{30}N_2O$
- Chemical Structure:



Uses

α-Methylfentanyl is not medically used per se, although it is a derivative of fentanyl with higher analgesic effects. α-Methylfentanyl is a designer drug that has been synthesized for its analgesic and euphoric effects. Due to its high potency (1000–2000 times more potent than heroin) and fast-acting narcotic analgesia, it has high abuse potential and is sold on the street as synthetic heroin. α-Methylfentanyl also has a high abuse potential in racing horses for its analgesic and stimulant actions. Therefore, α-methylfentanyl is a controlled substance listed in the US *Code of Federal Regulations*, Title 21, Part 1308.11 (1987).

Exposure Routes and Pathways

Most common exposure pathways to α-methylfentanyl are via intramuscular or intravenous injection.

Toxicokinetics

Limited information indicates that α-methylfentanyl is rapidly absorbed and distributed to the central nervous system (CNS). Elimination of this drug is primarily via the kidneys.

Mechanism of Toxicity

α-Methylfentanyl is believed to exert its toxic effects by binding to opiate receptors (μ-agonist) at many sites in the CNS.

Acute and Short-Term Toxicity (or Exposure)

Animal

In experimental horses, α-methylfentanyl induces locomotive responses (as quantified by counting the number of footsteps taken per unit of time), indicating that it is a morphine-like narcotic agonist in horses. The maximum effect can be seen $\sim$10 min after treatment of horses with greater than 4 $\mu g\,kg^{-1}$ body weight.

Human

α-Methylfentanyl is fast acting. At high doses it causes euphoria, marked muscular rigidity, and respiratory depression. The literature indicates that α-methylfentanyl overdose deaths are primarily due to respiratory paralysis. In addition to its high potency and fast action, further danger is due to its poor ability to mix with the cutting agents used with illicit drugs.

Chronic Toxicity (or Exposure)

Information on chronic toxicity in animal and human is not available.

Clinical Management

Since α-methylfentanyl exerts its toxicity as a μ-agonist, its toxicity can be managed with narcotic μ-antagonists such as naloxone and respiratory support with resuscitative equipment.

See also: Fentanyl; Fentanyl Derivatives, Illicit.

Methylmercury

Shayne C Gad and Kevin N Bayer

- Chemical Abstracts Service Registry Number: CAS 22967-92-6
- Synonyms: Alkyl, alkoxyalkyl mercury compounds
- Chemical/Pharmaceutical/Other Class: Organometals
- Chemical Formula: The term 'methylmercury' generally refers to monomethylmercury, but several organic forms can exist. CH_3Hg^+ (methylmercury cation) is usually associated with simple anions, that is, Cl^-. $(CH_3)_2Hg$ is dimethylmercury

Uses

Organomercury compounds, such as aryl and alkoxy-aryl, have been used in medicine, agriculture, and laboratory research. Their use in fungicides has been greatly reduced or eliminated.

Exposure Routes and Pathways

The major route of general population exposure to methylmercury is through the consumption of contaminated fish and fish products. This occurs as a result of inorganic mercury from natural or man-made sources being methylated by microorganisms in aquatic sediments. Methylmercury is then biomagnified in the food chain with relatively high concentrations accumulating in the edible tissues of fish. A minor route of exposure is through inhalation of vaporized methylmercury and organomercurials from the atmosphere or industrial workplace.

Toxicokinetics

Methylmercury from dietary and inhalation exposures is almost completely absorbed ($\sim$90%) into the bloodstream. Methylmercury may be converted to inorganic mercury in both experimental animals and humans by intestinal flora and macrophage cells. Glutathione and sulfhydryl peptide complexes have been observed in the bile. Methylmercury is rapidly distributed to all tissues, with high concentrations accumulating in the brain, the target organ of toxicity. Methylmercury moves readily across the placenta, and higher concentrations are found in cord blood compared to maternal blood. The fecal pathway is responsible for $\sim$90% of the total elimination of mercury following methylmercury exposure. The

majority of methylmercury resulting from biliary secretion is demethylated by intestinal flora and eliminated in the feces as inorganic mercury. The remaining methylmercury can enter the enterohepatic circulation, while a small percentage of inorganic mercury is absorbed and distributed to the tissues. The half-life of methylmercury in fish-eating humans is estimated to be between 39 and 70 days.

Mechanism of Toxicity

All mercury compounds exhibit high affinity for sulfhydryl groups in proteins. As a result, a variety of enzymes and structural proteins containing free sulfhydryl groups can be modified and their functions affected. Inhibition of protein synthesis is an early biochemical event following exposure. The integrity of the blood–brain barrier can be disrupted by methylmercury, which results in the alteration of amino acid uptake and subsequent brain metabolism. Methylmercury can alter cell division during critical stages of central nervous system (CNS) development, at least in part through inhibition of microtubule function. However, there is uncertainty whether methylmercury or the mercuric ion following cleavage from methylmercury is the ultimate toxicant.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} for methylmercury in rats is $58\,mg\,kg^{-1}$. Methylmercury causes mutations in rodents at $20–40\,mg\,l^{-1}$.

Human

Although methylmercury is generally recognized as a cumulative poison, acute effects, such as headache, gastrointestinal irritation (nausea, vomiting, abdominal pain, and diarrhea), and paresthesia of the extremities, have been reported. Severe neurologic toxicity, described below, may occur several weeks or months following exposure. Renal failure normally associated with inorganic mercury poisoning is seldom observed with methylmercury. At high concentrations, methylmercury is corrosive to the skin and eye.

Dimethylmercury is more toxic than the monomethyl form. In a well publicized case, it was associated with the fatality of a research chemist. The researcher was exposed by dermal absorption after spilling a small amount of the compound on her latex gloves. Dimethylmercury was found to penetrate disposable latex and polyvinyl chloride gloves in 15 s or less. Where possible, the use of inorganic mercury salts is recommended as a substitute in laboratory research. These compounds are less volatile and lipid soluble than dimethylmercury and scientists face a lesser risk of exposure to mercury.

Chronic Toxicity (or Exposure)

Animal

Methylmercury causes neurotoxicological effects in cats and dogs. Cats ingesting contaminated fish around Minamata Bay, Japan, died after paroxysmal fits (i.e., 'cat-dancing disease'). Mink are particularly sensitive to the toxicity of methylmercury. Methylmercury is also fetotoxic and teratogenic in laboratory mammals.

Human

Poisoning episodes in humans have occurred as a result of environmental contamination of fish due to industrial discharges (Minamata and Niigata, Japan) and through seed grains contaminated with a methylmercury fungicide (Iraq). In these episodes, most of the signs and symptoms of methylmercury poisoning were attributed to damage to the CNS; effects on nonnervous tissue were absent or negligible.

Characteristics of methylmercury poisoning in adults include a long latent period (several months) and a continuation of early nonspecific symptoms such as paresthesia, blurred vision, and malaise. A 5% increase in the incidence of paresthesia has been linked to a daily methylmercury intake of $3–7\,\mu g\,kg^{-1}$ body weight. Daily intakes of $0.4\,\mu g\,kg^{-1}$ body weight will not result in any detectable adverse effects. After time, additional signs may appear including concentric constriction of the visual field, deafness, speech difficulties, and ataxia (known as the Hunter Russell syndrome). Severely exposed patients may lapse into a coma and ultimately die, although there is no clear pattern of mercury-related deaths. Many effects in severe cases are irreversible due to destruction of neuronal cells. In less severe cases, some degree of recovery in each symptom may occur depending on the compensatory function of the CNS. At high doses, methylmercury also causes neuromuscular weakness from effects on the peripheral nervous system. The developing CNS is more sensitive to damage than the adult CNS. Some infants who have been exposed to high maternal blood levels of methylmercury were born with cerebral palsy. The main pattern of severe toxic effects includes microcephaly, hyperreflexia, and gross motor and mental impairment, sometimes associated with blindness and deafness. Milder degrees of the affliction show mainly as psychomotor impairment and persistence of pathological reflexes.

In Vitro Toxicity Data

In vitro exposure of primary cultures of rat cerebellar granule cells to methylmercury resulted in a time- and concentration-dependent cell death. Some *in vitro* models have shown that organic mercury may interfere with the bacteriocidal capacity of polymorphonuclear leukocytes.

Clinical Management

There is no known useful treatment for methylmercury poisoning. A variety of chelating agents, such as D-penicillamine, 1-acetyl-D,L-penicillamine, thiol resins, activated charcoal, BAL (British Antilewisite; 2,3-dimercaptopropanol), and meso-2,3-dimercaptosuccinic acid, have been used to treat methylmercury exposure but with limited to no success.

Thus, the best approach is prevention. Since young children appear to be the most sensitive to methylmercury toxicity, children under 7 years and pregnant or breast-feeding women should limit their consumption of fish that are known to have high levels of methylmercury in their edible tissues.

Environmental Fate

Inorganic mercury introduced as a pollutant into natural waters is scavenged by particulate matter and deposited into bottom sediments. Free Hg^{2+} is gradually released from this pool of slightly soluble inorganic mercury and is then transformed by microbial activity into methylmercury. Methylmercury diffuses into the water column and is taken up by fish and other organisms (either directly through water or through the food chain), and accumulated in their tissue. The degree to which mercury is transformed into methylmercury and transferred up the food chain through bioaccumulation depends on a variety of factors, including water chemistry and the complexity of the food web.

Exposure Standards and Guidelines

The US Environmental Protection Agency has set a criterion of 0.3 mg methylmercury per kg in fish tissue that should not be exceeded to protect the health of consumers of noncommercial freshwater/estuarine fish.

The joint expert committee for food additives and contaminants revised the Provisional Tolerable Weekly Intake for methylmercury, recommending that it be reduced to $1.6\,\mu g\,kg^{-1}$ body weight per week in order to sufficiently protect the developing fetus.

See also: Levothyroxine; Mercury; Metals; Neurotoxicity.

Further Reading

Castoldi AF, Coccini T, and Manzo L (2003) Neurotoxic and molecular effects of methylmercury in humans. *Reviews on Environmental Health* 18(1): 19–31.

Dourson ML, Wullenweber AE, and Poirier KA (2001) Uncertainties in the reference dose for methylmercury. *Neurotoxicology* 22(5): 677–689.

Tsubaki T and Irukayama K (1977) *Minamata Disease*. New York: Elsevier.

World Health Organization (WHO) (1989) *Environmental Health Criteria 86: Mercury-Environmental Aspects*. Geneva: WHO.

World Health Organization (WHO) (1990) *Environmental Health Criteria 101: Methylmercury*. Geneva: WHO.

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Methylmercury.

http://www.epa.gov – Methylmercury Criteria Document (from the US Environmental Protection Agency).

Methylnitrosourea

Robin C Guy

- Representative Chemicals: Some chemotherapeutic agents
- Chemical Abstracts Service Registry Number: CAS 684-93-5
- Synonyms: *N*-nitroso-*N*-methylurea; 1-Methyl-1-nitrosourea; MNU; *N*-Nitroso-*N*-methylcarbamide; Nitrosomethylurea; *N*-Methyl-*N*-nitrosourea
- Chemical/Pharmaceutical/Other Class: Alkylating agent; DNA alkylating agent; Chemotherapeutic agent

Uses

N-Nitroso-*N*-methylurea was once widely used to synthesize diazomethane in the laboratory; however, this use was replaced by other reagents. It has been studied as a chemotherapeutic agent in cancer treatment, either alone or in combination with

cyclophosphamide. Small quantities are used in research to study its mutagenic effects on plants.

Exposure Routes and Pathways

The potential for human exposure is limited because *N*-nitroso-*N*-methylurea is not produced or used in large quantities in the United States.

Occupational exposure may occur through oral, inhalation, or dermal contact at facilities where this chemical is used in research. In air, it exists solely as vapor where it is degraded (estimated half-life of 10 days) by reaction with photochemically produced hydroxyl radicals. It hydrolyzes in water (half-life of 1.2 h at pH 7 at 20°C). A limited number of research laboratory workers may also be possibly exposed; several accidents have been reported in which laboratory personnel were exposed when the compound exploded at room temperature.

The potential for direct exposure exists when injecting cancer patients with *N*-nitroso-*N*-methylurea in conjunction with cyclophosphamide, as a chemotherapeutic agent. Health professionals such as pharmacists, physicians, and nurses are potentially exposed to the compound during the preparation and administration of the pharmaceuticals or during clean-up.

Toxicokinetics

Methylnitrosourea is rapidly absorbed from the gastrointestinal tract. To form *N*-nitroso compounds *in vivo*, there may be a reaction of nitrite with secondary amines or amides in food or water. Whole body autoradiography showed that 2 min after an intravenous dose of [^{14}C]*N*-methyl-*N*-nitrosourea to rat, ^{14}C was fairly evenly distributed in most tissues. According to the World Health Organization, the biological half-life of *N*-nitroso compounds appears to be less than 24 h.

Mechanism of Toxicity

Methylnitrosourea is a DNA alkylating agent. Methylnitrosourea causes dermal sensitization. It is also an inhibitor of protein and nucleic acid synthesis in tissues.

Acute and Short-Term Toxicity (or Exposure)

Animal

As methylnitrosourea is a DNA alkylating agent, the major toxic effects result from severe damage to hematopoietic, lymphoid, and other tissues that have rapid rates of cell turnover. ICR male mice were given intraperitoneal injections at 5, 15, or 25 mg kg^{-1} day^{-1} for 5 days, then were mated on days 1–7, 8–14, 15–21, or 64–80 after the last dose and their progeny were observed on day 18 of pregnancy; there was an increased postimplantation loss, in addition to cleft palate, and fused ribs.

N-Nitroso-*N*-methylurea is carcinogenic in all animal species tested: mice, rats, Syrian golden, Chinese, and European hamsters, guinea pigs, rabbits, gerbils, pigs, dogs, and monkeys. It induces benign and malignant tumors following its administration by different routes, including ingestion. It produces tumors at different sites, including the nervous tissue, stomach, esophagus, pancreas, respiratory tract, intestine, lymphoreticular tissues, skin, and kidney. It is carcinogenic following its administration prenatally and in single doses.

It was positive for gene mutations (HGPRT and TK genes) as well as for chromosomal aberrations, sister chromatid exchange, and DNA repair-deficient bacterial assays.

Human

Nausea and vomiting were seen after an intravenous injection of 4 mg kg^{-1} to patients.

Chronic Toxicity (or Exposure)

Human

Methylnitrosourea causes dermatitis; however, it appears to be due to sensitization rather than primary irritation. Although carcinogenicity data are not available for humans, *N*-Nitroso-*N*-methylurea is reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in experimental animals.

Clinical Management

Affected persons should be decontaminated with caution because methylnitrosourea is probably carcinogenic to humans. Symptoms should be treated as they appear.

Ecotoxicology

Fish, mollusks, and phytoplankton are all adversely affected by methylnitrosourea.

See also: Chromosome Aberrations; Sensitivity Analysis; Sister Chromatid Exchanges; Skin.

Further Reading

International Agency for Research on Cancer (IARC) (1978) IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Some *N*-Nitroso Compounds. Vol. 17. 365 pp. Lyon, France.

International Agency for Research on Cancer (IARC) (1987) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Overall Evaluations of Carcinogenicity. Supplement 7. 440 pp. Lyon, France.

WHO Environmental Health Criteria 5: Nitrates and Nitroso Compounds, 1978.

Relevant Websites

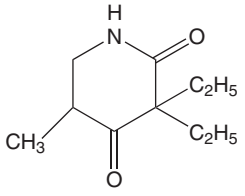
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Methylnitrosourea.

http://www.inchem.org – International Agency for Research on Cancer (IARC) – Summaries and Evaluations, *N*-nitroso-*N*-methylurea.

Methyprylon

S Rutherfoord Rose

- Chemical Abstracts Service Registry Number: CAS 125-64-4
- Synonyms: Methypryl; Methyprylone; 2,4-Dioxo-3,3-diethyl-5-methyl piperidine; 3,3-Diethyl-5-methyl-2,4-piperidinedine; 3,3-Diethyl-5-methyl-2,4-piperidinedione; Noludar; Noctan; Methyl-2,4-peperidine; 3,3-Diethyl-5-methyl-2,4-piperidinedione; Noludar; Noctan; Dimerin
- Chemical/Pharmaceutical/Other Class: Piperidinedione derivative
- Chemical Formula: $C_{10}H_{17}NO_2$
- Chemical Structure:



Uses

Methyprylon is a sedative–hypnotic agent that has been used in the treatment of anxiety, nervousness, and sleep disorders (insomnia). The drug was withdrawn from the US market in 1988. It was a commonly abused sedative–hypnotic agent.

Exposure Routes and Pathways

Methyprylon is only available in solid oral dosage forms. Poisoning occurs following ingestion.

Toxicokinetics

Following therapeutic doses, methyprylon is rapidly absorbed, with peak plasma levels occurring within 1 or 2 h. Greater than 95% of a dose undergoes oxidation and dehydrogenation in the liver; the remainder is excreted unchanged in the urine. At least two of four metabolites may have central nervous system (CNS) activity. The volume of distribution with therapeutic dosing is $\sim 1\,l\,kg^{-1}$. The extent of methyprylon binding to plasma proteins is unknown. Therapeutic plasma levels occur at $\sim 10\,mg\,l^{-1}$, with levels above $30\,mg\,l^{-1}$ considered toxic. The serum elimination half-life is ~ 3–5 h following therapeutic doses. The toxicokinetics of methyprylon are poorly understood. Both rates of absorption and elimination are prolonged following overdose. Pharmacokinetics appears to be dose dependent, and elimination becomes nonlinear at high doses.

Mechanism of Toxicity

Methyprylon produces depression of the CNS and decreases rapid eye movement sleep in a fashion similar to the barbiturates. Overdoses may also result in cardiovascular depression.

Acute and Short-Term Toxicity (or Exposure)

Human

Overdose results in dose-dependent CNS depression, ranging from mild lethargy to coma. Slurred speech, ataxia, nystagmus, headache, and gastrointestinal upset may occur. Paradoxical CNS stimulation (excitement) has been reported. Severe toxicity (ingestions exceeding 3 or 4 g) may produce hypotension, shock, or pulmonary edema. Death has occurred following ingestion of 6 g, and survival has occurred following ingestion of 30 g.

Chronic Toxicity (or Exposure)

Human

Chronic use may result in addiction, physical dependence, and withdrawal upon abrupt discontinuation of the drug.

Clinical Management

The basis of clinical management is supportive care. The airway should be secured and protected as needed. Symptomatic patients should have intravenous access and cardiac monitoring. Accidental ingestions exceeding 500–800 mg, and all intentional overdoses, should be treated with oral activated charcoal if patients present within 60 min of exposure. Seizures should be treated with benzodiazepines, or phenobarbital if refractory. Hypotension should be treated with intravenous fluids and vasopressors (dopamine or norepinephrine) if needed. Hemodialysis or hemoperfusion may enhance elimination of both the parent compound and metabolites, but the clinical value of extracorporeal drug removal is unknown. There is no known antidote.

Further Reading

Bailey DN and Shaw RF (1983) Interpretation of blood glutethimide, meprobamate, and methyprylon concentrations in nonfatal and fatal intoxications involving a single drug. *Journal of Toxicology. Clinical Toxicology* 20(2): 133–145.

Mandelbaum JM and Simon NM (1971) Severe methyprylon intoxication treated by hemodialysis. *Journal of the American Medical Association* 216: 139–140.

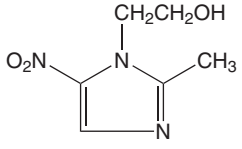
Reidt WU (1956) Fatal poisoning with methyprylon (Noludar), a nonbarbiturate sedative. *New England Journal of Medicine* 255: 231–232.

Metronidazole

David Eldridge and Christopher P Holstege

This article is a revision of the previous print edition article by Michael Shannon, volume 2, pp. 320–321, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 443-48-1
- Synonym: Flagyl
- Chemical/Pharmaceutical/Other Class: An antibiotic of the nitroimidazole class
- Chemical Structure:



Uses

Metronidazole is used as an antibiotic for certain bacterial and parasitic infections.

Exposure Routes and Pathways

Metronidazole is available as oral, topical, and parenteral preparations.

Toxicokinetics

Bioavailabilty of oral doses of metronidazole approaches 100% and peak serum concentrations occur within 1–2 h postingestion. The volume of distribution is $\sim 0.74\,l\,kg^{-1}$. Total protein binding is less than 20%. It readily crosses the placenta (contraindicated in the first trimester of pregnancy) and is excreted in breast milk at levels that approximate that of the serum concentration in the mother. Metronidazole is metabolized in the liver and undergoes biotransformation through hydroxylation, oxidation of side chains, and glucuronidation. Both unaltered metronidazole and its metabolites are excreted primarily by the kidney, although biliary excretion does occur. Metronidazole pigments may cause a dark brown discoloration of urine. The half-life of metronidazole is typically 8 h. The elimination half-life is unchanged except in patients with severe renal impairment or possibly in those with hepatic insufficiency.

Mechanism of Toxicity

Metronidazole possesses selective bactericidal and antiparasitic activity. Its mechanism of action is complex and not thoroughly understood but is thought to include interference with nucleic acid synthesis. Metronidazole is also capable of producing a disulfiram-type reaction with ethanol ingestion. This reaction is hypothesized to occur due to metronidazole inhibition of aldehyde dehydrogenase.

Acute and Short-Term Toxicity (or Exposure)

Animal

Metronidazole is also used as an antiparasitic in domestic animals. Manifestations of overdose in animals include vomiting, nystagmus, decreased appetite, and ataxia.

Human

There is no documented acute lethal dose in humans. Commonly seen acute effects include nausea, vomiting, and ataxia. When taken concurrently with ethanol, a disulfiram-type reaction can occur with nausea, flushing, hypotension, headache, and shortness of breath.

Chronic Toxicity (or Exposure)

Animal

Chronic feeding studies of rats and mice have demonstrated increased tumor development in treated animals compared to controls.

Human

With chronic toxicity, nausea, anorexia, headache, vomiting, and metallic taste in the mouth may occur. Chronic use has been associated with extremity numbness and parenthesis. Other neurological effects including insomnia, dizziness, and vertigo have been reported. White blood count suppression has been seen but is generally reversible.

Metronidazole use can lead to problematic drug interactions. It potentates the effects of oral anticoagulants and increases their physiologic effect. Acute psychosis and confusion has been reported in patients simultaneously receiving disulfiram and metronidazole. Its use can lead to elevated lithium and cyclosporine levels.

In Vitro Toxicity Data

Metronidazole produces inhibition of alcohol dehydrogenase *in vitro*.

Clinical Management

Clinical management of acute metronidazole ingestion is supportive. Administration of activated charcoal can be considered for patients with substantial ingestions that present within an hour of the exposure. There are no specific laboratory tests indicated for this isolated ingestion. With chronic metronidazole toxicity or toxicity resulting from interaction with other drugs, discontinuation of metronidazole is recommended. Supportive care is usually sufficient for the vast majority of patients.

See also: Disulfiram.

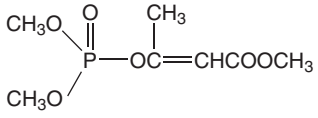
Further Reading

Kusumi RK, Plouffe JF, and Wyatt RH (1980) Central nervous system toxicity associated with metronidazole therapy. *Annals of Internal Medicine* 93: 59–60.

Mevinphos

Priya Raman*

- Chemical Abstracts Service Registry Number: CAS 7786-34-7
- Synonym: *O,O*-Dimethyl-1-carbomethoxy-1-propen-2-yl phosphate
- Chemical/Pharmaceutical/Other Class: Organophosphate pesticide
- Chemical Structure:



Uses

Mevinphos is used as a broad-spectrum insecticide for control of a variety of insects. It is also used as an acaricide that kills or controls mites and ticks. It acts quickly both as a contact as well as a systemic insecticide, being extremely effective at very low dosage rates.

Exposure Routes and Pathways

Oral and dermal routes are the most common routes of accidental and intentional exposure to mevinphos.

Toxicokinetics

Mevinphos is efficiently absorbed from the gut, through the skin, and across the pulmonary membrane. The compound is hydrolyzed in the body to alkyl phosphate. Mevinphos is rapidly degraded by the liver. Consequently, it is more toxic by peripheral exposure routes, such as dermal or intravenous, that bypass the liver.

*Clinical Management section prepared by Carey Pope.

Mechanism of Toxicity

The organophosphorus insecticide, mevinphos, exerts its acute toxicity by directly inhibiting the hydrolytic enzyme, acetylcholinesterase. This causes an increased accumulation of acetylcholine at the synaptic nerve terminals, thereby resulting in excessive stimulation of the cholinergic nerves. Recent studies have demonstrated that mevinphos intoxication may result from nitric oxide produced upon activation of the M2 subtype of muscarinic receptors by the progressive accumulation of acetylcholine.

Acute and Short-Term Toxicity (or Exposure)

Mevinphos is highly toxic through all routes of exposure, including ingestion, dermal absorption, and inhalation. Mevinphos poisoning affects the central nervous system (CNS), the cardiovascular system, the respiratory system, and the eyes.

Animal

Mevinphos has been reported to have an oral LD_{50} of 3–12 mg kg^{-1} in rats and 4–18 mg kg^{-1} in mice. It is highly toxic via the dermal route as well, with dermal LD_{50} value reported to be 4.2 mg kg^{-1} in rats and 40 mg kg^{-1} in mice. The 1 h LC_{50} for mevinphos was reported to be 0.125 mg l^{-1} in rats, indicating high inhalation toxicity. A 1 h exposure of rats to the above concentration of mevinphos resulted in acute pulmonary edema accompanied with changes in the structure or function of salivary glands.

Human

Effects of acute mevinphos exposure are similar to those due to exposure to other organophosphates. However, acute mevinphos toxicity may occur at much lower doses than with other organophosphates. Symptoms of acute exposure include numbness, tingling sensations, incoordination, headache, dizziness, tremor, nausea, abdominal cramps, sweating, blurred vision, respiratory depression, and slow heartbeat. Very high doses may result in unconsciousness, incontinence and convulsions, or fatality. The greatest occupational hazard of mevinphos is its absorption through the skin, lungs, and mucous membranes. Symptoms of mevinphos poisoning may appear as early as 15 min to 2 h following exposure to mevinphos. However, the onset of symptoms may be delayed for as long as 2 days. Persons with respiratory ailments, recent exposure to cholinesterase inhibitors, impaired cholinesterase production, or with liver malfunction are at increased risk from exposure to mevinphos.

An impairment of judgment in the ability to reason is an early and important symptom of mevinphos poisoning from dermal exposure. When inhaled, the first effects are usually respiratory and may include bloody or runny nose, coughing, chest discomfort, difficulty breathing, or shortness of breath. Following exposure by any route, systemic effects may begin within a few minutes or be delayed for up to 12 h. These may include pallor, nausea, vomiting, diarrhea, abdominal cramps, blurred vision, constriction or dilation of the eye pupils, etc. Severe poisoning will affect the CNS, producing incoordination, slurred speech, loss of reflexes, weakness, fatigue, involuntary muscle contracts, and eventually paralysis of the body extremities and respiratory muscles. Death may be caused by respiratory failure or cardiac arrest.

Chronic Toxicity (or Exposure)

Animal

The oral and dermal LD_{50} of mevinphos in male rats is 6.1 and 4.7 mg kg^{-1}, respectively. Following exposure to 50 ppm mevinphos for 60 days, rats showed reduced growth, slight tremors, and brain cholinesterase that was 20% of normal levels. Other signs and symptoms in rats following exposure to mevinphos include nonspecific degeneration of the liver and kidneys and degeneration of the epithelial cells lining ducts and acini of salivary, lacrimal, and other exocrine glands. Dogs exposed to mevinphos at a dietary level of 0.1 mg kg^{-1} day^{-1} for 14 weeks showed a reduction in both erythrocyte and plasma cholinesterase activity; however, the brain enzyme remained normal. Administration of 20 mg kg^{-1} day^{-1} of mevinphos to rats in their diets for 13 weeks resulted in death of the animals. Dietary doses of 10 mg kg^{-1} day^{-1} for 14 weeks proved to be lethal for dogs. Rats given dietary doses of 10 or 20 mg kg^{-1} day^{-1} for 13 weeks exhibited degeneration of the liver, kidney, and cells lining the salivary, tear, and other glands.

Human

The lowest oral dose of mevinphos responsible for toxic effects (peripheral nervous system effects) in humans was 690 μg kg^{-1} when given intermittently over 28 days. Repeated or prolonged low-level exposure to mevinphos may cause effects similar to those observed with acute exposure. Mevinphos is a compound of high toxicity, not only orally but also dermally. It is a direct inhibitor of

acetylcholinesterase. Signs and symptoms involving overstimulation of the muscarinic receptors include bronchoconstriction, increased bronchial secretion, bradycardia, salivation, lacrimation, diaphoresis, vomiting, diarrhea, and pupillary constriction (miosis). The nicotinic effects following exposure to mevinphos include tachycardia, hypertension, muscle fasciculations, weakness, muscle cramps, and respiratory paralysis. Excessive stimulation of the CNS receptors (both muscarinic and nicotinic) is responsible for some of the higher order symptoms such as anxiety, restlessness, CNS depression, agitation, confusion, delirium, coma, and seizures. Mevinphos-induced cholinesterase inhibition can persist for 2–6 weeks. Monitoring of cholinesterase levels through regular blood testing is highly recommended for individuals exposed to mevinphos.

Clinical Management

For exposure to eyes, eyelids should be held open and the eyes flushed with copious amounts of water for 15 min. For exposure to skin, affected areas should be washed immediately with soap and water. The victim should receive medical attention if irritation develops and persists.

For exposure through inhalation, the victim should be removed to fresh air and, if not breathing, given artificial ventilation. The victim should receive medical attention as soon as possible.

First aid for ingestion victims would be to induce vomiting, keeping in mind the possibility of aspiration of solvents. Gastric decontamination should be performed within 30 min of ingestion to be most effective. Initial management of acute toxicity is establishment and maintenance of adequate airway and ventilation. Atropine sulfate in conjunction with pralidoxime chloride can be administered as an antidote. Atropine by intravenous injection is the primary antidote in severe cases. Test injections of atropine (1 mg in adults and 0.15 mg kg^{-1} in children) are initially administered, followed by 2–4 mg (in adults) or 0.015–0.05 mg kg^{-1} (in children) every 10–15 min until cholinergic signs (e.g., diarrhea, salivation, and bronchial secretions) decrease. High doses of atropine over several injections may be necessary for effective control of cholinergic signs. If lavage is performed, endotracheal and/or esophageal control is suggested. At first signs of pulmonary edema, the patient should be placed in an oxygen tent and treated symptomatically.

Environmental Fate

Mevinphos does not readily adsorb to soil particles, having a soil half-life of 3 days. It is readily hydrolyzed resulting in loss of its insecticidal activity within 2–4 weeks.

Ecotoxicology

Mevinphos is highly toxic to birds, fish, and bees. Consequently, areas frequented by wildlife, including lakes or ponds inhabited by fish, should not be contaminated by use of mevinphos.

Exposure Standards and Guidelines

Mevinphos air concentration of 40 mg m^{-3} is immediately dangerous to life or health. The Occupational Safety and Health Administration (OSHA) time-weighted average (TWA) (skin), American Conference of Governmental Industrial Hygienists (ACGIH) TWA (skin) and National Institute for Occupational Safety and Health (NIOSH) recommended TWA (skin) for mevinphos is 0.1 mg m^{-3}. The OSHA short-term exposure limit (STEL), ACGIH STEL, and NIOSH recommended STEL for mevinphos is 0.3 mg m^{-3}. Based on a 2 year rat feeding study and a 10-fold safety factor, the acceptable daily intake (ADI) for mevinphos is 0.0025 mg kg^{-1} day^{-1}.

Miscellaneous

Pure mevinphos is a colorless liquid with a very mild odor or is practically odorless. Mevinphos has a molecular weight of 224.15 and a specific gravity of 1.25. It is completely miscible with water, alcohols, ketones, chlorinated hydrocarbons, aromatic hydrocarbons, and most organic solvents. Mevinphos has a melting point of 21°C (*cis*-isomer), 6.9°C (*trans*-isomer), and a boiling point of 99–103°C at 0.03 mmHg. Some commonly used trade names of mevinphos are Phosfene, Phosdrin, Mevinox, Meniphos, Fosdrin, and Apavinphos.

See also: Cholinesterase Inhibition; Neurotoxicity; Organophosphates; Pesticides.

Further Reading

Cochran RC, Formoli TA, Silva MH, Kellner TP, Lewis CM, and Pfeifer KF (1996) Risks from occupational and dietary exposure to mevinphos. *Reviews of Environmental Contamination and Toxicology* 146: 1–24.

Microarray Analysis

Kartik Shankar and Harihara M Mehendale

The genetic information stored in the DNA is first transcribed into message called RNA, which is further translated into proteins that carry on the various functions of living cells. Monitoring changes in messenger RNA (mRNA) in cells has been used as an indirect measure to characterize changes in proteins. Gene expression changes have been traditionally monitored using Northern blot analyses and more recently by reverse-transcriptase polymerase chain reaction (PCR) and Rnase protection assays. DNA microarrays provide an unprecedented and revolutionary platform to perform genome-wide expression analyses. There are basically two types of DNA microarrays: oligonucleotide-based arrays and cDNA arrays. Oligonucleotide arrays are made either by spotting prefabricated oligonucleotides or by using specific chemical synthesis steps using photolithographic mask, light, or other methods to generate the sequence order in the synthesis. This results in the formation of high-density short oligonucleotide probes that are synthesized at specific predefined positions. cDNA microarrays, on the other hand, can be used to study differences in gene expression between a control and experimental group. Partial cDNAs (500–2000 bp) that correspond to unique gene sequences are spotted onto surfaces of treated glass using a high-speed robotic printer. Spotted cDNAs represent either known genes or collections of partially sequenced cDNA derived from expressed sequence tags (ESTs) corresponding to mRNAs of genes of unknown function. It is possible to print greater than 25 000 cDNAs on a single microarray.

A standard microarray experiment using a cDNA microarray is summarized in **Figure 1**. RNA from either experimental or control (tissue or cells) sample is extracted. Fluorescent cDNA probes are generated from control and experimental RNA samples in a single round of reverse-transcription in the presence of fluorescently labeled dUTP, such that control and test products are labeled with different fluorescent labels (e.g., control Cy3-dUTP and test is Cy5-dUTP). The labeled cDNAs from both control and test groups are then mixed and hybridized to a glass microarray. The fluorescent signal is detected using a scanning confocal microscope equipped with lasers for fluorescence excitation. This method eliminates the need for a separate hybridization for control samples. The data acquired are generally represented as ratios of the fluorescent signals (Cy3/Cy5). The methodology for analyzing gene expression via

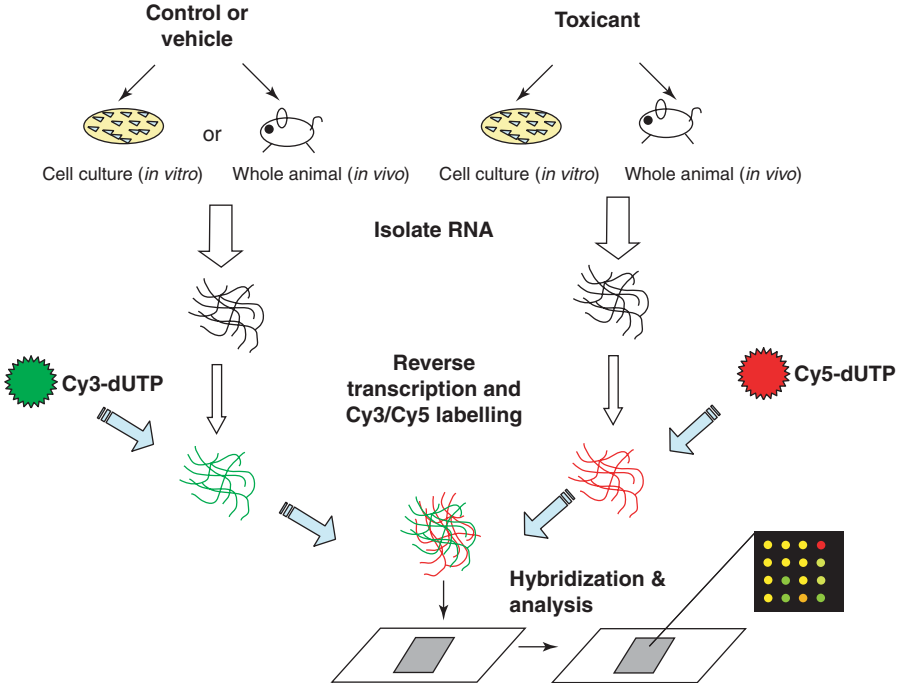


Figure 1 Schematic of a standard microarray experiment using a cDNA microarray.

cDNA arrays and oligonucleotide arrays is different. In case of oligonucleotide microarrays sample preparation involves generation of ds-cDNA from either total RNA or poly(A) + RNA followed by antisense RNA synthesis in an *in vitro* transcription reaction with biotinylated or fluortagged nucleotides. The RNA probe is then fragmented to facilitate hybridization and visualization done using a confocal scanner. Data generated via microarray analyses is difficult to analyze without the utilization of computational tools that handle large volumes of data. A large variety of bioinformatic software is now available both as free and commercial software for data acquisition and analysis.

See also: Bioinformatics; Genomics, Toxicogenomics.

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Micronucleus Assay

Robin C Guy

Background Information

The micronucleus assay is an *in vivo* or *in vitro* assay for the detection of chromosome damage. An *in vitro* micronucleus test with mammalian cell culture and a modified *Salmonella* reverse mutation (Ames) assay are frequently used for screening purposes early in the development and evaluation of a new chemical. Further, the micronucleus assay is one of the three assays that usually constitute the minimum test battery satisfying global regulatory requirements for evaluation of mutagenic potential. This set includes an *in vivo* rodent bone marrow micronucleus test, a bacterial reverse mutation assay, and an *in vitro* cytogenetic test with mammalian cell culture, or an *in vitro* gene mutation assay in mammalian cell cultures; supplementary studies might be conducted as a follow-up to the findings from this initial testing battery and/or to satisfy a regulatory requirement. *In vivo* assays have an advantage over *in vitro* assays, as metabolism and other physiological functions and interactions are able to occur as part of the study.

Reticulocytes, or polychromatic erythrocytes, are newly formed, immature red blood cells. These are larger than red blood cells and have retained some ribosomal RNA. These cells are detectable from normochromatic erythrocytes, mature erythrocytes that lack ribosomes, by their staining properties. Micronuclei are small nuclei, separate from and additional to the main nuclei of the cell. Micronuclei are formed due to breakage of chromatin or chromosomes, from spindle fiber or chromosome abnormalities, or from an entire chromosome that may have lagged behind in anaphase. After an insult that would increase the frequency of micronuclei, micronucleated polychromatic erythrocyte levels would increase at ~10–12 h and remain elevated for 20 h, with a possible twofold increase at 24 h. Micronucleated polychromatic erythrocyte levels may take longer to be at their peak, if there is a mitotic delay or slower uptake of the material or a metabolite due to metabolism. Therefore, it is prudent to have dose groups sacrificed over more than 1 day.

The *in vitro* assay primarily utilizes human lymphocytes, but other mammalian cells have been used. One technique incorporates a cytokinesis-block technique. Cytoplasmic division is inhibited, thereby resulting in binucleated and multinucleated cells.

In the *in vivo* assay, animals are dosed with the test materials by the appropriate route, and subsequently cells are collected for cytogenic analyses. The primary animal species for these tests are mice and rats. Chinese hamsters are also used for this assay; however, the US Food and Drug Administration (FDA) *Redbook* requires justification for any other species used besides the mouse and rat.

Rodents are typically administered a single dose of the test material. To obtain micronucleus information from a subgroup of a larger, repeated daily dose toxicity study lasting over 4 weeks, the ratio of micronucleated mature (normochromatic) erythrocytes in the peripheral blood to mature erythrocytes are determined. As long as there is no proof that the test material or a metabolite cannot act on the bone marrow, this assay may be used.

The US Environmental Protection Agency (EPA) and the US FDA *Redbook* state that there should be five analyzable animals per sex per group. The International Conference on Harmonisation (ICH) guidelines recommended by the FDA's Center for Drug Evaluation and Research state males are the most sensitive gender and that unless there are obvious metabolism differences between male and females, or that a gender-specific material is to be tested, males should be used. Gender differences have been shown in at least one experiment, so studies utilizing different genders are important.

The EPA and FDA state that there is no standard treatment schedule; therefore, one, two, or more doses every 24 h is acceptable as long as toxicity has been demonstrated, or that a limit dose has been achieved. In addition, if a large volume of material needs to be administered to the rodents, it may be administered as a divided dose, as long as the doses are not separated by more than a few hours. Doses may be selected from the results of a range finding study, or any preliminary tests done with the rodents after administration via the same route. The highest dose selected for the main assay would produce some bone marrow toxicity, including, in the bone marrow or peripheral blood, a decreased number of immature erythrocytes to total erythrocytes. In the case of a nontoxic test article, the limit dose is defined as $2000\,\mathrm{mg\,kg^{-1}}$.

Negative (solvent) and positive controls must be utilized for a valid study. A historical database must be maintained for these results. A positive control must produce a noticeable increase in micronucleated cells as compared to the solvent controls. Examples of positive control substances include:

- Ethyl methane sulfonate (CAS 62-50-0).
- Ethyl nitrosourea (CAS 759-73-9).
- Mitomycin C (CAS 50-07-7).
- Cyclophosphamide (CAS 50-18-0 (monohydrate form: CAS 6055-19-2)).
- Triethylenemelamine (CAS 51-18-3).

After sacrifice, bone marrow cells are extracted from femurs or tibias, prepared and placed on slides, and then stained for microscopic evaluation. When peripheral blood is used, the blood is collected at appropriate times after treatment and smear preparations are made and stained. If using peripheral blood, care should be taken to ensure that the species selected for study had a spleen that cannot remove micronucleated erythrocytes. The mouse was the species of choice for the measurement of micronucleated immature (polychromatic) erythrocytes in peripheral blood, but the rat has also been shown to have good results in this test.

EPA and the FDA's Center for Food Safety and Applied Nutrition toxicology study guidance and requirements (*Redbook* 2000) state that the proportion of immature erythrocytes among the total erythrocytes for each animal is determined by counting at least 200 erythrocytes for bone marrow and 1000 erythrocytes for peripheral blood. At least 2000 immature erythrocytes per animal should be scored for a micronucleated immature erythrocyte count. Statistical studies have been performed that show between 4000 and 5000 polychromatic erythrocytes are needed to detect a doubling of spontaneous frequencies that fall into the 1–3% range. Micronuclei may also be counted in the mature erythrocytes.

Many compounds that are positive in the micronucleus assay are mammalian carcinogens; however, there is no exact correlation between the micronucleus assay and carcinogenicity. Correlation may be dependent on chemical class.

The micronucleus assay is one type of study recommended by the FDA *Redbook* and ICH guidelines as part of a standard genetic toxicology battery. The other assays include the Ames (bacterial reverse mutation) and mouse lymphoma tests.

To ensure that the results of an assay are valid, specific criteria have been determined. The frequency of micronucleated cells need to be within the normal historical control range. The frequency of micronucleated cells in the positive controls need to be significantly increased over the vehicle control. In addition, there must be five surviving animals per group.

There are several criteria for determining a positive result, such as a dose-related increase in the number of micronucleated cells, or a statistical, repeatable increase in the number of micronucleated cells in a single dose group at a single sampling time. Biological relevance of the results should be considered even if there is statistical significance. Negative results indicate that the test substance does not produce chromosomal or spindle damage leading to the formation of micronuclei in the immature erythrocytes of the test species. An equivocal study may result from only one group with a statistically significant increase and should be elucidated by further testing, preferably using a modification of experimental conditions.

See also: Ames Test; Federal Insecticide, Fungicide, and Rodenticide Act, US; Food and Drug Administration, US; Good Laboratory Practices (GLP); International Conference on Harmonisation; Micronucleus Assay; Genetic Toxicology; Toxicity Testing, Mutagenicity; Redbook.

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Microtox

Gary P Bond and John Martin

Published by Elsevier Inc.

Overview

Microtox is an *in vitro* test system which uses bioluminescent bacteria for the detection of toxicity. It is used as a screening system to provide an indication of the relative toxicity of a sample. Applications which have been published include the testing of air, samples containing biological toxins, industrial effluent, industrial process waters, municipal effluent, drinking water, ecotoxicological samples, hazardous waste, soil, sediments, storm water, and medical products for bioreactivity, among others. The Microtox test provides both acute toxicity and genotoxic analyses. (Microtox is a registered trademark of Strategic Diagnostics, Inc., Newark, DE.)

The Microtox test system utilizes a strain of naturally occurring luminescent bacteria – *Vibrio fischeri*. Exposure to a toxic substance causes a disruption of the respiratory process of the bacteria resulting in reduced light output. The effective concentration (EC_{50}) is determined as the concentration of a toxicant that causes a 50% reduction in light output over a prescribed period of time (typically 5, 15, or 30 min). The test is fast, fairly simple to conduct, uses small sample sizes, and is relatively inexpensive. Results correlate well with those from other toxicity bioassays such as fish and *Daphnia*. The test is used extensively in the measurement of toxicity of effluent and drinking waters, as well as an early screening tool for relative toxicity as part of a test battery.

Principle of Operation

Vibrio fischeri are nonpathogenic, marine, luminescent bacteria which are sensitive to a wide range of toxicants. The organisms are supplied for use in a standard freeze-dried (lyophilized) state, which serves to maintain the sensitivity and stability of the test organisms. Disruption of the respiratory process, by exposure to a toxicant affects the metabolic pathway that converts chemical energy via the electron transfer system of the bacteria to visible light. The process occurs in and close to the cell membrane so target sites are close to the cell surface. Each test utilizes approximately 1 million organisms and each organism is less than 1 μm in diameter, so a very high surface to volume ratio is presented. Sensitivity and statistical significance are therefore high; the response being an integrated effect of the toxicant on the entire population, which is very large.

The Microtox equipment includes a self-calibrating analyzer which incorporates a photomultiplier tube, a data collection and reduction system, and software. The temperature-controlled analyzer maintains the test organisms and samples at a standard temperature of 15°C. It also detects the light intensity at 490 nm, the wavelength emitted by the bacteria.

Advantages

The Microtox test has many advantages over other bioassays which makes it a useful tool in monitoring programs and screening studies. These include cost-effectiveness, simplicity, rapidity, precision, statistical significance, and the requirement for a small sample volume. The Microtox test is one of the least volume-intensive toxicity assays, using only 2.5 ml of sample per test. The rapidity of the test means that real-time data can be generated as part of a monitoring program, allowing fast corrective actions to be taken. Acute test protocols take ~45 min from start to finish when conducted by a trained operator (genotoxic protocols are conducted in 16, 20, and 24 h periods). Twenty-nine individual samples may be tested at one time for determination of relative toxicity or three complete serial dilution bioassays can be run simultaneously to provide effect concentrations.

Use as Part of a Test Battery and Correlation with Other Bioassays

No single test can ensure the detection of all toxic effects in a complex mixture. A test battery should have good sensitivity to a broad range of toxicants, while possessing unique characteristics making it a useful detector of certain types of toxicity. The Microtox test has been extensively evaluated for its correlation with other bioassays. Regression analyses of the inhibitory effects of chemicals on *Vibrio fischeri* with their acute toxicities to a variety of aquatic species show a high degree of collinearity over several orders of magnitude. Species tested include, *Daphnia magna* (water flea), *Pimephales promelas* (fathead minnow), *Leuciscus idus melanotus* (goldorfe), *Poecilia reticulata* (guppy), *Lepomis macrochirus* (bluegill sunfish), *Ictalurus punctatus* (channel catfish), *Carassius auratus* (goldfish), *Gambusia affinis* (mosquito fish), *Salmo trutta* (brown trout), *Cyprinodon variegatus* (sheepshead minnow), and *Oncorhynchus mykiss* (rainbow trout). Additional studies have also shown significant relationships between the Microtox EC_{50} and rat and mouse LD_{50} values.

Effluent test results support the use of Microtox as part of a test battery. Uniform response of sublethal effects to 50 effluents from pulp and paper mills was obtained with the Microtox test compared to the results with fish and algae, which varied greatly with age and genetic variation within the population. Microtox has also been used to detect other sublethal effects (e.g., chronic toxicity). For example, the bacterial response can be used to quantify the stress on the immunological defense systems of mussels exposed to toxins in polluted rivers or wastewaters. In this case, the bacteria are exposed to hematocytes extracted from the mussels for 30 min at standard temperature. The phagocytic activity of the hematocytes on the bacteria decreases bioluminescence by a quantifiable amount.

While it alone cannot substitute for acute or sublethal hazard assessment which covers all species, Microtox can be useful in a battery of screening tests or to supplement data obtained in other toxicity bioassays.

Regulatory Status

The Microtox test is recognized as an approved protocol or under consideration for acceptance by the regulatory agencies of many nations including the United States, Canada, and countries from the European Union, South America, and the Pacific Rim. The US Environmental Protection Agency has specifically referred to Microtox as an inexpensive tool to test effluent toxicity. Test protocols involving Microtox have been approved by the International Standards Organization, American Society for Testing and Materials, the Organization for Economic Cooperation and Development (OECD) and are included in Standard Methods for the Examination of Water and Wastewater ('Standard Methods').

See also: Biomonitoring; Effluent Biomonitoring; *In Vitro* Test; Toxicity Testing, Aquatic; Toxicity Testing, Mutagenicity.

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Relevant Websites

http://caat.jhsph.edu – John Hopkins University Center for Alternatives to Animal Testing Workshop that includes Microtox as a valuable ecotoxicology test.

http://www.sdix.com – Microtox is registered trademark of Strategic Diagnostics, Inc.

http://www.wrrc.hawaii.edu – Honolulu Board of Water Supply proposed use of Microtox for first tier monitoring in event of contamination as a result of terrorism.

Minamata

Stephen C Bondy

History

The Chisso Corporation of Japan used inorganic mercury as a catalyst in the production of acetaldehyde. A fraction of this mercury was discharged within waste water into Minamata Bay. This contamination occurred between 1932 and 1968, with an estimated total of 150 tons of mercury delivered to the bay. This led to a gradual but severe poisoning of a considerable fraction of the 50 000 people living in a town adjacent to the bay, where the primary industry was fishing. Concentrations of mercury in the fish (such as mullet, sea beam, rockfish, and mackerel) were found to be over 10 ppm mercury.

Around 900 people died as a result of mercury poisoning and over 3000 were severely affected. Hair concentrations as high as 705 ppm were present in some victims. It is likely that well over 15 000 people were adversely affected by ingestion of mercurials within fish. Symptoms were both neurological and systemic. In adults, the lowest levels affected individuals by causing parasthesia. Symptoms resulting from higher exposures led to other sensory disorders such as pain, paralysis, headaches, tremors, and also deficits in hearing, vision (concentric visual field constriction and blindness), and speech. In addition, motor deficits such as poor coordination were manifested. Postmortem pathological findings revealed cerebellar atrophy and enlarged cerebellar folia. The cerebellar granule cell population was most affected. Diffuse cortical and subcortical atrophy with gliosis and macrophage infiltration was also apparent. Hypothalamus and substantia nigra were relatively unaffected.

Fetal damage following intrauterine poisoning was especially pronounced. Affected infants were born with both physical malformations and profound mental retardation. Symptoms resembled cerebral palsy. Several infants were born completely blind. Pathology in these cases involved the cerebral hemispheres to a major extent and microcephaly was found in several instances. Cerebral cortices were not only underdeveloped but their convolutions were also narrowed. The corresponding maternal toxicity in these cases was minimal and rapidly reversible. The observable postnatal effects at the lowest levels of fetal exposure, was psychomotor retardation expressed as delayed behavioral development, often after a latent period of apparently normal maturation.

After an extended period of controversy, some corporate liability was admitted and limited compensation was paid to victims of this exposure. Discharge into the bay was stopped, and dredging was commenced to recover mercurials in the sediment at the bottom of the bay. A net was placed across the bay to prevent exodus of contaminated fish. This net was removed in 1997 and fishing was again allowed, since mercury levels in fish were found to be below 0.4 ppm and this was deemed safe.

Commentary

The Minamata Bay tragedy, whereby many inhabitants of the region died or were permanently injured as a result of methylmercury poisoning is a classic example of a harmful neurotoxic exposure. A series of unforeseen circumstances combined to make this a very severe outbreak with long lasting consequences. Although the harmfulness of methylmercury had long been known, the severity of the toxic exposures involved can be attributed to several factors:

1. The environmental fate of mercury was incompletely understood
   a. *Assumed:* The mercurial wastes that were discharged into the bay were not considered potentially harmful since they contained relatively low concentrations of much less toxic inorganic mercury, and it was believed that tidal action would be sufficient to flush the bay and prevent the accumulation of excess mercury.
   b. *Unanticipated:* The bacterial conversion of a large fraction of effluent mercury to an organic form led to an extensive bioaccumulation of methylmercury within the food chain. The consumption of microorganisms including phytoplankton, was followed by their consumption by zooplankton, which in turn were the prey of marine invertebrates and other plankton filtering species. The progression up the food chain then proceeded by ingestion of contaminated animals by small fish and ultimately larger species of fish which provided a nutritional source for humans. This led to extensive contamination of an important food source for inhabitants living in fishing villages around the bay.
2. The cause of the problem was initially misidentified
   a. *Assumed:* Signs of toxicity had a gradual onset and occurred over an extended period. This

occluded the association between the development of neurological disease and methylmercury for a considerable time. When an epidemic of severe neurological disorders, especially associated with newborn infants, became apparent, an epidemiological study revealed the derangement to be confined to low lying marshy areas around the bay. Since drier areas at higher altitudes and more removed from the bay were much less affected, an insect-borne disease of viral origin was suspected.
b. *Unanticipated:* Some clues that might have helped make fish suspect as disease vectors, were ignored. Fish in the preceding years had become rather sluggish and easier to catch than usual. Abnormal hyperreactive behavior in domestic cats which lived primarily on fish wastes, were humorously noted but the critical association was not made. Smaller mammals have higher metabolic rates so that these changes may have preceded signs of human toxicity.

3. Assumptions about recovery were incorrect being based on a different exposure scenario
   a. *Assumed:* The extent of recovery from methylmercury poisoning can be quite marked in cases of more acute poisoning that occurred in Iraq in 1971 following ingestion of grain treated with methylmercury as a fungicide. These doses were high and thus signs of intoxication arose shortly after exposure. The time frame of any recovery was relatively short in such circumstances.
   b. *Unanticipated:* The very extended low-level exposure to methylmercury following fish consumption led to a slow development of symptoms. The recovery from this exposure scenario was very gradual and often very limited. Any partial improvement observed took place over an extended time. Thousands of victims still remain handicapped by the exposure.
4. Appropriate warnings and other actions to protect public health were delayed
   a. *Assumed:* Safeguarding the welfare of the general population is a primary responsibility of government. It also a duty of industrial enterprises to protect its workers and other individuals living adjacent to manufacturing sites. Thus, when evidence for a causal relation between an industrial product and an adverse effect on the environment is noted, prompt action to mitigate the hazard, taken by both industry and the state, is necessary and to be expected.
   b. *Unanticipated:* There was a great reluctance on the part of both the Chisso Corporation and the Japanese governmental authorities to acknowledge the relation between mercurials in Minamata Bay and the prevalent and serious disease outbreak within the town. This delay, in part due to misplaced traditional concepts of loyalty, greatly expanded the scope of the outbreak. For example, the fishermen of Minamata were at one point allowed to eat but not to sell suspect fish. This regulation, apparently an attempt by government to absolve itself of responsibility, led to increased local fish consumption by inhabitants of Minamata and worsened the condition of the most high-risk families.
5. The magnitude of the outbreak was underestimated
   a. *Assumed:* Upon investigation by medical authorities, large number of exposed victims were found to exhibit marked neurological deficits. It was recognized that there might be a larger number of people who might experience subclinical deficits and that these would be difficult to locate.
   b. *Unanticipated:* A significant number of the exposed population initially exhibited either no or very minor symptoms, but experienced a progressive worsening of their health. It was not understood that a lesser exposure to methyl mercury could lead to delayed onset of toxicity. Thus, in some cases toxicity could first be manifested many years after cessation of exposure. This has now been confirmed in animal models where exposure of young animals to methylmercury, was found to lead to behavioral deficits that were only apparent as the animal aged. This is a classic example of a prolonged period of asymptomatic latency following toxic exposure.

See also: Methylmercury; Pollution, Water.

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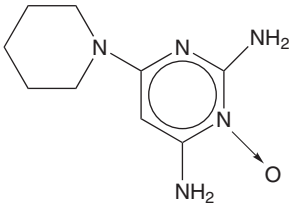
Minoxidil

Elizabeth J Scharman

- Chemical Abstracts Service Registry Number: CAS 38304-91-5
- Synonyms: 2,6-Diamino-4-piperidinopyrimidine; 6-(1-Piperidinyl)-2,4-pyrimidinediamine-3-oxide; Rogaine®; Loniten®
- Chemical/Pharmaceutical/Other Class: Hypotensive agent; Vasodilating agent
- Chemical Formula: $C_9H_{15}N_5O$
- Chemical Structure:



Uses

Minoxidil is used for severe hypertension, and used when the patient is symptomatic or when end-organ damage is present. Minoxidil is also used topically to stimulate hair regrowth in patients with alopecia androgenetica (male pattern baldness).

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposures to minoxidil. Toxicity may also occur via dermal exposure. Minoxidil is available in an oral dosage form and in a 2% or 5% topical formulation.

Toxicokinetics

Minoxidil, given orally, is almost completely absorbed ($\geqslant$90%). Peak plasma concentrations are reached within 1 h and fall rapidly afterwards. Pharmacodynamic effects do not mirror the drug's pharmacokinetics. With administration of a single dose, hypotensive effects begin within 30 min, peak within 2–8 h, and may continue for 75 h. Following oral administration, ~90% of the drug is metabolized, primarily by glucuronidation. The metabolite, minoxidil-*o*-glucuronide, is active but is not as active as the parent drug. The metabolite, but not the parent drug, may accumulate when renal impairment is present. Minoxidil is widely distributed with a volume of distribution of 2–3 l kg^{-1}. Minoxidil is found in breast milk; it is not protein bound. Dialysis and peritoneal dialysis will remove minoxidil. The half-life is 4.2 h. Percutaneous absorption is low through intact skin (average $<$2% of the applied dose).

Mechanism of Toxicity

Minoxidil acts as a direct vasodilator of vascular smooth muscle which decreases peripheral vascular resistance and blood pressure. Veins are affected to a lesser extent than arterioles. The resulting hypotensive effect induces a reflex increase in heart rate, cardiac output, and stroke volume. Sodium and water retention also occur leading to edema; plasma renin activity is increased.

Acute and Short-Term Toxicity (or Exposure)

Human

Experience with acute toxicity is limited. A minimum toxic dose is not defined. Headache, dizziness, hypotension, tachycardia, sodium and water retention, and cardiac dysrhythmias may be seen following overdose. Severe hypotension may result in myocardial ischemia; other end-organs may also be affected.

Chronic Toxicity (or Exposure)

Animal

Chronic therapy in animals has caused cardiac hypertrophy, cardiac dilation, and epicarditis. The characteristic lesion in rats and dogs is focal necrosis of the papillary muscle and subendocardial areas of the left ventricle.

Human

Side effects associated with chronic therapy include tachycardia, sodium and water retention, and hypertrichosis. Sodium and water retention can cause complications secondary to the expanded fluid volume; for example, congestive heart failure. Pericardial effusion has been documented in 3% of patients. Topical therapy may cause contact dermatitis.

In Vitro Toxicity Data

Minoxidil has not been shown to be mutagenic in a variety of studies including the Ames *Salmonella*

assay, DNA damage alkaline elution assay, or mouse bone marrow micronucleus assay.

Clinical Management

Activated charcoal will adsorb minoxidil following recent oral ingestions. Serum levels are not readily available and do not guide treatment. Standard supportive therapies, such as vasopressors, should be utilized as clinically necessary; however, epinephrine and norepinephrine should be avoided. Hemodialysis is of theoretical value if standard therapies fail; however, clinical experience is unavailable to support use.

See also: Ames Test.

Further Reading

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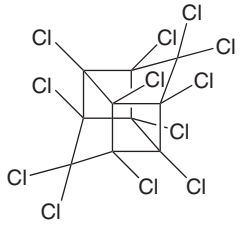
Mirex

Carey N Pope

- Chemical Abstracts Service Registry Number: CAS 2385-85-5
- Synonyms: GC-1283; Dechlorane
- Chemical/Pharmaceutical/Other Class: Chlorinated hydrocarbon insecticide
- Chemical Formula: $C_{10}Cl_2$
- Chemical Structure:



Uses

Mirex has not been manufactured or used in the United States since 1978. Mirex was used to control fire ants, and as a flame retardant from 1959 to 1972.

Exposure Routes and Pathways

Exposure to mirex occurs primarily from direct contact with contaminated soil or from consumption of contaminated food (e.g., fish). Mirex has been found in at least seven sites on US Environmental Protection Agency's National Priorities List of contaminated waste sites. Thus, since the use of mirex was eliminated in the United States in 1978, exposure would be most likely around contaminated waste sites. Mirex may still be used in other countries, however, leading to more exposure in the general population.

Toxicokinetics

Mirex is poorly absorbed from the oral tract. About half of an oral dose in rats was recovered unchanged in the feces. After an initial relatively rapid rate of excretion, the half-life was estimated at 100 days. Storage in tissues is high, often failing to reach a plateau with long-term exposures. To a very limited degree, some mirex is converted into 2,8-dihydromirex and 5,10-dihydromirex. Mirex is excreted unchanged in milk, thus posing a hazard for nursing infants. Approximately 90% of mirex residues are in fat.

Mechanism of Toxicity

Mirex is a potent microsomal enzyme inducer. Mirex may inhibit Na,K-ATPase and interfere with energy production and utilization. Mirex is also a nonphorbol ester tumor promoter.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} in rats ranged from 600 to $2000\,mg\,kg^{-1}$. The acute oral LD_{50} in dogs was $>1\,g\,kg^{-1}$. A single dose of mirex in rats was reported to elicit hepatic centrilobular hypertrophy with a marked increase (twofold) in liver weight. High levels of mirex can affect the stomach, intestine, liver, kidneys, eyes, thyroid, and nervous and reproductive systems. Multiple doses over a number of days appear to elicit more extensive toxicity.

Human

There is little information on effects of acute exposure to mirex in humans.

Chronic Toxicity (or Exposure)

Animal

Mirex is more toxic with repeated dosing. Lethality was increased in adult male rats at dosages as low as $5\,mg\,kg^{-1}\,day^{-1}$ for 30 days and in adult females at dosages as low as $6.2\,mg\,kg^{-1}\,day^{-1}$ for 90 days. In mice, 100% mortality occurred following exposure at $1.3\,mg\,kg^{-1}\,day^{-1}$ for 60 days. Dietary mirex for 148 days in rats caused reduced motor activity, irritability, and tremors. Similar dosing led to decreased litter sizes and decreased mating in rats. A number of studies reported cataract formation in offspring from reproductive toxicity studies. Dietary exposure with mirex ($2\,mg\,kg^{-1}\,day^{-1}$ for 3 months) led to impaired reproductive performance in male mice.

Human

There are no reports of chronic toxicity in humans with mirex exposure. On the basis of findings in animal studies, mirex should be considered a potential carcinogen for humans.

Environmental Fate

Mirex degrades slowly in the environment. Residues can remain in soil and water for years. Mirex does not evaporate to any degree from surface water or soil. Mirex is practically insoluble in water, but adsorbs to soil and sediment. Mirex does not appreciably leach into groundwater. Bioaccumulation in fish and other aquatic organisms and animals that eat these organisms is possible.

Ecotoxicology

Mirex is highly toxic to a number of aquatic organisms with crustaceans including commercially important species of shrimps and crabs being particularly sensitive.

Further Reading

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Hayes WJ (1982) Chlorinated hydrocarbon insecticides. In *Pesticides Studied in Man*, pp. 172–283. Baltimore, MD: Williams and Wilkins.

Relevant Websites

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Mirex.

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Mirex.

http://www.inchem.org – International Programme on Chemical Safety.

Mistletoe

Christopher P Holstege

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- SYNONYMS: American mistletoe: *Phoradendron flavescens*, *Phoradendron juniperinum*, *Phoradendron leucarpum*, *Phoradendron macrophyllum*, *Phoradendron serotinum*, *Phoradendron tomentosum*; European mistletoe: *Viscum album*

Uses

Mistletoe has been advocated for the treatment of hypertension, cancer, muscular spasms, arthritis, and as an abortifacient. It is also widely used in Christmas decorations.

Background Information

The term mistletoe is used for a number of different parasitic plants from the Genus *Phoradendron* and *Viscum*. It is a semiparasitic shrub with ovate, opposite leaves. It is found growing on trees, especially oaks. The berries grow in grapelike clusters and are typically white, round, and translucent.

Exposure Routes and Pathways

All parts of the plant may cause toxicity when ingested, with berry ingestion being the most common

source of exposure. Exposure may also occur by herbal extracts derived from mistletoe.

Mechanism of Toxicity

Viscum contain lectins that are cytotoxic by inhibiting protein synthesis on the ribosomal level in a manner similar to the toxalbumins ricin and abrin. Viscotoxin and phoratoxin are cardiac toxins and vasoconstrictors. Both produced reflex bradycardia, negative inotropic effects, and, in high doses, vasoconstriction of skin and skeletal muscle vessels in animals.

Acute and Short-Term Toxicity (or Exposure)

Human

Reported human toxicity occurs from either ingestion of the plant itself or from ingestion of a herbal remedy derived from the mistletoe. Ingestion of the plant, most commonly the berries, may be associated with the development of gastrointestinal distress consisting of nausea, vomiting, abdominal cramps, and diarrhea. Mistletoe berry exposures most commonly occur in children during the Christmas season; development of symptoms is rare.

Rare cases of cardiovascular collapse have been reported. It is unclear if this effect is due to direct cardiotoxicity or due to hypovolemic shock caused by protracted vomiting and diarrhea. Rare cases of central nervous system effects have also been reported and include drowsiness, ataxia, and seizures.

Chronic Toxicity (or Exposure)

Human

Extracts of *Viscum album* have been used as a traditional remedy for diabetes.

In Vitro Toxicity Data

Extracts of mistletoe have demonstrated antitumor qualities in numerous tumor cell lines.

Clinical Management

The mainstay of treatment is supportive care. Fluid and electrolyte replacement may be needed in patients manifesting gastrointestinal signs and symptoms. Seizures can be managed with common anticonvulsant therapy. Pregnant patients should be monitored for premature uterine contractions.

See also: Plants, Poisonous.

Further Reading

Krenzelok EP, Jacobsen TD, and Aronis J (1997) American mistletoe exposures. *American Journal of Emergency Medicine* 15: 516–520.

Mithramycin

Christopher P Holstege

- Chemical Abstracts Service Registry Number: CAS 18378-89-7
- Synonyms: Aurelic acid; Aureolic acid; Mithracin; Mithramycin A; Mitramycin; Plicamycin
- Chemical/Pharmaceutical/Other Class: Antineoplastic

Uses

Mithramycin is used as a DNA binding fluorescent dye, as an antineoplastic agent, and to reduce hypercalcemia, especially due to malignancies. Mithramycin is a potent inducer of fetal hemoglobin production in erythroid cells and is being investigated to alleviate the symptoms underlying beta-thalassemia and sickle cell anemia.

Exposure Routes and Pathways

Intravenous infusion is the only reported exposure pathway.

Toxicokinetics

Mithramycin peak levels are achieved immediately through the intravenous route. It is rapidly cleared from the blood within the first 2 h. Nearly 70% is excreted in the urine within the first 4 h and over 90% is recovered within the first 24 h. There is no evidence of protein binding.

Mechanism of Toxicity

Mithramycin inhibits RNA and protein synthesis by adhering to DNA. Mithramycin appears to effect bone resorption by stimulating osteoclast activity and results in hypocalcemia and hypophosphatemia.

Acute and Short-Term Toxicity (or Exposure)

Human

Mithramycin is toxic to bone marrow, liver, and kidneys.

Chronic Toxicity (or Exposure)

Animal

Mithromycin is occasionally used in veterinary practice as an antineoplastic. Expected toxicities are related to bone marrow suppression and bleeding.

Human

Mithromycin produces hemorrhagic diathesis in up to 10% of patients treated daily. These hemorrhagic diathesis may manifest early with epistaxis or gastrointestinal bleeding, with laboratory findings significant for thrombocytopenia, increased coagulation times, leukopenia, and/or anemia. Adverse gastrointestinal, cutaneous, and neurological manifestations may occur and include anorexia, nausea, vomiting, diarrhea, stomatitis, fever, malaise, headache, facial flushing, and skin rash. Mithramycin-induced hypocalcemia may result in fatigue, depression, muscle cramps, fasciculations, and tetany.

In Vitro Toxicity Data

Mithramycin was found to be mutagenic in the Chinese hamster ovary cell assay.

Clinical Management

In patients manifesting clinical toxicity, discontinuation of mithramycin should be considered and supportive care instituted. Administration of calcium and blood products may be necessary depending upon the degree of toxicity.

See also: Blood; Veterinary Toxicology.

Further Reading

Ashby MA and Lazarchick J (1986) Acquired dysfibrinogenemia secondary to mithramycin toxicity. *The American Journal of the Medical Sciences* 292(1): 53–55.

Mitomycin C

Samantha E Gad

- Chemical Abstracts Service Registry Number: CAS 50-07-7
- Synonyms: Ametycine; MMC; Mutamycin; Mit-C; Methylmitomycin
- Chemical/Pharmaceutical/Other Class: Fungal metabolite
- Chemical Formula: $C_{15}H_{18}N_4O_5$

Uses

Mitomycin C is used as an antineoplastic agent and for slowing of fibroblast formation in open-angle glaucoma. Recently, mitomycin C has been used to induce tumor responses in patients with many types of cancer. For example, mitomycin C is used in the palliative treatment of various solid tumors such as non-small cell lung, cervical, colorectal, breast, bladder, pancreatic, and esophageal carcinomas. In addition to its systemic use in combination regimens for these tumors, mitomycin has been used as a single agent given by intrahepatic infusion for hepatic metastases from colorectal carcinoma and by intravesical instillation for carcinoma *in situ* of the bladder.

Exposure Routes and Pathways

Mytomycin C is administered intravenously as a therapeutic agent.

Toxicokinetics

Mitomycin C is absorbed inconsistently from the gastrointestinal tract. The volume of distribution is $16–56\,l\,m^{-2}$. The primary means of elimination is by hepatic metabolism, but it is also excreted in urine. $T_{1/2} = 8$ min.

Mechanism of Action

Mitomycin C inhibits DNA synthesis and cross-links DNA at the N6 position of adenine and at the O6

and N2 positions of guanine. In addition, single-strand breakage of DNA is caused by reduced mitomycin C (this can be prevented by free radical scavengers). Its action is most prominent during the late G1 and early S phases of the cell cycle. Mitomycin C can inhibit RNA and protein synthesis at high concentrations.

Mechanism of Toxicity

Mytomycin C is an aneuploidy-inducing agent. Oxygen and radiation therapy have shown to enhance the development of toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

The intravenous LD_{Lo} is $1500\,\mu g\,kg^{-1}$. In rats the intravenous TD_{Lo} is $2.6\,mg\,kg^{-1}$ and the oral LD_{50} is $68\,mg\,kg^{-1}$. Oral mouse LD_{50} is $88\,660\,\mu g\,kg^{-1}$; and oral LD_{Lo} in dogs and monkeys is $\sim 10\,mg\,kg^{-1}$. At the highest concentration of mitomycin C, in a rabbit experiment, the cornea was inflamed, with stromal necrosis and marked endothelial loss. Hemorrhagic iris necrosis was also observed.

Human

Hemolytic anemia, thrombocytopenia and renal dysfunction, leading to potentially fatal hemolytic uremic syndrome, can occur in patients given tamoxifen with or shortly after mitomycin C. Mitomycin C is contraindicated in patients with preexisting myelosupression and anemia.

Vinblastine and vindesine can increase the pulmonary toxicity of mitomycin C. Severe and life-threatening bronchospasms and two cases of fatal acute respiratory failure have been reported.

Chronic Toxicity (or Exposure)

Animal

Mitomycin C is a carcinogen (sarcomas and other cancers) in mice and rats after intraperitoneal, intravenous, or subcutaneous injections. It is teratogenic in mice (including skeletal defects). It is nephrotoxic. It is mutagenic in sister chromatid exchange assays.

Human

Intravenous administration of mitomycin C has resulted in dyspnea and lung fibrosis. Other effects include dermatitis, nausea, myelosuppression, fever, malaise, and glomerular damage. On rare occasions, interstitial pneumonitis, hemolytic uremic syndrome, and pulmonary fibrosis have occurred. It is a nephrotoxin and a male reproductive toxin. Mitomycin C causes alopecia and pulmonary damage and can cause severe tissue damage if it escapes from vasculature.

Serious and potentially life-threatening intravascular hemolysis and kidney failure may develop after long-term use of mitomycin and fluorouracil. The International Agency for Research on Cancer concluded that antimony trioxide was possibly carcinogenic to humans (group 2b) on the basis of the animal studies noted above.

In Vitro Toxicity Data

Mitomycin C has exhibited mutagenic properties *in vitro*. It induced chromosomal aberrations in drosophila oocytes and dominant and recessive mutations in wasp habrobracon.

Clinical Management

Early withdrawal of the drug and administration of corticosteroids appear to significantly improve the outcome. Topical application of dimethyl sulfoxide may be effective for management of mitomycin C extravasation.

Miscellaneous

Drug Interactions

Aclarubicin The bone marrow depressant effects of aclarubicin can be increased by previous treatment with mitomycin.

Doxorubicin (adriamycin) An increased incidence of late onset congestive heart failure has been seen in patients treated with mitomycin that had previously been given doxorubicin.

See also: Kidney; Reproductive System, Male; Sister Chromatid Exchanges; Toxicity Testing, Mutagenicity.

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Mixtures, Toxicology and Risk Assessment

Glenn Rice, Linda K Teuschler, Jane Ellen Simmons, and Richard C Hertzberg

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Human health risk assessment of chemical mixtures is the process of evaluating the biological consequences of human exposures to groups of chemicals. This entry provides a broad overview of chemical mixtures risk assessment, introducing fundamental concepts and describing approaches for evaluating the risks mixtures pose. Here, a mixture is defined as a combination of two or more chemicals that influences a population's risk of chemical toxicity. We note that exposures to mixtures could occur at different times and through different routes; chemicals forming a mixture may originate from different sources. We encounter many different mixtures in our environment; for example, there may be mixtures of small quantities of dioxins in our food. Exposures to some of these mixtures can be deleterious to our health. Identifying these potentially harmful mixtures and quantifying the risks they pose are among the goals of mixture risk analysis. While we focus on the evaluation of human health risks, most of the concepts are applicable to ecological risk assessment. For a detailed treatment of mixtures risk assessment, read the US Environmental Protection Agency's (EPA's) Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures or the Health Council of the Netherlands Exposure to Combinations of Substances: A System for Assessing Health Risk.

Basic Mixtures Concepts

For environmental health hazards, we typically rely on the US National Research Council's Risk Assessment Paradigm to guide the evaluation of whether or not a risk to human health is posed. This Paradigm is composed of a series of interconnected processes, including hazard identification, dose–response assessment, exposure assessment, and risk characterization. Mixtures are evaluated through the same processes used in single chemical risk assessment, but with ancillary considerations such as interactions between compounds, changes in the composition of the mixture over time, and the similarity between mixtures that have been tested toxicologically and the mixtures we encounter in our environment.

Specific terms are used to describe potential interactions between and among the chemical components of mixtures. Additivity implies that the effect caused by a mixture can be estimated directly from the sum of the scaled exposure levels (dose addition) or the sum of the risks (risk or response addition) of the individual components in the mixture. When evaluating the risk posed by a mixture, it is initially assumed that the components act in an additive manner. Mixtures producing risks greater than expected from additivity are described as synergistic, those producing risks less than expected from additivity are described as antagonistic. The designation of synergism and antagonism, therefore, depends on the definition of additivity.

Assessing exposures to mixtures can be complex. The initial composition and physical characteristics of a mixture can differ depending on how the mixture is produced; even the composition of mixtures produced by the same general process can differ dramatically (e.g., the components formed in chlorinated drinking water vary by temperature and source of the water). Once released into the environment the composition of a mixture typically changes. Some components may be selectively retained in one environmental medium (e.g., certain dioxin congeners released from the stacks of combustion sources appear to be selectively taken up and retained in plant tissues. See the work of Lorber). Cacela shows that other components such as polychlorinated biphenyls may be transformed by biological, chemical, or physical processes in the environment. These differences in the composition of mixtures result in differences in the exposures experienced by the population, potentially resulting in different biological consequences. Given the large number of mixtures and the variability of their components, we cannot test the toxicity of every mixture to examine the biological consequences.

The term 'similar mixtures' describes test mixtures that differ slightly in composition from an environmental/toxicological mixture of interest, but that are expected to share comparable environmental fates, and toxicokinetic and toxicodynamic processes. Similar mixtures may have the same components but in slightly different proportions, or have most components in nearly the same proportions with only a few different (more or fewer) components than an environmental mixture of interest. Risk assessors judge whether or not the toxicity of a test mixture is sufficiently similar to that of the environmental mixture of interest. If judged to be 'sufficiently similar', then the toxicity exhibited by the test mixture is used as a surrogate for the environmental mixture of interest (i.e., the biologic consequences of exposure to the two mixtures are judged indistinguishable).

Chemical Mixtures Toxicology

Chemical mixtures toxicity test strategies may analyze a whole mixture, a defined mixture, or individual mixture components. Samples of whole mixtures may be comprised of both known and unknown component chemicals, where the fraction of the mixture mass represented by known chemicals may often be smaller than that of the unknown chemicals. The whole-mixture approach offers a considerable advantage because it captures the toxicity of all of the components and all interactions between and among the known and unknown component chemicals. However, toxicological evaluation of the whole mixture is often fraught with technical difficulties. As concentration of environmental mixtures is typically required prior to conducting a toxicologic evaluation, careful consideration must be given to the environmental realism of the resulting sample. Have toxicologically important chemicals been lost or chemical artifacts been introduced into the sample during the concentration process? Are the relative proportions (the mixing ratios) of the resulting concentrate similar to those in environmental samples? Is the sample matrix compatible with the biological test system? Given that careful attention must be paid to sample quality, and sample preparation is often time-consuming and costly, toxicological evaluation of complex environmentally realistic mixtures may best be focused on those classes of mixtures, such as mixtures resulting from chlorination of drinking water, for which there is widespread human exposure, significant portions of the mixture mass are unknown and there is some, even if limited, epidemiologic information suggestive of adverse human health effects.

The toxicological testing of defined mixtures includes the preparation of chemical mixtures by adding individual components known to comprise whole mixtures. The component concentrations are designed to mimic the component ratios observed in whole mixtures (or a range of such ratios). The defined mixture approach has suffered historically from lack of experimental designs and corresponding statistical analysis techniques that compensate for the fact that traditional full-factorial experimental designs become technically infeasible as the number of both component chemicals and dose levels increase. Increasingly, less-than-full factorial designs, such as those described by Teuschler, are being developed and used. Toxicological evaluation of defined mixtures is most useful when: the research effort targets data collection either on mixtures of component chemicals that frequently occur together in the environment or on toxicologically significant combinations of chemicals; the experimental design includes environmentally-relevant mixing ratios; the experimental design includes data points at the low end of the dose–response curve; and, efficient experimental designs are employed and predictive modeling is incorporated into the study.

Toxicity tests conducted on individual components of whole mixtures also provide useful information to risk assessors. These tests may be conducted in isolation, in combination with other components of the mixture, or as an element of a defined-mixture study. Individual component approaches can be used to examine the assumptions underlying component-based risk assessment methods described in the next section. Toxicity tests on individual components are less expensive and faster than whole mixture and defined mixture approaches but lack information on interactions and unknown components.

Mixtures Risk Assessment Methods

When possible, risk assessors prefer to make assessments using epidemiologic or toxicologic data on the environmental mixture to which people are exposed. A second approach, described above, uses data from a test mixture judged to be sufficiently similar to the environmental mixture. A third option is to use data from a group of sufficiently similar mixtures; for example, a group of similar mixtures could be generated by the same commercial process or from similar emissions sources (e.g., diesel engines from emergency generators or from off-road vehicles). These mixtures may be readily available for testing, but may vary in composition. Based on toxicity test results from any of these three sources of whole mixtures, a safe level (e.g., a reference dose (RfD)) could be developed from an experimental no-observed-adverse-effect level or a benchmark dose, as could a cancer risk slope.

Component methods include those based on the assumption of response addition (e.g., addition of probabilistic cancer risks) or dose addition (e.g., relative potency factors (RPFs), hazard indexes (HI)). The advantages of component methods include an ability to utilize single chemical exposure and dose–response information to estimate a mixture risk and the flexibility to compare mixtures containing the same chemicals, but in different concentrations and proportions.

The RPF method is based on dose addition and assumes that the chemicals in a mixture share a common toxic mode of action; this means that when tested in the same bioassay, the dose response curves of each component should be similarly shaped. The components in this 'similarity group' are assumed to be true 'toxicologic clones' of each other and have

similar toxicokinetics, so that isoeffective doses differ by a fixed proportionality constant. The RPF method also requires the identification of an index chemical (IC). The IC is the mixture component that best represents the toxicity of the other members of the mixture and preferably has the highest quality dose–response information.

In the RPF method (eqn (1)), the user must identify the constraints of the application of a set of RPFs. For example, the health effect, dose range of component doses, route(s) of exposure, and duration(s) of exposure for which the RPFs can be applied must be specified (e.g., a set of RPFs may be constrained to oral exposures and not be used for exposures to the same mixture through the inhalation route). To apply the method, an RPF is estimated for each mixture component; the RPF estimates the toxicity of the component relative to that of the IC. RPFs commonly are estimated from a ratio of equally toxic doses of the individual dose–response functions for the component and the IC. For example, the quotient of the effective dose at which ten percent of a test population exhibits an effect (ED_{10}) of the IC and the component could serve as a value for the component's RPF; obviously, the RPF for the IC equals 1. The index chemical equivalent dose of an individual component is the product of the component dose and the RPF of the component. These equivalent doses are summed across all components. The risk posed by the mixture is estimated by comparing the summed index chemical equivalent doses of the mixture to the dose–response function of the IC:

$$R_{\mathrm{m}} = f_1\left(\sum_{i=1}^{n} \mathrm{RPF}_i \times D_i\right) \qquad (1)$$

where R_{m} is the risk posed by chemical mixture, f_1 (*) is the dose–response function of index chemical, D_i is the dose of the ith mixture component ($i = 1,\ldots,n$), and RPF_i is the toxicity proportionality constant relative to the index chemical for the ith mixture component ($i = 1,\ldots,n$). n is the number of mixture chemicals in the similarity group.

The hazard index, as described by Svensgaard, is another commonly used component method based on dose addition, where the component doses are scaled by their relative toxicity and then summed. HI serves to indicate concern rather than predict risk. When HI is less than 1, the mixture exposure is usually considered to pose no appreciable risk. Typically, the inverse of an acceptable, safe level (e.g., an RfD) is used to scale the component's toxicity. As described by the US EPA in 1989, the HI formula for oral exposures using RfDs appears in eqn (2):

$$\mathrm{HI} = \sum_{i=1}^{n} \frac{E_i}{\mathrm{RfD}_i} \qquad (2)$$

where HI is the hazard index, E_i is the oral exposure dose for the ith chemical, and RfD_i is the reference dose for the ith chemical. E_i and RfD_i have the same units and n is the number of chemicals in the mixture.

A more general component formula can be applied to mixtures of synergistic or antagonistic chemicals. US EPA (2000) describes a formula incorporating available toxicological interaction studies for pairs of component chemicals, based on the formula in eqn (2). For each chemical pair, a determination is made of the weight-of-evidence (WOE) that an observed toxicological interaction is relevant to human risk. The WOE classification is converted to a score that is used along with the component doses to estimate the mixture response. The formula for this interaction-based HI ($\mathrm{HI}_{\mathrm{INT}}$) is in eqn (3).

$$\mathrm{HI}_{\mathrm{INT}} = \sum_{j=1}^{n}\left(\mathrm{HQ}_j * \sum_{k\neq j}^{n} f_{jk} M_{jk}^{(B_{jk}g_{jk})}\right) \qquad (3)$$

where B_{jk} is the WOE interaction score for the influence of chemical k on chemical j, M_{jk} is the interaction magnitude of the influence of chemical k on chemical j, f, and g are functions of the component exposure levels, and HQ_j, the hazard quotient (equal to E_i/RfD in eqn (2)), represents the toxicity contributed by the jth component chemical if there were no interactions.

This formula adjusts each chemical's toxicity by the information on pairwise toxicological interactions involving that chemical. ATSDR has posted several interaction profiles with WOE determinations. If no interactions existed, then the second sum is always 1 and the formula reduces to the HI formula based on dose addition given in eqn (2); Hertzberg provides additional details about this equation.

While it appears complicated, this formula is the sum of chemical toxicities adjusted by pairwise interactions. Because there is no extrapolation parameter that can be gradually varied to move from the component and pairwise data to the mixture response, this interaction formula can be viewed as an extrapolation from dose addition to the mixture response. Comparing eqns (2) and (3), the pairwise interactions can be viewed as 'correction steps' roughly accounting for all the interactions (pairwise, three-way, etc.) in the mixture. (See the work of Hertzberg). As long as each pairwise interaction magnitude is fairly small, the estimated mixture

response will be a minor extrapolation away from dose addition. Few interaction studies attempt to quantify the interaction magnitude. Among those that do, the magnitude, expressed as a change in effective dose, is usually less than fivefold; the US EPA (2000) correspondingly set the default interaction magnitude at 5. This default magnitude limits the interaction-based HI to five-fold change in the additive HI. The strongest influence of the interactions will be when the evidence is excellent so the WOE scores are highest ($B = 1$), when all the pairs are at equitoxic levels (so every $g = 1$), and when all interactions are in the same direction. For example, if all pairwise interactions were synergistic, then the resulting calculation gives a maximum interaction-based index of $HI_{INT} = 5^{*}HI$.

Note that the interaction WOE score is based on judgment: the weaker the evidence, the closer the score is to zero, and the less impact the interaction has on the estimated mixture response. For most mixtures, no data exist on the whole mixture, so that accuracy checks are usually not possible. As a consequence, the quality of the component-to-mixture extrapolation is then judged by the quality and relevance of both the component data and pairwise interaction data.

See also: Aggregate Exposures; Dose–Response Relationship; Risk Assessment, Ecological; Risk Assessment, Human Health.

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US EPA (1989) Risk Assessment Guidance for Superfund. Vol. 1. Human Health Evaluation Manual (Part A). EPA/540/1-89/002.

Relevant Websites

http://www.epa.gov – Environmental Protection Agency.

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Chemical Mixtures.

Mode of Action

Lynne Haber

Mode of action refers to how a chemical exerts its toxic effects. Its meaning is similar to 'mechanism of toxicity' or 'mechanism of action'. Some scientists use the terms interchangeably, while others draw distinctions, with 'mechanism' indicating a more detailed understanding. For example, the International Programme on Chemical Safety (IPCS) framework for evaluating the mode of action for chemical carcinogenesis states "a supported *mode of action* would have evidence provided by robust mechanistic data to establish a biologically plausible explanation. *Mechanism of action*, in contrast, relates to sufficient understanding of the molecular basis to establish causality; it is at the other end of the continuum from little or no evidence of *mode of action* to scientific proof of *mechanism of action*."

The US Environmental Protection Agency (EPA) focuses more on the level of detail, rather than the degree of scientific support, in distinguishing mechanism and mode of action, stating that mechanism of action is used to imply a detailed understanding, often at the molecular level, of how a chemical exerts its toxic effect, while mode of action refers to a more general understanding of the process. The US EPA defines mode of action as "a sequence of key events starting with interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation."

Although the term mode of action appears sometimes in the context of noncancer effects, it finds particular use in the context of cancer risk assessment, where mode of action forms the basis for: (1) determining whether tumors observed in animals are relevant to humans, and (2) determining the approach for quantitative cancer risk assessment. IPCS has developed a conceptual framework for evaluation mode of action for chemical carcinogenesis, based partly on a modification of the Bradford–Hill criteria for causality. A similar approach is used by the US EPA. Under this framework, each mode of action is analyzed separately, noting that multiple modes of action may contribute to the development of a given tumor type, and that a single chemical may cause tumors in different tissues by different modes of action. This framework includes:

1. Introduction – identifies the observed cancer endpoints, and which one(s) are being addressed in the analysis.
2. Postulated mode of action – brief description of the sequence of events on the path to cancer.
3. Identification of the 'key events', measurable events that are critical to the induction of tumors as hypothesized in the mode of action.
4. Dose–response relationship – evaluation of whether the dose–response for the key events parallels the dose–response for tumors.
5. Temporal association – evaluation of the sequence of events, and whether the key events precede the tumor response.
6. Strength, consistency, and specificity of association of tumor response with key events – discussion of the weight of evidence linking the key events, precursor lesions, and tumors.
7. Biological plausibility and coherence – consideration of whether the postulated mode of action is consistent with current understanding of carcinogenesis in general (biological plausibility) and the specific chemical (coherence).
8. Other modes of action – discussion of alternative modes of action that logically present themselves.
9. Assessment of postulated mode of action – overall conclusion, with the level of confidence in the postulated mode of action.
10. Uncertainties, inconsistencies, and data gaps.

There are a variety of possible carcinogenic modes of action. The following examples of modes of action are not intended to be exhaustive, but they do provide examples of the major modes of action relevant to carcinogenesis:

- Mutagenicity – interacting with DNA to cause DNA changes that can be inherited by daughter cells. The reaction with DNA may be direct or indirect.
- Mitogenesis – stimulation of cell division. Examples include disruption of hormone homeostasis and interfering with the cell signaling pathways.
- Inhibition of cell death. Programmed cell death (apoptosis) is used by organisms to eliminate damaged cells. Inhibition of this process can allow cells with damage to the cell cycle control system to proliferate.
- Cytotoxicity with reparative cell proliferation.
- Immune suppression. Decreased activity of the immune cells that monitor for damaged cells (NK, or natural killer cells) can allow the damaged cells to proliferate.

Once a chemical's mode(s) of action has been identified, the risk assessor can proceed with the evaluation of carcinogenic potential, including consideration of the relevance of the mode of action to humans. Further consideration of mode of action distinguishes between a genotoxic (or sometimes more rigorously described as DNA-reactive) mode of action, for which there may be some risk at every nonzero dose, and nongenotoxic (or nonmutagenic) modes of action, for which threshold or nonlinear dose–response curves may apply.

See also: Carcinogenesis; Risk Assessment, Human Health.

Further Reading

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Models *See* Animal Models.

Modifying Factors of Toxicity

Frank C Lu and Sam Kacew

The nature and extent of the toxic manifestations in an organism that is exposed to a toxic substance depend on a variety of factors. Exposure to a substance in certain instances produces a reaction in one individual but not in another. While there may be a difference in sensitivity between these individuals, it is also possible that the responsive person was previously exposed (i.e., this individual is actually subjected to a higher concentration of chemical). Two of the factors known to modify the toxic consequences of exposure to chemicals are the dose and duration. This section will focus on the modification of toxicity by other factors including the species and strain of the animal, its sex and age, and its nutritional and hormonal status. Various physical factors also play a part. In addition, the toxic effect of a chemical may be influenced by simultaneous and consecutive exposure to other chemicals. The toxic effects may be modified in a number of ways: alteration of the absorption, distribution, or excretion of a chemical; an increase or decrease of its biotransformation; and changes of the sensitivity of the receptor at the target organ.

It should be remembered that the outcome of adverse effects predicted in humans is based on data generated in animals since drugs released on the market for prescription in humans must be tested on animals. Hence, a clear understanding of the existence of these factors and of their mode of action is important in designing the protocols of toxicologic investigation. It is equally important in evaluating the significance of the toxicologic data and in assessing the safety/risk to humans under specified conditions of exposure. This is not always possible, however, because in certain circumstances the testing in animals is improper (i.e., a test for toxicity is carried out in an animal, but the human responds differently). The use of thalidomide in pregnant women as a sedative resulted in fetal malformations in humans, but this drug did not produce these effects in rats. Another example of a lack of specific conditions of human exposure is related to a long latency period. Pregnant mothers were administered diethylstilbesterol to prevent miscarriage, only to discover that 20 years later female offspring developed vaginal cancer. An industrial accident involving the release of methyl isocyanate, an intermediate in carbaryl synthesis, by Union Carbide in Bhopal, India, resulted in toxicity and death. The toxicological profile of methyl isocyanate was not known, and the chemical was released under uncontrolled conditions. Industrial accidents by definition are not specified conditions of exposure but must be borne in mind as a factor to consider. With increased knowledge of chemicals and the factors that affect these chemicals, the ability to assess and react to adverse conditions is enhanced.

Modifying Factors

Species

Differences of adverse effect from one species to another have long been recognized. Knowledge in this field has been used to develop, for example, pesticides, which are more toxic to pests than to humans and other mammals. Among various species of mammals, most effects of toxicants are somewhat similar. This fact forms the basis for predicting the toxicity to humans from results obtained in toxicologic studies conducted in other mammals, such as the rat, mouse, dog, rabbit, and monkey. There are, however, notable differences in toxicity even among mammals.

Some of these differences can be attributed to variations in detoxication mechanisms. For example, the loss of consciousness induced in several species of laboratory animals by hexobarbital (a barbiturate derivative that depresses the central nervous system (CNS)) shows marked differences; these are attributable to the activity of the detoxication enzyme that inactivates this chemical. In the mouse, the activity of the detoxifying enzyme is 16-fold greater than that in the dog, which is reflected by 12 min of hexobarbital-induced sleep in the mouse versus 315 min of sleep in the dog. There are other examples of species-related differences in the ability to detoxify chemicals that consequently result in differences in toxicity. Other examples include the industrial chemicals, ethylene glycol and aniline. Ethylene glycol is metabolized to oxalic acid, which is responsible for its toxicity, or to carbon dioxide. The rank order of ethylene glycol toxicity in animals is as follows: cat $\geqslant$ rat $\geqslant$ rabbit; this is the same for the extent of oxalic acid production. Aniline is metabolized in the cat and dog mainly to *o*-aminophenol, and these species are more prone to toxicity; however, in the rat and hamster aniline is metabolized mainly to I-aminophenol and thus these species are less susceptible to aniline toxicity.

A more serious example in which animal data are of little clinical relevance is the release of butadiene gas in the production of rubber products.

Butadiene is converted to butadiene monoepoxide, which is believed to be responsible for carcinogenesis in rodents but not in humans. In humans, butadiene monoepoxide is further converted to butenediol and conjugation with glutathione results in no toxicity. In rodents, however, there is direct conjugation of butadiene monoepoxide with glutathione, which presumably is not adequate, and thus cancer initiation occurs. In this example, it can be seen that rodent data are a poor indicator for prediction of risk in humans because the detoxification pathways differ.

Differences in bioactivation also account for dissimilarities in toxicity. A notable example is 2-naphthylamine, which can produce bladder tumors in the dog and human but not in the rat, rabbit, or guinea pig. Dogs and humans, but not the others, excrete the carcinogenic metabolite 2-naphthyl hydroxylamine. Acetylaminofluorene (AAF) is carcinogenic in many species of animals but not in the guinea pig. The *N*-hydroxy metabolite of AAF, however, is carcinogenic to all animals including the guinea pig, demonstrating that the difference between the guinea pig and the other animals is not in their response to the toxicant but in the bioactivation, that is, the guinea pig lacks the ability to form the toxic metabolite.

There are other factors, including absorption, distribution, and excretion of chemicals, to consider in trying to understand differences in toxicity between individuals. Paraoxon is the active metabolite of the organophosphate insecticide parathion. Hence, a difference in the rate of formation of this metabolite is attributable to a difference in toxicity. This has been reported in rats, in which females are more susceptible to parathion neurotoxicity than males and in which paraoxon formation occurs faster in the female resulting in greater toxicity in this sex. This phenomenon has been widely studied in humans with the drug isoniazid. For example, there are 'slow inactivators', who are deficient in acetyltransferase. Such individuals acetylate isoniazid only slowly and are thus likely to suffer from peripheral neuropathy resulting from an accumulation of isoniazid. On the other hand, people with more efficient acetyltransferase, termed 'fast metabolizers', require larger doses of isoniazid to obtain its therapeutic effect. Since large amounts of drug are needed, these individuals are more likely to suffer from hepatic damage caused by isoniazid because isoniazid also acts on the liver.

Strain

It is essential to determine the safety of new pharmaceutical agents for humans using the appropriate mammalian toxicity tests mandated by governmental regulatory agencies (e.g., US Food and Drug Administration and Health and Welfare Canada). This necessitates the use of a species that can be compared to humans for factors such as pharmacokinetics, metabolism, excretion, absorption, and distribution of the test material. The rat is one of the species that has proven to be extremely useful in pharmacologic and toxicologic research because there are many similarities between rat and human metabolic pathways; many anatomical and physiological characteristics are similar, allowing for comparisons in absorption, excretion, and pharmacokinetics. The rat is also of a convenient size, is relatively docile, has a short life span and gestation period, and is economical to maintain. There is a large database of its characteristics which is invaluable in the interpretation of the relevance of animal data for humans.

There are three main classes of rats used in research; these are inbred strains, outbred stocks, and mutants (including transgenic stocks). It is very important that research workers understand the characteristics of these three classes of stock because they may have a profound influence on the quality of their research. For example, outbred stocks such as Wistar rats may be segregating at many genetic loci, which are important in drug metabolism, so that different individuals within a colony will react differently. In many cases it does not make much sense to do detailed pharmacological studies against such a variable genetic background. The characteristics of the three main classes of stock are briefly summarized in the following sections.

Inbred Strains

Inbred strains are produced by at least 20 generations of brother $\times$ sister mating, with all individuals being derived from a single breeding pair in the 20th or subsequent generation (this eliminates parallel sublines). For most practical purposes, an inbred strain can be regarded as an immortal clone of genetically identical individuals. Inbred strains have a number of properties that make them the animal of first choice for most types of research. Because the strain is isogenic (i.e., all individuals are genetically virtually identical), the genotype of the whole colony can be determined at a particular genetic locus by typing a single individual. Many genetic markers are fixed in each strain so that the authenticity of the strain can be determined. This can now be done using DNA markers detected by the polymerase chain reaction. This contrasts sharply with outbred rats, in which currently there are not even any genetic markers that can be used to distinguish between Wistar and Sprague–Dawley stocks.

All inbred animals are homozygous at all genetic loci, so there are no 'hidden' recessive genes that could cause confusion in experiments involving breeding. As a result of this homozygosity, the strain stays genetically constant for many generations. This is valuable because it makes it possible to build up background data on genetic characteristics that should remain valid for a long period of time. Of course, the phenotype (but not the genotype) may alter if the diet, environment, or associated microorganisms change. However, over a period of several generations, an inbred strain will remain much more constant than an outbred stock.

The isogenicity and homozygosity together tend to lead to greater phenotypic uniformity of inbred animals. This is important because greater uniformity leads to more statistically powerful experiments that are able to detect a given biological effect with fewer animals. The degree of the contrast with outbred stocks depends on the character being studied. Clearly, for characters controlled by a single or small numbers of genetic loci, such as the major histocompatibility complex or the drug metabolizing enzymes, animals within an inbred strain will be uniform, whereas animals of an outbred stock will usually not. However, the greater uniformity of inbred animals may not be apparent for characters such as body and organ weights (which also depend on environmental and chance factors), unless very large numbers are studied.

Each inbred strain has its own unique pattern of behavior, growth patterns, reproductive performance, spontaneous disease (including tumors), and response to xenobiotics. Differences between strains are an indication that the observed character is under genetic control. Currently, there are over 200 inbred rat strains.

Outbred Stocks

So-called 'outbred stocks' are usually maintained as closed colonies of rats of undefined genotype and sometimes known by generic names, such as Wistar, Sprague–Dawley, or Long–Evans, which indicate their historical origin. The amount of genetic variation present in any given colony depends on its history. At one extreme, if the colony has been maintained as a closed colony for many years with small numbers of breeding animals each generation, it may be genetically highly uniform to the extent that it will closely approximate an inbred strain. If the colony has become inbred, it may have gone through a period of rapid genetic drift so that it will differ from other colonies with the same historical origin. At the other extreme, a colony that has recently been crossed to an unrelated stock should be genetically highly variable.

Mutants or Transgenics

Over 300 genetic loci associated with mutants and polymorphisms of various sorts have been described in the rat. A mutant can be created by insertion of DNA from an external source, be it a virus, recombinant DNA, etc. The inserted DNA is referred to as a transgene and the recipient host a transgenic animal, and the methodology for insertion of DNA is termed transgenic technique. The sequence of DNA in the transgene can be similar to sequences already present in the host or the sequence can be different. If the inserted DNA sequence is not novel, the new mutant created is termed a 'knockout' mutant or animal. Some of these, such as the polymorphisms associated with drug metabolizing enzymes and mutants such as acholuric jaundice (widely known as the 'Gunn' rat) and the Rowett, a thymic nude, are important in pharmacological and toxicological research. Recently, 'mutants' such as the 'Big Blue' rats have been produced using transgenic techniques. Transgenic and knockout rats produced by gene targeting techniques are likely to be of increasing importance in toxicological research. Mutants and transgenes can be placed on any genetic background by suitable breeding techniques. Thus, the jaundice gene from the Gunn rat is available on the inbred ACI, LEW, R/A, and RHA genetic background, as well as on a number of outbred genetic backgrounds. For this reason, it would be incorrect to discuss drug metabolism in 'the Gunn rat' (or any other mutant or transgenic) without specifying its genetic background because drug metabolism will depend on many genes in addition to the specific locus that is abnormal in the Gunn rat.

Choice of Strain in Research and Screening

There seem to be no serious disadvantages (apart from cost) and many advantages in the use of inbred strains rather than outbred stocks in academic research. These animals offer the nearest equivalent to pure reagents that is possible when using animals in research, particularly if they are also of a high health status. In disciplines other than toxicology, there has been a relentless trend toward the increased use of inbred strains. It is not entirely clear why their use is not more widespread in toxicological research. Any disadvantage in terms of initial cost should be amply compensated for by improved research quality and the need for fewer animals. In toxicological screening

the relative merits of inbred strains versus outbred stocks has been debated for more than 50 years without reaching a consensus. An inbred rat strain F344 is used in the National Toxicology Program Carcinogenesis Bioassay, but most commercial screening is done using outbred stocks.

Sex

Male and female animals of the same strain and species usually react to toxicants similarly. It must be borne in mind, however, that there are marked differences in the hormonal makeup between sexes, and this can result in notable differences in responses. Chloroform produces damage to liver and kidney in humans and mice. In mice, however, chloroform produces nephrotoxicity only in males. Furthermore, administration of testosterone (male hormone) to the female mouse followed by chloroform results in kidney damage. Clearly, there are androgen (male) receptors in the kidney that sensitize males to chloroform-induced nephrotoxicity. In rats, exposure to the hydrocarbon decalin results in a renal nephropathy and tumor formation in the male but not female, and this is associated with an α_2-globulin protein accumulation. Treatment of females with testosterone followed by decalin also produces renal toxicity and protein accumulation. These examples demonstrate that kidney function differs between the sexes and, consequently, toxic manifestations will vary between males and females.

There are metabolic differences between the sexes. Many barbiturates induce more prolonged sleep in female rats than in males. The shorter duration of action of hexobarbital in male rats is related to the higher activity of the liver microsomal enzymes stimulated by testosterone to hydroxylate this chemical. This higher activity can be reduced by castration or pretreatment with estrogen (female hormone).

Female rats are also more susceptible than males to such organophosphorus insecticides as azinphosmethyl and parathion. Castration or estrogen treatment of the male reverses this difference. The male rat is far more susceptible to carcinoma than the female as shown in the following examples: Males are more susceptible to the induction of pancreatic tumors by azaserine, colonic carcinoma by dimethylhydrazine, intestinal tumors by dimethylnitrosamine, renal tumors by decalin, and liver cirrhosis by AAF. In the case of hydroquinone, which is present in photographic material, acute exposure produced renal toxicity in the female; but in a chronic 2 year study, the male and not the female was found to have tubular degeneration and adenoma.

Imbalances of nonsex hormones can also alter the susceptibility of animals to toxicants. Hyperthyroidism, hyperinsulinism, adrenalectomy, and stimulation of the pituitary–adrenal axis have all been shown to be capable of modifying the effects of certain toxicants. One of the functions of thyroid hormone involves the maintenance of normal heart activity; in hyperthyroidism, however, there is tachycardia and hypertension. In normal circumstances, ingestion of caffeine, which is a cardiac stimulant, does not affect heart function but large doses of caffeine produce cardiac arrhythmias. It is thus evident that a hyperthyroid patient drinking excess coffee would be more prone to cardiac dysfunction than a normal individual. Hyperinsulinism is manifested by a hypoglycemic coma through a depletion of carbohydrate stores and lack of CNS energy supply. The insecticide DDT in toxic doses is known to produce CNS excitability, tremors, and convulsions and is associated with carbohydrate store depletion. Thus, it can be seen that in conditions of hyperinsulinemia, exposure to DDT or the heavy metal cadmium, which acts in a similar fashion, results in a greater sensitivity of the CNS to toxicity.

Age

The pharmacokinetic principles applied in pediatric drug therapy are, in general, similar to those utilized for adults. Data obtained in adult studies, however, are not always applicable to rational therapy in infants or young children. The infant must be regarded as a distinct organism (not a small adult), and lack of appreciation of this fact can result in serious harm and potentially in death.

A number of important characteristics exist that distinguish drug therapy in infants from adult medication protocols. For example, after intramuscular administration, drug absorption is partially dependent on blood flow in the muscle bed. Abnormal drug absorption following intramuscular injection can occur in premature infants, in whom muscle mass is small and blood flow to the musculature is poor. Examples of adverse effects attributed to altered drug absorption are the reactions of infants to cardiac glycosides and anticonvulsants.

In the infant, absorption from the gastrointestinal tract of an orally administered drug differs from that in adults. Certain toxicants are absorbed to a greater extent by the young than by the adult. For example, young children absorb four or five times more lead than adults and 20 times more cadmium. In both adults and infants, the rate and extent of drug absorption depend on the degree of ionization, which, in turn, is influenced by pH. Within the first

24 h of life, gastric acidity increases rapidly, and this is followed by an elevation in alkalinity over the next 4–6 weeks. These conditions result in drugs existing in the infant gastrointestinal tract in states of ionization other than might be observed in adults. The higher incidence of methemoglobinemia in young infants has been explained on the basis that their lower gastric acidity allows upward migration of intestinal microbial flora and the reduction of nitrates to a greater extent. Furthermore, infants have a higher proportion of fetal hemoglobin, which is more readily oxidized to methemoglobin. Other factors that modify gastrointestinal drug absorption in the young infant include an irregular neonatal peristalsis, a greater gastrointestinal tract surface to body ratio, and enhanced β-glucuronidase activity in the intestinal tract. The significance of the β-glucuronidase is that it converts drug-bound glucuronide to the free form and thus increases drug bioavailability.

Differences exist in the organ distribution of drugs between newborns and adults. The greater susceptibility of the young to morphine is attributable to a less efficient blood–brain barrier. In the newborn, a higher percentage of body weight is represented by water, so extracellular water space is proportionally larger. To initiate a receptor response, the distribution of drugs must occur predominantly in the extracellular space, so the amount of drug reaching the receptor sites is higher in neonates. Furthermore, the ability of newborn infants to bind drugs in plasma is significantly less than that in adults. This again suggests that neonates could be expected to be more susceptible to the effects of drugs. Differences also exist with respect to drug-metabolizing enzymes. It has been clearly demonstrated that the drug inactivation rate is generally slower in newborns. The available information indicates that the greater susceptibility of the young animals to many toxicants can be attributed to deficiencies of various detoxication enzyme systems. Both phase I and phase II reactions may be responsible. For example, hexobarbital at a dose of $10\,mg\,kg^{-1}$ induced a sleeping time of longer than 360 min in 1-day-old mice compared to 27 min in the 21-day-old mice. The proportion of hexobarbital metabolized by oxidant in 3 h in these animals was 0% and 21–33%, respectively. On the other hand, chloramphenicol (an antibiotic) is excreted mainly as a glucuronide conjugate. When a dose of $50\,mg\,kg^{-1}$ was given to 1- or 2-day-old infants, the blood levels were $15\,\mu g\,ml^{-1}$ or higher over a period of 48 h. In contrast, children aged 1–11 years maintained such blood levels for only 12 h.

Not all chemicals, however, are more toxic to the young. Certain substances, notably CNS stimulants, are much less toxic to neonates. The acute toxicity of DDT was reported more than 20 times smaller in newborn rats than in adults, in sharp contrast to the effect of age on malathion.

Furthermore, the ability of the neonate to eliminate drugs via the kidney, the major excretion pathway, is significantly limited by the state of development of these organs. Penicillin and tetracycline (two antibiotics) are excreted more slowly and hence are more toxic in the young. Consideration of these factors indicates that the susceptibility and responsiveness of newborns to drug therapy are different from those of adults.

Old animals and humans are also more susceptible to certain chemicals. This problem has not been studied as extensively as in the young. The available evidence indicates that the aged patients are generally more sensitive to many drugs. A prime example is the use of antibiotics to treat infections in geriatric patients. Since the detoxification of antibiotics is dependent on renal clearance, which is generally slower in the aged, drug accumulation and toxicity are higher in the older patient. The possible mechanisms include reduced detoxication and an impaired renal excretion. In addition, the distribution of chemicals in the body may also be altered because of increased body fat and decreased body water. A number of drugs have been found to be likely to induce more severe signs of toxicity. These include most CNS depressants, certain antibiotics, cardiac glycosides, and hypotensive agents.

Pregnancy

During the course of a pregnancy a mother is likely to take a number of drugs for therapeutic reasons. In addition, with many more women in the work force, there is an increased potential of exposure to a variety of chemicals under occupational conditions. Furthermore, a large number of women indulge in a variety of recreational chemicals including cigarettes and alcohol. The consequences attributed to exposure to a pharmaceutical product can be advantageous to the mother; however, in many instances the effects are deleterious to the fetus. Exposure to occupational and/or environmental chemicals is more likely to result in adverse effects than to be beneficial. Thus, it may be stated that the fetus is at some jeopardy as a result of exposure to foreign chemicals.

Nutrients essential for fetal growth and development require an active transport system to be moved from the maternal circulation to the fetal circulation against a concentration gradient. By contrast, drugs and other chemicals cross the placenta by simple diffusion. The amount of a chemical that is transferred to the fetus is dependent on lipid solubility,

the degree of ionization, and the molecular weight. Lipophilic chemicals tend to diffuse across the placenta readily, while highly ionized compounds penetrate the placental membrane slowly. The molecular weight of a chemical affects placental transfer, with the larger molecules crossing the placental barrier less readily. Protein binding of a chemical or its metabolites will affect the rate and the amount transferred to the fetus. Exposure of fetal target tissues to chemical entities may also be influenced by metabolism in the placenta or the fetal liver.

An important component to consider is the stage of fetal development at the time of chemical exposure. During the first week of development after fertilization, the embryo undergoes the process of cleavage and gastrulation. Exposure to chemicals or drugs such as antimetabolites, ergot alkaloids, or diethylstilbestrol at this stage can result in termination of pregnancy. Organogenesis is the next developmental stage covering weeks 2–8 of gestation. Exposure to drugs or other chemicals including thalidomide, alcohol, and phenytoin during this phase can result in serious structural abnormalities. Drugs and chemicals such as cigarette smoke, heavy metals, or carbon monoxide may affect development during the remaining gestational period ranging from 9 weeks to 9 months. Predominant effects are alteration in the differentiation of the reproductive system and CNS. Consequently, altered brain function and growth retardation are some of the principal adverse effects due to exposure at this stage.

Lactation

The nursing mother can serve as a source of exposure for the neonate to drugs and environmental chemicals. Most drugs are detectable in breast milk regardless of whether they are over-the-counter medication or something prescribed by a physician. In addition, exposure of the nursing mother to environmental pollutants can result in chemical contamination of breast milk. The presence of a drug or chemical in maternal milk may be construed as a potential hazard to the infant even though only 1% or 2% of total intake is likely to be found here. Hence, the primary consideration in maternal drug therapy or exposing a lactating mother to industrial chemicals is the risk to the nursing infant rather than the mere presence of a xenobiotic in the milk.

Several factors play a role in determining the quantity of a drug or chemical that will be transferred to breast milk. The amount of drug or chemical that is actually available for transfer to milk is dependent on certain maternal factors including amount of drug or chemical absorbed, frequency and route of exposure, xenobiotic biotransformation, and protein binding and excretion.

Drug utilization can to a large extent be controlled, so the prudent use of drugs during lactation is imperative because of the potential transfer of these agents or their metabolites into the milk. Certain drugs should be totally avoided during lactation. Certain foods or nutritional supplements have also been shown to cause adverse effects in the infant as a result of lactational exposure.

It is much more difficult to control exposure to environmental chemicals. For example, a mother has no knowledge of what pesticides may have been used on the fruits or vegetables purchased for consumption. Nor is there an easy way to protect oneself from ambient industrial pollution. Some examples of environmental agents known to produce adverse effects in the nursing infant include lead and tetrachloroethylene (dry cleaning solvent).

Nutritional Status

The principal biotransformation of toxicants is catalyzed by the microsomal mixed function oxidase system (MFO). A deficiency of essential fatty acids generally depresses MFO activities. This is also true with protein deficiency. The decreased MFO has different effects on the toxicity of chemicals. For example, hexobarbital and aminopyrine are detoxified by these enzymes and are thus more toxic to rats and mice with these nutrient deficiencies. On the other hand, the toxicity of aflatoxin is lower in such animals because of their depressed bioactivation of this toxicant. MFO activities are decreased in animals fed high levels of sugar.

A number of carcinogenesis studies have demonstrated that restriction of food intake decreases tumor yield. Deficiency of protein generally lowers tumorigenicity of carcinogens, such as aflatoxin and dimethylnitrosamine. It is well known that enzymes, derived from protein, are required to produce reactive, toxic metabolites of aflatoxin or dimethylnitrosamine. Hence, with protein deficiency the toxic metabolite cannot be generated. The importance of diet on carcinogenesis is further demonstrated by the fact that rats and mice fed diets rich in fats have higher tumor incidences compared to those that are given a restricted diet.

Chemical Interaction

The toxicity of a chemical in an organism may be increased or decreased by a simultaneous or consecutive exposure to another chemical. If the combined effect is equal to the sum of the effect of each

substance given alone, the interaction is considered to be additive, for example, combinations of most organophosphorus pesticides on cholinesterase activity. If the combined effect is greater than the sum, the interaction is considered to be synergistic, for example, carbon tetrachloride and ethanol on the liver and asbestos exposure and cigarette smoking on the lung. In the latter example, there can be a fivefold increase in lung cancer incidence among asbestos workers, an 11-fold increase among cigarette smokers, and a 55-fold increase among asbestos workers who are cigarette smokers. The term potentiation is used to describe the situation in which the toxicity of a substance on an organ is markedly increased by another substance that alone has no toxic effect on that organ. For example, isopropanol (a solvent) has no effect on the liver, but it can increase considerably the hepatotoxicity of carbon tetrachloride (another solvent).

The exposure of an organism to a chemical may reduce the toxicity of another. Chemical antagonism denotes the situation wherein a reaction between the two chemicals produces a less toxic product, for example, chelation of heavy metals by dimercaprol. Functional antagonism exists when two chemicals produce opposite effects on the same physiologic parameters, such as the counteraction between CNS stimulants and depressants. Competitive antagonism exists when the agonist and antagonist act on the same receptor, such as the blockade of the effects of nicotine on ganglia by ganglionic blocking agents. Noncompetitive antagonism exists when the toxic effect of a chemical is blocked by another not acting on the same receptor. For example, atropine reduces the toxicity of acetylcholinesterase (AChE) inhibitors not by blocking the receptors on the AChE, but by blocking the receptors for the ACh accumulated.

Chemical interactions are achieved through a variety of mechanisms. For instance, nitrites and certain amines can react in the stomach to form nitrosamines, the majority of which are potent carcinogens, and thus greatly increase the toxicity. On the other hand, the action of many antidotes is based on their reactivity with the toxicants; for example, thiosulfate is used in cases of cyanide poisoning. Furthermore, a chemical may displace another from its binding sites on plasma protein and thereby increase its effective concentration. A chemical may modify the renal excretion of weak acids and weak bases by altering the pH of urine. Competition for the same renal transport system by one chemical can hinder the excretion of another. A notable example is the administration of the drug probenecid along with the antibiotic penicillin to reduce the renal excretion of the antibiotic, thereby prolonging its duration of action.

One important type of interaction involves the binding of chemicals with their specific receptors. An antagonist blocks the action of an agonist, such as a neurotransmitter or a hormone, by preventing the binding of the agonist to the receptor.

Another important type of interaction results from alterations of the biotransformation of a chemical by another. Some chemicals are inducers of xenobiotic-metabolizing enzymes. They augment the activities of these enzymes, perhaps mainly by *de novo* synthesis, a fact that is consistent with the finding that repeated administrations are necessary. The common inducers include phenobarbital, 3-methylcholanthrene (3-MC), polychloro biphenyls, DDT, and benzo(*a*)pyrene. The inducers may lower the toxicity of other chemicals by accelerating their detoxication. For example, pretreatment with phenobarbital shortens the sleeping time induced by hexobarbital and the paralysis induced by zoxazolamine. In addition, 3-MC pretreatment greatly reduces the liver injury produced by bromobenzene, probably by increasing the activity of the epoxide hydrase. On the other hand, pretreatment with phenobarbital augments the toxicity of acetaminophen and bromobenzene, apparently by increasing the toxic metabolites formed. Repeated administration of a chemical may induce its metabolizing enzymes, as has been shown with the industrial chemical vinyl chloride.

Piperonyl butoxide, isoniazid, and SKF 525A and related chemicals are inhibitors of various xenobiotic-metabolizing enzymes. For instance, piperonyl butoxide increases the toxicity of pyrethrum (an insecticide) by inhibiting MFO activity in insects that detoxifies this agent. Isoniazid, when taken along with phenytoin, lengthens the plasma half-life of the antiepileptic drug and increases its toxicity. Iproniazid inhibits monoamine oxidase and increases the cardiovascular effects of tyramine, which is found in cheese and which is normally readily metabolized by the oxidase.

See also: Absorption; Analytical Toxicology; Biotransformation; Distribution; Exposure; Mechanisms of Toxicity; Mixtures, Toxicology and Risk Assessment; Pharmacokinetics/Toxicokinetics; Resistance to Toxicants.

Further Reading

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Mold

Martha Boss

Mold is part of the taxonomic fungi kingdom. These kingdoms (Monera, Protista, Fungi, Plantae, Animals) represent the known life-forms on the planet Earth.

Fungi reproduce through alteration of generations, from asexual to sexual to asexual, and so on. Their vegetative body may be unicellular (yeasts) or composed of microscopic threads called hyphae. The general term for the latter is molds, and in certain environmental conditions, yeasts being dimorphic can grow using hyphae. Fungi are eukaryotic, nonvascular, and reproduce by means of spores. Both sexual (meiotic) and asexual (mitotic) spores may be produced.

Fungi are heterotrophic (must feed on preformed organic material) rather then being autotrophic (make their own food by photosynthesis). Unlike animals (also heterotrophic), which ingest then digest, fungi digest then ingest. Fungi secrete exoenzymes to digest their food.

Fungi are not dependent on light and can grow in dark habitats in any direction. As they grow, the fungi can imbed their absorptive filaments (mycelia) into their growth substrate surface and, thus, obtain nutrients and water.

In our outdoor environment, fungi interacts with the other life-forms and inanimate objects of our planet. Molds are present in various geographic regions of our world and in the microhabitats in these regions.

Fungi Classification

Fungi are classified into phyla based on the morphology of their spores produced for sexual and asexual reproduction. This classification system has been heavily dependent on how the spores looked under a microscope. Fungi may be reclassified as sexual spores are identified or the genetic structure of the fungi is determined.

Currently, four major phyla are recognized. Chytridiomycota, which has sexual and asexual spores. The gametes have posterior flagella and, thus, are motile in a liquid environment. Zygomycota, which has sexual spores called zygospores that are thick-walled and asexual spores contained in a sac structure called a sporangium. Ascomycota, which has spores borne internally in a sac called an ascus. The asexual spores may be borne at the tips or sides of hyphae. These asexual spores (if dust like in size) may be termed conidia and are borne on a conidiophore. Basidiomycota, which has spores borne externally on a club-shaped structure called a basidium. The majority of Basidiomycota do not produce asexual spores.

Fungal Cellular Structures

The cell walls of fungi are structurally similar to plants; however, the chemical composition is different. Fungi cell walls are composed mostly of chitin plant walls whereas plant cell walls are composed mostly of cellulose. Only one subgroup of Chytridiomycota, the Hyphochytrids, has cellulose as well, a trait unique among living fungi. The cytoplasmic ultrastructure of fungi is broadly similar to plant cells; however, the types of organelles present are significantly different.

Most fungal cell walls contain glucose polymers as structural components. These glucans can be chemically bound to chitin. Glucans have been implicated in toxicological effects. A-(1-3)-D-Glucan is a known and potent T-Cell adjuvant, simulates macrophages and neutrophils, plays a role in organic dust toxic syndrome, and may be involved in hypersensitivity pneumonitis.

Microbial Volatile Organic Compounds

Molds while growing produce various chemicals as a result of their primary metabolic processes. These processes are needed to ensure the continuation of the mold's life cycle. The gaseous metabolic products are collectively referred to as microbial volatile organic compounds (mVOCs). Some mVOCs are primary solvents and are chemically identical to those originating from solvent-based building materials and cleaning supplies (e.g., alcohols, aldehydes, ketones, hexane, methylene chloride, benzene, and acetone).

Health effects from mVOCs have not been comprehensively studied. Some mVOCs are implicated in trigeminal nerve irritation and odor-related health complaints.

Mycotoxins

Molds also produce complex products of secondary metabolism. These secondary metabolites include chemicals used to ensure that the molds maintain their niche within their current habitat. These chemicals may suppress the growth of bacteria or other molds (antibiotic effect), or may be toxic to other eukaryotic cells (mycotoxic effect),. Mycotoxins may function as inhibitors of DNA, RNA, and protein synthesis. The production of secondary metabolites

takes energy away from the growing fungi, and is a process used only as needed.

Mycotoxin formation is more likely when a mixture of microorganisms is present. This is often the case when molds grow indoors. Conversely, molds isolated and grown in pure cultures (cultures containing only one species/strain of organisms) stop making mycotoxins after a few generations.

The growth substrate, temperature, humidity, the species and strains of fungi, and the presence of competitive organisms determine the potential and rate of mycotoxin formation. The mycotoxins vary in toxic potency, mechanism, target species, and target organs.

Over 200 mycotoxins have been identified as being produced by some 300 different genera of mold. Some of the mycotoxins and the mold genera that produce them, include:

- Aflatoxin and sterigmatocysti form *Aspergillus* (various species).
- Anthoquinones (e.g., rugulosin), from *Penicillium islandicum*.
- Substituted coumarins (aflatoxins), from *Aspergillus flavus* and *A. parasiticus*.
- Epipolythiodioxoperazines (gliotoxin), from at least six species of *Aspergillus*, *Penicillium*, and *Stachybotrys*.
- Ergot alkaloids, from *Claviceps purpurea* and species of *Aspergillus, Rhizopus*, and *Penicillium*.
- Substituted furans (e.g., citreoviridin), from *Penicillium citreoviride*.
- Griseofulvins, from *Aspergillus*, *Memnoniella,* and *Penicillium*.
- Ochratoxins, from several species of *Aspergillus*, *Memnoniella*, and *Penicillium*.
- Quinones (citrinins), from several species of *Aspergillus* and *Penicillium*.
- Trichothecenes from *Fusarium*, *Stachybotrys*, and *Trichoderma*, among others. (*Note*: Trichothecenes include sesquiterpenes with a trichothecane skeleton, olefinic groups at C-9 and C-10, and epoxies at C-12 and C-13. Macrocyclic trichothecenes have a carbon chain between C-4 and C-15 in an ester or ether linkage (e.g., T-2 toxin, DON, satratoxins G and H; verrucarins B and J, trichoverrins A and B)).

Mycotoxins may include: butanol, estrogenic compounds (e.g., zearalenone), heptanone, lactones, lactams (patulin), stachybotrylactones, stachybotrylactams, 2-pentylfuran, 2-hexanone, 2-methyl-1-propanol, 3-methylfuran, 2-methylisoborneol, 3-methyl-2-butanol, and macrocyclic trichothecenes (Satratoxins F, G. and H, Roridine, Verrucarinj, and Trichoverrols).

Different strains of the same mold may make differing amounts and types of mycotoxins. Certain strains may not always produce mycotoxins, making it impossible to predict mycotoxin levels based solely on spore concentrations in air.

Mycotoxin Exposure Routes

Some mycotoxins cling to the mold spores' surfaces and may be found in dust as adsorbed or absorbed chemically on the dust particulates' matrix. Thus, the exposure pathways for mycotoxins include inhalation of mVOC vapors and inhalation of associated dusts or mold spores. Ingestion and dermal exposure through initial skin contact is also possible.

Aspergillus flavus and *A. parasiticus* produce the mycotoxin aflatoxin B1. Aflatoxin B1 is a carcinogenic chemical that can cause liver cancer. Both ingestion and inhalation are proven exposure routes. Aflatoxin B1 has been found on contaminated grains, peanuts, and other foodstuffs. *A. flavus* and *A. parasiticus* are not commonly considered an indoor contaminant, unless the grains, peanuts, cereal-based animal food, or other foodstuffs are stored in an indoor environment.

Studies that have investigated inhalation of mold and mold products found that inhalation produces more potent effects than ingestion. These effects are as potent as intravenous administration. Mycotoxins upon inhalation may produce immunosuppression, carcinogenesis, cytotoxicity, neurotoxicity (including acute or chronic central nervous system damage), mucous membrane irritation, skin rash, nausea, acute or chronic liver damage, and endocrine effects. These effects may be independent of infection or stimulation of antibodies (in contrast to the *Mycobacterium* mycotoxins).

Ingestion of moldy foods may also cause health effects such as liver damage, nervous system damage, and immunological effects.

Studies have included the effects from various exposure routes, including intravenous, intradermal, intramuscular, and intraperitoneal routes, as well as more natural dermal, ingestion, or inhalation routes.

Mycotoxin Toxicity

The mycotoxins differ in their absorption, toxicokinetics, toxicodynamics, target organs, metabolism, detoxification, and elimination due to differences in chemical structure. Mycotoxins also differ in potency, ranging from a lethal dose 50% (LD_{50}) in fractions of milligrams per kilogram to hundreds of milligrams per kilogram.

Some mycotoxins have only been tested for cytotoxicity, which is a relatively crude measure of effect

involving testing the toxin against isolated cells or tissues in culture.

The US Food and Drug Administration has set regulatory limits for aflatoxins produced by *Aspergillus flavus*. Health Canada has set limits for zearalenone (from *Fusarium* and some other molds). However, the majority of mycotoxins do not have regulatory limits.

The US Army Medical Research Institute for Infectious Disease has investigated a number of toxins for their potential to be used as weapons. Other toxins have been investigated because of their large economic impact on agricultural animals and crops.

With the exception of mycotoxins examined for military use, the bulk of research with animals has focused on the ingestion route given the potential for contaminated feed and fodder. The World Health Organization has investigated and sought control of mycotoxin exposure to humans.

More research is needed on other mycotoxins, including penicillic acid, roquefortine, cyclopiazoic acid, verrucosidin, rubratoxins A and B, PR toxin, luteoskyrin, cyclochlorotine, rugulosin, erythroskyrine, secalonic acid D, viridicatumtoxin, kojic acid, xanthomegnin, viomellein, chaetoglobosin C, echinulin, flavoglaucin, versicolorin A, austamide, maltoyzine, aspergillic acid, paspaline, aflatrem, fumagillin nigragillin chlamydosporol, and isotrichodermin.

Exposure Routes and Pathways

Effect often varies, depending on the degree of access of the exposure route to blood or lymph pathways. These pathways provide a means of distribution to target tissues for the specific poisons.

Dose and Effect

The doses necessary to establish effect levels for most types of mold exposures have not been established. Risk assessments are difficult due to the complex effects of mixtures of toxins and other physiologically active molecules produced by molds.

Animal experiments are unlikely to detect the incidence of toxic effects because of the low power of such studies. Low power is defined herein as investigations involving a small number of animals and animal homogeneity. This resultant small degree of test population variability requires that high doses of challenge agents be administered to observe an effect (e.g., 10 animals per dose group).

Allergic Responses

Adverse respiratory effects have been documented due to allergic responses to mold vegetative growth and spore proteins. Asthma can be triggered by a specific allergy, including an allergy to fungi.

Mucus within the respiratory tract traps fungi. If the fungi particulate is inhaled into the bronchi, the cilia of the bronchi attempt to move the mucus and entrapped mold particulate to the mouth and nose.

Chronic respiratory conditions are diagnosed by the examination of this mucus. However, for conditions requiring surgical intervention, the mucus is routinely suctioned out and discarded. Consequently, this mucus has not been available for examination and the subsequent examination of excised tissue may underestimate the fungi present.

Some research has concluded that in certain patients a cascading effect occurs. Eosinophils cluster around the fungi in the respiratory tract and produce toxins to subdue the fungi. In sinusitis, these toxins also destroy the outer lining of the sinus tissue, clearing the way for a bacterial infection that causes inflammation and sinusitis.

See also: Aflatoxin; Immune System; Mycotoxins; Penicillin; Respiratory Tract.

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Molecular Toxicology–Recombinant DNA Technology

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Molecular technology and recombinant DNA technology have revolutionized the field of toxicology. These techniques are powerful tools for better understanding and assessment of the mechanisms of action of substances that may adversely affect human and environmental health.

The application of recombinant DNA technology has accelerated scientific discovery in all areas of biological research to an unprecedented rate. This technology has enabled researchers to examine the molecular mechanisms and structures responsible for such complex processes as cell growth, metabolism, differentiation, and development. More significantly, it provides a means to manipulate molecules critical to these processes and an opportunity to examine the effects of these manipulations in living systems and elucidate the physiological roles of the protein under investigation. There is also a general consensus among researchers that the application of recombinant DNA techniques to problems in toxicology will have a profound impact on the future direction of scientific research.

Molecular toxicology has enabled toxicologists to understand events at the molecular level and examine alterations in fundamental biological processes that lead to the manifestation of toxic responses. As a result, toxicologists are examining the mechanisms of action of toxic substances in order to identify molecular changes predictive of exposure to harmful substances. This information can be used to identify susceptible groups within a population or establish safe levels of exposure using a mechanistic approach as an alternative to association of risk based on extrapolation of 'high-dose' studies in rodent models to low-level human exposures. This molecular or 'reductionist' approach is not intended to circumvent *in vivo* studies but to introduce mechanistic data into risk assessment in order to define the possible implications of exposure to potentially harmful substances. The inclusion of recombinant DNA technology in toxicological research has facilitated our understanding of the mechanisms of action of several toxic substances. This information has subsequently been used by toxicologists in risk assessment and in the development of assays that identify and assess the potential adverse effects posed by uncharacterized toxic substances.

Perhaps the single most important advance in the last 40 years of medical science was the development of the polymerase chain reaction (PCR) protocol. PCR exploits features of DNA replication, which provide researchers endless possibilities in the manipulation of DNA. The most powerful advantage of this technology is its ability to produce enormous numbers of copies of a specific DNA sequence from a minute sample. The technique involves two primers that are designed to be complementary to the boundaries of the desired DNA segment and DNA polymerase that produces copies of the DNA sequence between the primers (**Figure 1**). The cycling of this replicative reaction results in the exponential production of a DNA fragment whose boundaries are determined by the primers. PCR can also be used to isolate, characterize, or quantify mRNA through the use of reverse-transcriptase PCR (RT-PCR), which uses viral reverse transcriptase enzymes to produce complimentary DNA (cDNA) sequences from isolated mRNA. PCR has applications not only in research

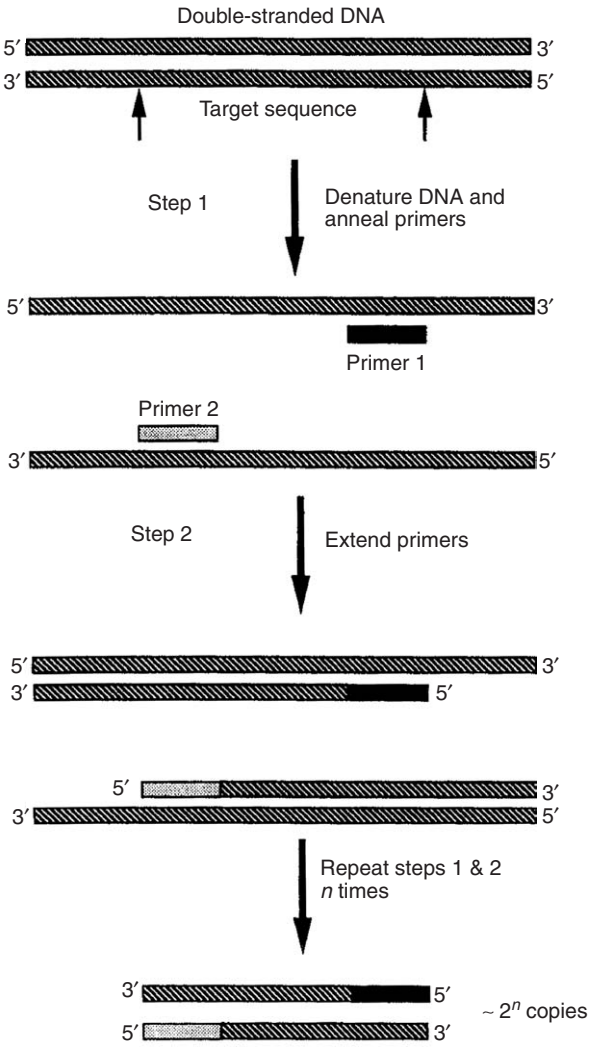

Figure 1 A schematic representation of the polymerase chain reaction (PCR). Repetitive cycles of DNA denaturation followed by annealing of the primers and primer extension results in the exponential amplification of the DNA fragment whose boundaries are determined by the primers.

but also in forensic toxicology, evolutionary studies, and in the diagnosis of infectious diseases, genetic abnormalities, and cancer. This technique is so sensitive that forensic scientists can obtain sufficient amounts of DNA extracted from saliva left on postage stamps or discarded cigarettes to implicate individuals in criminal activities. More recent advances in PCR technology have produced 'real-time' PCR, in which the PCR reaction can be monitored in real-time with the use of fluorescent dyes or labeled DNA probes. This technique is even more sensitive than standard PCR, and has proved to be significantly more effective for quantification of gene expression.

Recombinant DNA technology has provided a dramatic expansion in our knowledge of the structure, function, multiplicity, and regulation of xenobiotic metabolizing enzyme (XME) superfamilies. Its use has resulted in the sequencing of the entire human genome, and the cloning of cDNA sequences that encode or act as the blueprint for the synthesis of proteins that are important in toxicology. The increased availability of cDNA and deduced protein sequences has provided a rational foundation for the development of standardized nomenclatures based on amino acid sequence similarities. These systems are a welcome alternative to the multiple, laboratory-specific classifications that have led to considerable confusion particularly in the P450 superfamily, in which over 200 unique cDNAs have been identified. In addition to the characterization of the human enzymes, XMEs from a variety of other species have been cloned using the same techniques. Comparisons of the XME sequences between species can enhance our understanding of the critical amino acids for enzyme functionality, and has led to rapid advances in the field of evolutionary toxicology.

A major emphasis in drug metabolism research has been focused on determining the role of individual XMEs in the *in vivo* biotransformation of drugs and chemicals in order to understand their role in eliciting adverse drug reactions. The identification of specific XMEs that are responsible for adverse drug effects has been difficult since multienzyme superfamilies made up of enzymes with diverse structure and overlapping substrate specificities are involved in metabolism. A further complication has been the relatively low abundance of individual forms of XMEs. This limitation has been overcome by using heterologous expression systems to produce large quantities of specific XMEs. These systems use cloned XME cDNAs and take advantage of the protein production machinery in bacteria, yeast, insect, or mammalian cells in culture to overexpress large quantities of a desired protein. Depending on the system that is utilized, proteins can be generated by simply introducing the appropriate vector containing the desired cDNA into the cells of choice. Expression of these proteins can be greatly enhanced through the use of a vector containing a strong enhancer element, such as the SV40 enhancer. The enzymatic activity of recombinant proteins can be directly measured from whole cells or isolated subcellular fractions. Heterologous expression systems have also been used to produce sufficient quantities of desired protein for subsequent purification to homogeneity and biophysical investigations. Many heterologous expression systems are commercially available and provide all the necessary materials to express the desired product in a variety of organisms.

Bacterial, yeast, and mammalian cells have been utilized in novel ways to develop or improve assays

for the identification of drug metabolizing enzymes involved in the bioactivation and detoxification of xenobiotics. In addition to the use of immortalized cell lines, more effective techniques of isolating and culturing primary cell lines have enabled the use of these cell types as well. Protocols have been developed which allow for the expression of XMEs in these cell culture systems, as well as manipulation of XME structure, function, and expression levels (**Figure 2**). However, these studies are limited by the use of a single cell type, which may not properly model the *in vivo* toxicity. For example, xenobiotics which are bioactivated in one organ system or tissue, but exert their toxic effects in another, require the use of two different cell lines for proper understanding of the overall mechanism. Another complication in the use of these expression systems can be altered physiology brought on by *in vitro* culturing. Maintenance of cells under these artificial growth conditions can lead to changes in gene and protein expression. This is particularly important when considering toxicants that may effect the regulation of cellular proliferation, which is typically altered in immortalized cell lines. In addition, results produced using human cell lines do not reflect the overall genetic variability of our species, but rather only the particular XME genotype of the individual(s) from which the cell line was produced.

Cell lines have also been produced which stably express a specific XME isozyme. For example, cDNAs encoding for detoxification enzymes, such as *N*-acetyltransferase and glutathione *S*-transferase, have been introduced into Ames tester strains and mammalian cells, which are naturally devoid of these enzymatic activities. This has enabled researchers to examine the mutational specificity of a chemical following metabolism or bioactivation by specific detoxification enzymes. Furthermore, a series of bioengineered lymphoblastoid cell lines expressing various human P450s and phase II enzymes have been prepared and are commercially available. Results from these *in vitro* test systems combined with data gained from experiments investigating differential regulation, tissue-specific expression, and interindividual variation in human XMEs should provide a sound mechanistic basis for assessing human risk following procarcinogen and promutagen exposures.

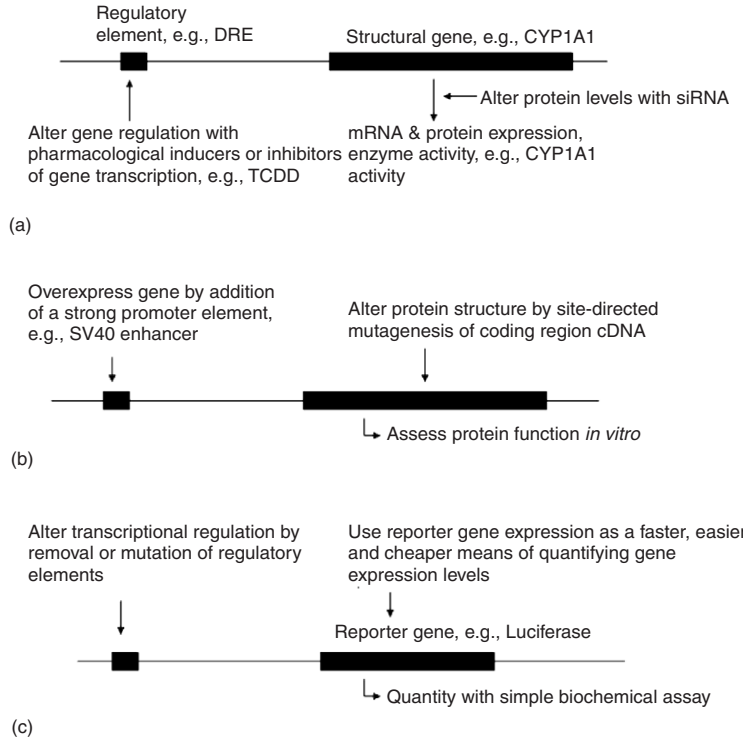

Figure 2 Methods of altering XME gene expression *in vitro*. (a) Modulation of endogenous gene activity. Gene transcription can be induced by the application of pharmacological inducers, such as TCDD for CYP1A1, or inhibitors. siRNA can be used to 'knock down' protein expression. (b) Expression of functional cDNAs. Vectors containing XME cDNAs can be introduced to a cell culture expression system. Strong positive regulators such as the SV40 enhancer can be used to drive overexpression of the XME gene product. XME structure and function can be altered by site-directed mutagenesis of the cDNA. (c) Expression of regulatory region and reporter gene. The 5′ regulatory region or 'promoter' can be fused to a reporter gene such as luciferase, which allows for the rapid analysis of gene expression using a simple biochemical assay. Specific regulatory elements within the promoter can then be removed or mutated, allowing for identification of the critical elements for control of XME expression.

The recent development of short-inhibitory RNA (siRNA) has added even more power to the use of cell-culture-based expression systems. siRNA takes advantage of an unexpected mechanism by which a short, double-stranded RNA of known sequence will initiate the degradation of complimentary RNA. This allows researchers to 'knock-down' the expression of proteins by introducing these siRNAs via the same protocols commonly used to introduce exogenous cDNA expression or reporter constructs. Although some difficulties in design and effectiveness of siRNAs still exists, this technique, together with traditional expression vectors, allows for the inhibition or overexpression of most genes whose mRNA sequences have been characterized. Importantly, these techniques allow toxicologists the opportunity to modulate the function of endogenous XMEs within an expression system.

In addition to the controlled expression of genes made possible by available expression systems and siRNA, specific control of gene expression in the whole animal has been made possible by recombinant DNA technology. These transgenic and 'knock-out' animals have been invaluable in the dissection of a number of important toxicological pathways, and are discussed further elsewhere in the encyclopedia.

Recombinant DNA technology has also led to techniques in which individual bases in a cDNA sequence can be changed with great precision in the laboratory. This allows for site-directed mutagenesis, which in addition to altering the sequence of a cDNA, can alter the amino acid sequence of the protein produced in expression systems. This is of great importance in the study of the molecular mechanisms of XMEs, as individual amino acids within the active sites of these enzymes can be changed sequentially to determine their relative importance in the functionality of the enzyme. Heterologous expression technology has also been an important technique in assessing the functional consequences of XME polymorphisms. Expression of cDNAs with variant DNA sequences using heterologous systems allows one to identify protein products with altered catalytic activity without having to perform lengthy, labor-intensive purification protocols from tissue samples from multiple phenotypic populations. In addition, these studies may assist in the identification of critical residues within XMEs that are required for optimal catalytic activity. This information may lead to improved drug design and efficacy by introducing modifications that minimize metabolic transformations to reactive metabolites that are responsible for eliciting adverse reactions, or facilitate synthesis of prodrugs that exhibit better pharmacokinetic properties.

The advancement of recombinant DNA technology has also allowed for the study of proteins in a 'modular' sense. Individual domains of proteins can be removed entirely by manipulation of the cDNA sequence to determine their function. Furthermore, domains from entirely different proteins can be fused together to create fusion proteins. One example of the usefulness of this technique is the creation of GST-fusion proteins, in which a small domain from the glutathione-*s*-transferase (GST) gene is fused to a gene of interest before introduction into an expression system. The small GST domain usually does not impact the functionality of a protein, but it allows for the fast and simple purification of the fusion protein using an affinity column. A second example of the usefulness of fusion proteins is the yeast two-hybrid system. In this system, a gene of interest is fused to the DNA binding domain of a yeast transcription factor, such as Gal-4, and a library of cDNA sequences from the same species or cell type are fused to the transactivation domain of the same yeast transcription factor. These fusion cDNAs are then introduced into an expression system in yeast, and proteins that physically interact with the protein of interest bring the DNA binding domain and the transactivation domain of Gal-4 into close proximity, activating expression of a reporter gene. The cDNA sequences can then be isolated, cloned, and the identity of the interacting proteins discovered, even if they were not previously characterized. This technique is a powerful tool for identification of proteins that interact directly with XMEs or transcription factors that regulate XME expression.

In addition to investigating the structural features of drug metabolizing enzymes, molecular biology has also enhanced our understanding of how the expression of these enzymes is regulated. The XME whose transcriptional regulation is the most well characterized is CYP1A1. Its expression has been linked to the bioactivation of a number of carcinogens and studies have found that it is inducible by a variety of structurally diverse compounds, including 3-methylcholanthrene, β-naphthoflavone, and halogenated aromatic hydrocarbons such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). The induction of CYP1A1 expression is mediated by a specific cytosolic protein referred to as the aryl hydrocarbon receptor (AhR). The AhR is a ligand-inducible transcription factor that contains separable and distinct domains for ligand binding, DNA binding, and transcriptional activation for gene expression. The AhR is a member of PAS (PER, ARNT, SIM) family of proteins which also includes its dimerization partner, the Ah receptor nuclear translocator (ARNT), hypoxia inducible factor-alpha (HIF1-Alpha), and period

(PER1). The members of this protein family tend to act as environmental sensors. For instance, while the AhR mediates responses to xenobiotics, HIF1-Alpha mediates responses to low oxygen levels, and PER1 and MOP3 are involved in the circadian rhythmicity, which, in part, is a response to light.

Ligand binding to the AhR causes the release of cytoplasmic cofactors, which enables the receptor to move into the nucleus and heterodimerize with ARNT. The resulting complex exhibits high affinity for DNA and seeks out specific DNA sequences referred to as dioxin or xenobiotic response elements (DREs or XREs) located in the 5′ regulatory region of the CYP1A1 gene. Binding of the complex to DREs results in the recruitment of factors that facilitate the increased rate of CYP1A1 gene transcription. Analysis of the CYP1A1 5′ regulatory region has identified six DREs, all of which contain the core sequence, 5′-GCGTG-3′, and have a role in the dramatic increase in CYP1A1 activity following treatment with TCDD. In addition to CYP1A1, other TCDD-inducible genes such as the rat glutathione S-transferase Ya subunit, CYP1A2, and quinone oxidoreductase also possess DREs in their 5′ regulatory regions. Although the mechanism by which TCDD and related compounds cause toxicity has not been fully elucidated, it is generally believed that the AhR plays a role. In support of this, mice lacking the AhR are virtually immune to the effects of TCDD, and CYP1A1 null mice are resistant to some, but not all, of the toxic effects of this toxicant. Molecular toxicology has also provided great advances in our understanding of the role of XME and XME-regulating genes in normal physiology. For instance, mice lacking the AhR exhibit a number of cardiovascular and reproductive defects that suggest that this protein plays a role in normal physiology, in addition to its control of XME induction.

Receptors are not the only mechanisms used by toxic substances to affect gene regulation. Reactive oxygen intermediates (ROIs), such as superoxide and hydroxyl radicals, are continuously produced in cells as side products of electron-transfer reactions. Oxidative stress occurs when abnormally high levels of ROIs are produced within a cell following exposure to physical, chemical, and biological agents including UV radiation, alkylating agents, hydrogen peroxide, metals, cytokines, and other natural ligands for cell surface receptors. In addition to causing cellular damage, ROIs also induce the expression of a number of genes by the induction and activation of transcription factors such as c-*fos*, c-*jun*, and NF-kappa-B. c-*Fos* and c-*jun* are members of the AP-1 transcription factor family, which forms *fos-jun* or *jun-jun* dimers prior to binding to specific DNA sequences located in the 5′ regulatory region of target genes. These specific DNA sequences or response elements have been found to regulate the expression of numerous genes, including enzymes with radical scavenging or DNA repair activities, which serve to protect the cell from oxidative damage and repair damage that has already occurred.

Methods for investigating the control of XME gene expression by the AhR, AP-1, and other transcription factors have also been greatly enhanced through the use of recombinant DNA technology. The 5′ regulatory region, or promoter sequences, of XME genes can be linked to reporter genes that are then controlled in an expression system via the same mechanisms by which XME expression is controlled. This allows for the study of critical response elements that are involved in the control of XME expression. Examples of reporter genes include firefly luciferase, bacterial chloramphenicol acetyltransferase, bacterial β-galactosidase, green fluorescent protein, and heat-resistant mammalian alkaline phosphatase. Reporter genes provide sensitive, rapid, and easily measured enzymatic activities that are usually absent in the host cell. The XME promoter can then be altered by the same site-directed mutagenesis methods that are used to alter amino acid sequence in the coding region of the XME for functional analysis. Results of these studies can pinpoint the regulatory elements involved in controlling gene expression. For instance, removal of the DRE sequences in the CYP1A1 promoter eliminates the ability of TCDD to induce expression of this gene.

Reporter gene technology has also been exploited to develop bioassays that assist toxicologists in the detection and assessment of potentially toxic substances. These bioassays consist of reporter genes whose expression is controlled by the 5′ promoter of a target gene. This allows for identification of substances that activate gene expression with a simple biochemical assay, and without direct mRNA quantification. Several different reporter gene bioassays are currently being used to assess the potential toxicity of individual chemicals as well as complex mixtures. These bioassays can detect a number of different substances including halogenated aromatic hydrocarbons, sex steroid mimetics, peroxisome proliferators, metals, and inducers of oxidative stress.

Pharmacogenetics, the study of genetically determined variations in drug response, has been profoundly transformed by molecular biology. Genetic polymorphisms have been traditionally defined as the occurrence in a population of more than one allele of a particular gene with the prevalence of the less common form being at least 1%. Prior to recombinant DNA technology, genetic polymorphisms were

identified by familial and population studies following the observation of an atypical drug reaction in a population. Classification of individuals as poor or extensive metabolizers was determined by measuring drug clearance of a substance that was metabolized by a specific XME. On this basis it became evident that there were genotypic variants in a wide variety of human XMEs. Recombinant DNA technology has advanced the characterization and study of these polymorphisms tremendously. Literally hundreds of polymorphisms in cytochrome P450s and other drug metabolism enzymes have now been identified (**Table 1**). It is believed that adverse drug reactions account for up to 5% of all hospital admissions in the United States, and many of these reactions may be due to XME polymorphisms. Also, polymorphisms in proteins which are not involved in xenobiotic metabolism, but which have substantial effects on the therapeutic effectiveness or tolerance of certain drugs have also been identified. For example, polymorphisms in glucose-6-dehydrogenase, which reduce the activity or expression of this enzyme affect more than 400 million people worldwide, and can cause adverse reactions to primaquine and sulfonamides.

In addition to altering the metabolism of, and causing adverse reactions to pharmacological agents, XME polymorphisms also have the potential to alter the susceptibility of individuals to environmental toxicants. Many xenobiotics are metabolized by XMEs to form toxic metabolites which then led to toxicity, and functional alterations of these XMEs could lead to altered production of these toxic metabolites. For example, a number of XME polymorphisms are linked to increased risk for smoking-related cancers. However, the link between XME polymorphisms and cancer risk is poorly understood. Epidemiological studies of large populations have shown only a moderate increase in risk for the development of these cancers in individuals with XME polymorphisms. These results are confounded by the involvement of multiple polymorphisms, the genetic variability of the human population in general, and in difficulties in assessing exposure levels. Transgenic mice may add to our understanding of the role of XME polymorphisms in potentiation or protection from xenobiotic toxicity. For example, mice lacking the AhR are immune to benzo(*a*)pyrene carcinogenicity, presumably due to an inability to induce CYP1A1 expression.

While these results are intriguing, there are no known human polymorphisms that completely disrupt the function of the AhR or CYP1A1. Interestingly, however, persons with 'high-inducibility' of P4501A1 have been shown to be at a higher risk for the development of certain smoking-related cancers.

By combining allele-specific DNA sequence information with PCR, noninvasive assays have been developed that can be rapidly performed to identify individuals susceptible to adverse drug reactions. The three strategies that have been successfully employed include the amplification of a DNA sequence that contains a restriction fragment length polymorphism (RFLP) which identifies a variant phenotype, the use of allele-specific PCR primers designed to hybridize to a sequence only if a known variation is present, and the use of allele-specific DNA probes during real-time PCR (**Figure 3**).

RFLPs involve the digestion of DNA with purified bacterial enzymes, known as restriction endonucleases, which recognize and cleave specific DNA sequences. The digested DNA fragments are subsequently separated based on size using gel electrophoresis and the resultant DNA fragment lengths are determined by comparison to a known standard. These fragments can then be further amplified and sequenced for exact characterization of the polymorphism. RFLPs have been used in the identification of DNA sequence changes that are responsible for genetic diseases as well as in forensic science in order to establish an association between an individual and the human tissues such as blood, saliva, or semen collected at a crime scene. Genetic polymorphisms that involve changes in DNA sequence at restriction enzyme recognition sites can be directly detected following restriction enzyme digestion of both normal and variant DNA. However, polymorphisms that alter

Table 1 Some common XME polymorphisms and their clinical importance

Enzyme	*Substrates*	*Functional defect*	*Adverse clinical effects*
CYP1A2	Olanzapine	Reduced enzyme induction	Enhanced side-effects
Cyp2C8	Taxol	Reduced metabolism	Altered pharmacokinetics, toxicity
CYP2C9	Phenytoin warfarin	Reduced metabolism	Lower therapeutic doses, toxicity
CYP2C19	Diazepam	Reduced metabolism	Prolonged sedation
CYP2D6	Perhexilene	Reduced metabolism	Hepatotoxicity and neuropathy
CYP3A4	Nefedipine	Reduced metabolism	Altered pharmacokinetics
NAT2	Sulfonamides	Reduced activity	Hypersensitivity
Aldehyde dehydrogenase 2	Ethanol	Inactive enzyme	Decreased tolerance for alcohol

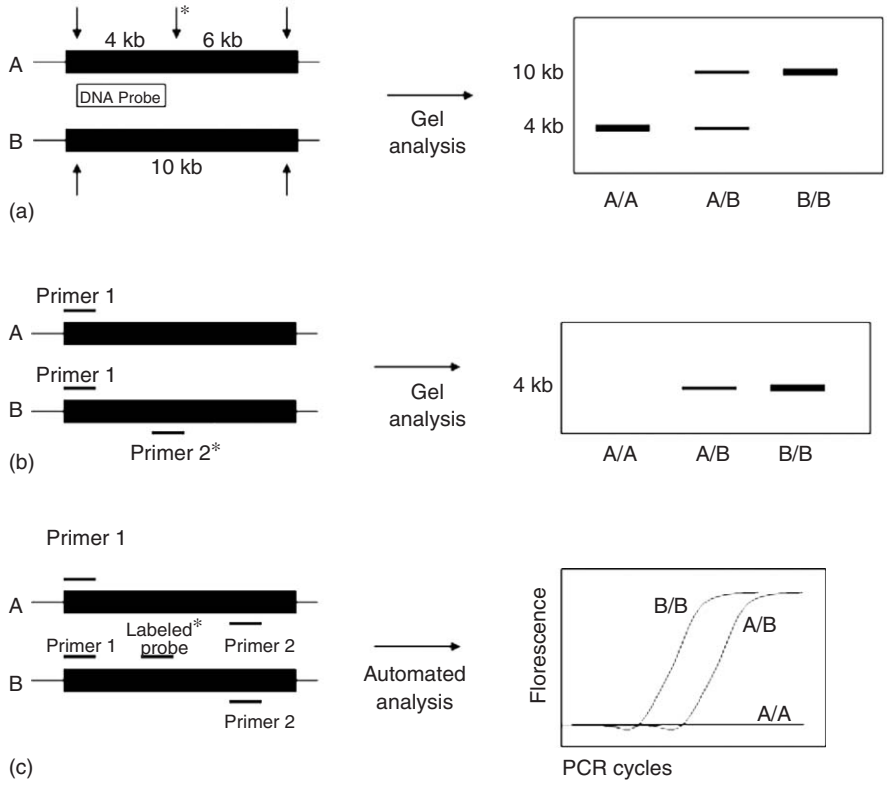

Figure 3 Methods for identification of XME polymorphisms. (a) RFLP analysis. Genomic DNA is digested with restriction endonucleases, hybridized to a radioactive DNA, and run on a gel. The asterisk indicates a restriction endonuclease site which is found only in variant A. Gel analysis demonstrates three different banding patterns, corresponding to the three possible genotypes. (b) XME polymorphism identification using allele-specific primers. Standard PCR is performed using a primer that only recognizes one allele. The resulting products are run on a gel, and visualized as bands following staining with an intercalating dye. No bands are seen in A/A individuals, a strong band in B/B individuals, and an intermediate band in A/B individuals. (c) XME polymorphism identification using real-time PCR. A florescent-labeled DNA probe which only recognizes variant B is used to monitor the progress of the PCR reaction. A/A individuals produce no florescence, because the probe will not bind variant A. B/B individuals will produce a rapid increase in florescence as the product is produced, and A/B individuals will produce a slower increase in florescence, due to the 50% reduction in available B-alleles at the start of the PCR reaction.

restriction endonuclease recognition sites and cause abnormalities in protein function are rare. It should also be emphasized that the majority of the DNA sequence variations located in or around a gene do not necessarily explain the observed phenotypic variants since many of the differences detected by RFLP analysis are silent polymorphisms that do not enchanges at the protein level and, therefore, have no functional consequences. For example, in the CYP2D6 polymorphism, digestion of genomic DNA with 20 different restriction enzymes identified the presence of 14 different RFLPs. However, only two of these RFLPs correlated with specific DNA sequence variations that were associated with the poor metabolizer phenotype.

In contrast to RFLP analysis and standard PCR techniques, real-time PCR analysis can easily and rapidly detect a single nucleotide polymorphism (SNP) in a gene of interest without the need to run the products on a gel. This technique uses a florescent dye-labeled probe that specifically recognizes and binds to polymorphic sequences during the PCR reaction, and is detected in real-time using a sensitive camera. Real-time PCR is extremely fast and sensitive, and has been used successfully to genotype a number of important polymorphic genes.

The development of these techniques for rapid analysis of XME polymorphisms may eventually lead to routine, detailed genotyping in a clinical setting. Indeed, the use of PCR-based genotyping to determine the proper pharmacological intervention in patients who may be treated with drugs which are metabolized by polymorphic XMEs has been widely suggested. This may be particularly useful prior to the use of drugs metabolized by CYP2C9, CYP2D6, and CYP3A4, which are responsible for metabolism of 60–70% of all therapeutic drugs, and are highly polymorphic. However, the use of these technologies

to determine individual XME genotypes within a clinical setting must be balanced with ethical concerns regarding patient confidentiality.

The ability of toxic substances to induce gene expression has been successfully used to develop assays that assist in the identification of substances that may cause adverse effects. In the past, toxicologists relied on physiological responses, such as decreased body weight or lethality, to assess the effects of a toxic substance. As more information regarding the mechanism of action of compounds was acquired, biochemical responses such as enzyme activities were used to determine the potential adverse health effects resulting from exposure to toxic substances. Molecular toxicology provides techniques such as RT-PCR and real-time PCR, which have enabled toxicologists to directly measure subtle changes in the expression of specific target genes. Recombinant DNA technology has also provided strategies for toxicologists to identify target genes that are directly involved in eliciting the observed toxic responses. Differential hybridization to microarrays containing thousands of genes has been successfully used to identify structurally unrelated genes that are regulated by a common mechanism such as exposure to a specific chemical agent. These powerful techniques are discussed in a separate chapter.

The development of recombinant DNA technology and molecular biology techniques has accelerated the rate of discovery in all major areas of biological research. The incorporation of this technology into the field of toxicology will enhance both the basic and applied aspects of the discipline. These technological advances have already enabled toxicologists to elucidate the mechanisms of action of toxic substances at the molecular level and this information has been successfully used to engineer and improve the sensitivity and specificity of a number of assays commonly used to assess the potential toxicity of a substance. Furthermore, the use of this technology has also contributed to our understanding of the normal physiological roles of proteins and enzymes that are disrupted by toxic substances. In conclusion, the use of recombinant DNA technology will extend the comprehensive nature of toxicology and will assist toxicologists in identifying and predicting the potential risks an unknown substance may pose to human health and environmental quality.

See also: Analytical Toxicology; Carcinogen–DNA Adduct Formation and DNA Repair; Developmental Toxicology; Genetic Toxicology; Genomics, Toxicogenomics; GF; Mechanisms of Toxicity; Microarray Analysis; Risk Assessment, Human Health; Toxicity Testing, Mutagenicity; Transgenic Animals.

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Molinate

Danny Villalobos

- Chemical Names: *S*-Ethyl azepane-1-carbothioate; *S*-Ethyl perhydroazepine-1-carbothioate; *S*-Ethyl perhydroazepine-1-thiocarboxilate; *S*-Ethyl hexahydro-1*H*-azepine-1-carbothioate (9CI)
- Chemical Abstracts Service Registry Number: CAS 2212-67-1
- Synonyms: Hydram; Ordram; Yalan
- Chemical/Pharmaceutical/Other Class: Thiocarbamate herbicide
- Chemical Formula: $C_9H_{17}NOS$

Uses

Molinate is a selective thiocarbamate herbicide used to control broad-leaf and grassy plants primarily in rice production. Molinate is available in granular and emulsifiable liquid formulations. Recently, US manufacturers of molinate have requested voluntary cancellation of all uses of molinate by 2009.

Exposure Routes and Pathways

Potential routes of exposure to molinate include inhalation (for mixers, applicators, field workers, and residents of rice-growing regions), dermal (for mixers, applicators, field workers, and anyone exposed to drift of spray droplets or residues on plants), and dietary (from drinking water sources contaminated with molinate and from residues on rice and rice products).

Toxicokinetics

Molinate is well absorbed by the oral route. It is widely distributed with highest concentrations remaining in the circulation. There is no evidence for accumulation of molinate. It is nearly completely excreted within 48 h, primarily via the urine. The primary biotransformation pathway for molinate in rats is *S*-oxidation to sulfoxide, followed by hydrolysis (hexamethyleneimine) or conjugation (mercapturate). In humans, ring hydroxylation is the primary route followed by glucuronidation.

Mechanism of Toxicity

Reproductive effects of molinate may depend on inhibition of neutral cholesterol ester hydrolase, leading to disruption of mobilization of cholesterol from high-density lipoprotein, a process selective for rodents. However, the selectivity of this mechanism or others in rodents relative to reproductive toxicity remains unclear. Molinate can inhibit acetylcholinesterase as well as aldehyde dehydrogenase.

Acute and Short-Term Toxicity (or Exposure)

Animal

Molinate is moderately toxic by ingestion, with reported oral LD_{50} values of 369–720 mg kg^{-1} in rats and 530–795 mg kg^{-1} in mice. Dermal LD_{50} values are 4000–4800 mg kg^{-1} in rats. It is mildly irritating to rabbit skin and moderately irritating to rabbit eyes, and is not a skin sensitizer. A 4 h inhalation LC_{50} of 1.36 mg l^{-1} indicates moderate toxicity by this route as well. Some formulations show a lower degree of acute toxicity. Molinate is more acutely toxic to developing organisms.

Human

Persons poisoned by contaminated well water demonstrated rapid onset signs and symptoms including abdominal and gastrointestinal disorders, fever, weakness, and conjunctivitis. These signs and symptoms disappeared rapidly and there was no evidence of persistent sequelae.

Chronic Toxicity (or Exposure)

Animal

Molinate has been shown to adversely affect reproduction/fertility in the rat. Administration of molinate to young male rats at a dose of 3.6 mg kg^{-1} day^{-1} for 2 months caused changes in spermatozoa but did not decrease sperm fertility. When these rats were mated to normal females, many of the embryos were resorbed and postnatal mortality was increased. Sertoli cells appeared to be directly affected as neither gonadotropin nor androgen levels were affected. Female reproductive function also appeared sensitive to molinate. Molinate also led to developmental defects in rats including reduced/retarded uterine growth, incomplete ossification, and dilated brain ventricles, but a potential role for maternal toxicity was unclear. Molinate is a cholinesterase inhibitor and elicits signs of neurotoxicity, with excess salivation being a relatively sensitive response. Molinate did not cause segmental demyelination as do some other thiocarbamates (e.g., disulfuram). Molinate has been reported to potentiate the delayed neurotoxicity of some

organophosphorus toxicants. Interestingly, while not an organophosphorus molecule, there are data suggesting that molinate can cause delayed neurotoxicity in the hen.

Human

In a study of male workers between 1980 and 1982 at a molinate production facility, measurements were made on reproductive parameters including sperm concentration, motility, morphology, and serum follicle-stimulating hormone (FSH), leuteinizing hormone (LH), and testosterone levels. The study provided little evidence of an effect of molinate on sperm or serum hormone levels. Subsequent analysis by US Environmental Protection Agency concluded there was a slight decrease in number of children especially for the high exposure classification, both between production cycles and during peak production exposure. Molinate showed no evidence of skin sensitization in agricultural workers.

In Vitro Toxicity Data

Molinate was positive in the mouse lymphoma (with metabolic activation) and mouse micronucleus assays. It was negative in other mutagenesis tests including Ames assays.

Clinical Management

Symptoms of exposure to molinate include skin sensitization, nausea, diarrhea, abdominal pain, fever, weakness, and conjunctivitis.

Molinate has the potential to interact with the endocrine system. The primary target organ affected by molinate is the thyroid.

Environmental Fate

Chlorination during water purification treatment converts molinate to molinate sulfoxide. Complete degradation to noncarbamate compounds requires other oxidants, such as chloramination, potassium permanganate, or ozone. Molinate is of low persistence in the soil environment, with a half-life of 5–21 days. It is poorly bound to soils, relatively soluble in water, and thus may be mobile. Soil microorganisms are responsible for most degradation of molinate. Molinate may rapidly volatilize if not plowed into the soil, and may undergo degradation by sunlight. Molinate may be degraded by hydrolysis. Molinate is rapidly taken up by plants and transported to leaves. In the leaves, molinate inhibits leaf growth and development.

Ecotoxicology

Molinate appears to be practically nontoxic to birds. It poses an acute risk to fish, amphibians, and aquatic invertebrates, limited to organisms in small streams and tributaries that are subject to high exposure from rice drainage. Chronic risk to freshwater fish is possible. Molinate also poses a chronic risk to freshwater invertebrates that live in agricultural drains and small rivers. Molinate poses low acute risk to estuarine fish and invertebrates. It poses a risk to nontarget aquatic plants.

Exposure Standards and Guidelines

The reference dose for molinate is $0.002\,\mathrm{mg\,kg^{-1}\,day^{-1}}$.

Miscellaneous

Based on currently available animal data, molinate is shown to be a reproductive toxicant. The registrant (Syngenta) and the California Rice Commission are developing mechanistic data that they believe will support their conclusion that this effect is rodent specific.

See also: Carbamate Pesticides; Cholinesterase Inhibition.

Further Reading

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Relevant Websites

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.

http://www.epa.gov – US Environmental Protection Agency.

Molybdenum

Robert Kapp

- Related Compounds: Molybdenum dioxide, MoO_2 (CAS 18868-43-4); Molybdenum trioxide, MoO_3 (CAS 1313-27-5); Molybdenum pentachloride, $MoCl_5$ (CAS 10241-05-1)
- Chemical Abstracts Service Registry Number: CAS 7439-98-7
- EINECS No.: 231-107-2
- Synonyms: Amperit (105.054); Amperit (106.2); MChVL Metco (63); Molybdenum, metallic; TsM_1
- Chemical/Pharmaceutical/Other Class: Transition metal.

Uses

Molybdenum occurs naturally in various ores, the most important being molybdenite (MoS_2), which is converted to molybdenum trioxide (MoO_3) for use in ferro- and manganese alloys, chemicals, catalysts, ceramics, and pigments. Molybdenum is a valuable alloying agent because it contributes to the hardening and resilience of quenched and tempered steels. It also increases the strength of steel at high temperatures. Molybdenum is used in electrodes for heated glass furnaces, in nuclear energy applications, in missile and aircraft parts, in the production of tungsten, in glass to metal seals, and in colloidal form as a lubricant additive. It has additional applications in petroleum refining as a catalyst. Generally, molybdenum is prepared from the powder made from hydrogen reduction of purified molybdic trioxide or ammonium molybdenate. Molybdenite is also recovered as a by-product of copper and tungsten mining operations.

Exposure Routes and Pathways

The primary pathway for molybdenum exposure is ingestion by water or food. Molybdenum is found in leafy vegetables, legumes, meat, and many grains. Molybdenum does not appear to be absorbed dermally. Dusts and fumes may be inhaled. In ambient air in urban areas, molybdenum ranged from 0.01 to 0.03 $\mu g\,m^{-3}$, and in nonurban areas it varied between 0.001 and 0.0032 $\mu g\,m^{-3}$.

Toxicokinetics

Water-soluble molybdenum compounds are readily taken up through the lungs and the gastrointestinal tract; but insoluble compounds are not. Following absorption, molybdenum is distributed throughout the body with the highest levels generally found in the liver, kidneys, spleen, and bone. Limited data suggest that 25–50% of an oral dose is excreted in the urine, with small amounts also eliminated in the bile. The biological half-life may vary from several hours in laboratory animals to as much as several weeks in humans. The vast majority of molybdenum found in the liver is concentrated in the outer membrane of the mitochondria where it is readily available as a cofactor in enzyme reactions. Molybdenum is excreted rapidly primarily as in the urine as molybdate.

Mechanism of Toxicity

The physical and chemical state of molybdenum, route of exposure, and compounding factors such as dietary copper and sulfur levels may all affect toxicity. The mechanism of molybdenum toxicity is not yet understood, but is assumed that a primary factor is the formation of a copper–tetrathiomolybdate complex in the reduction medium of the gastrointestinal tract, reducing the biological utility of copper. Molybdenum is considered an essential trace element. It functions as an electron transport agent in the molybdenum–flavoprotein enzyme, xanthine oxidase, and is also a cofactor for aldehyde oxidase, NADH-dehydrogenase, xanthine dehydrogenase, and sulfite oxidase. The molybdate ion (MoO_4^{2-}) inhibits glutaminase and sulfoxidase. The absence of molybdenum creates an interruption of sulfur-containing amino acid metabolism.

Molybdenum deficiency is manifest by alterations in the uric acid and sulfite metabolism, often noted by the development of mouth and gum abnormalities, hypouricemia, hyperoxypurinemia, and eventually coma.

Acute and Short-Term Toxicity (or Exposure)

Animal

Severe gastrointestinal irritation, diarrhea, coma and death from cardiac failure can be symptoms of acute exposure to molybdenum (molybdenosis). The rat oral LD_{50} values are 188 $mg\,kg^{-1}$ (125 $mg\,Mo\,kg^{-1}$) for molybdenum trioxide, and 680 $mg\,kg^{-1}$ (370 $mg\,Mo\,kg^{-1}$) for ammonium molybdate. Oral LD_{100} values of 2200 $mg\,kg^{-1}$ (1200 $mg\,Mo\,kg^{-1}$), 1870 $mg\,kg^{-1}$ (1020 $mg\,Mo\,kg^{-1}$), and 2400 $mg\,kg^{-1}$ (1310 $mg\,Mo\,kg^{-1}$) have also been reported for

guinea pigs, rabbits and cats, respectively, dosed with ammonium molybdate. Inhalation exposures to molybdenum compounds have resulted in respiratory tract irritation, pulmonary hemorrhages, perivascular edema, and liver and kidney damage. Other effects reported in animals include muscle incoordination, loss of hair, loss of weight, changes in electrocardiograms, increased arterial blood pressure, increased serum lactate dehydrogenase, increased cardiac adrenaline and noradrenaline levels, and inflammation of the uterine horns with necrotic foci and endometrial atrophy. Some molybdenum compounds, such as molybdenum trioxide and sodium molybdate (Na_2MoO_4), are strong eye and skin irritants; however, others such as calcium and zinc molybdate are not primary irritants. Molybdenum was one of seven metals reported to cause abnormalities in chick embryos following injection of 4–1000 g sodium molybdate into the air sac of the egg on day 2 of incubation. Neck defects, hemorrhages and reduced body size were the most common abnormalities; however, there was no clear dose–response relationship. Diffuse pneumoconiosis with interstitital pneumonia was observed after 9 months upon histological examination in rabbits that had been given a suspension of powdered molybdenum intratracheally in doses of 70–80 mg kg^{-1}.

Human

In general, molybdenum and its compounds are considered to be of low toxicity to humans; however, molybdenum dust and fumes can cause irritation of the eyes, nose, throat, and respiratory tract. The trioxide and ammonium molybdate are more toxic than the ore molybdenite, the metal or the dioxide. It is not irritating to the skin, and is not a sensitizer. Mild cases of molybdenosis may be clinically identifiable only by biochemical changes (e.g., increases in uric acid levels due to the role of molybdenum in the enzyme xanthine oxidase). Excessive intake of molybdenum causes a physiological copper deficiency, and conversely, in cases of inadequate dietary intake of copper, molybdenum toxicity may occur at lower exposure levels.

Chronic Toxicity (or Exposure)

Animal

Water-insoluble molybdenite (MoS_2) is practically nontoxic; rats dosed with up to 500 mg molybdenite daily for 44 days exhibited no adverse effects. In contrast, animals dosed subchronically with water-soluble molybdenum compounds exhibited gastrointestinal disturbances, growth retardation, anemia, hypothyroidism, bone and joint deformities, liver and kidney abnormalities, and death. Fifty percent mortality was reported in rats maintained for 40 days on molybdenum-enhanced diets containing 125 mg Mo kg^{-1} (as molybdenum trioxide, MoO_3), 100 mg Mo kg^{-1} (as calcium molybdate, $CaMoO_4$), or 333 mg Mo kg^{-1} (as ammonium molybdate, $(NH_4)_2MoO_4$). A dietary level of 0.1% sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$) for several weeks was lethal to rabbits. Growth retardation was observed in rats maintained on diets containing 0.04–0.12% molybdenum. Evidence that the toxic effects of molybdenum might be caused by a secondarily acquired copper deficiency was shown in a study where a significant reduction in growth occurred in rats after 11 weeks on a diet containing 20 ppm molybdenum and 5 ppm copper; whereas, growth was not affected by molybdenum dietary levels as high as 80 ppm when the dietary level of copper was increased to 20 ppm. Hypothyroidism, as evidenced by decreased levels of plasma thyroxin, was found in rabbits maintained on a diet containing 0.3% Mo (as sodium molybdate) for several weeks or longer.

Anemia, as well as anorexia, weight loss, alopecia, and bone deformities occurred in young rabbits maintained for 4–17 weeks on a diet containing 0.1% molybdenum (as sodium molybdate). Anemia was also observed in rats maintained on a diet containing 0.04% Mo (as sodium molybdate) for 5 weeks, in rabbits on a dietary level of 0.2% sodium molybdate for 5 weeks, and in chicks on a dietary level of 0.4% sodium molybdate for 4 weeks. Signs of anemia and marked erythroid hyperplasia of the bone marrow were observed in rabbits maintained for 11 days on a diet containing 0.4% sodium molybdate. Bone and connective tissue disorders observed in animals receiving dietary levels of molybdenum 0.04% for 4 weeks or longer included mandibular exostoses, joint deformities, detachment of tendons, epiphyseal line fractures, and epiphyseal plate widening.

The liver can be affected to varying degrees by excessive intake of molybdenum. Significantly elevated levels of serum bilirubin were observed in dogs receiving 20 mg kg^{-1} of ammonium molybdate in their diet for 5.5 months. Fatty changes in the liver occurred in rabbits dosed with 50 mg kg^{-1} day^{-1} of ammonium molybdate for 6 months, and in guinea pigs dosed with 25 mg kg^{-1} day^{-1} of molybdenum dioxide for 14 days. Histological changes in the liver and altered glycolytic enzyme activity were observed in rats dosed with 289 mg Mo kg^{-1} day^{-1} (as ammonium molybdate) in drinking water for 28 days. Severe liver damage, consisting of perilobular necrosis, nuclear clumping and an increase in Kupfer cells, occurred in rats receiving 489 mg Mo kg^{-1} day^{-1}

(as ammonium molybdate) in their diet for 20 days. A 72% reduction in glycogen levels occurred in rats receiving the same dietary level for 30 days. An increase in kidney weight and indications of mild renal failure (decreased glomerular filtration as measured by a reduction in creatinine clearance) occurred in rats dosed for 8 weeks by gastric intubation with 80 mg Mo kg^{-1} day (as $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$). Histological changes in kidneys were also observed in rats dosed with 289 mg Mo kg^{-1} day^{-1} (as ammonium molybdate) in drinking water for 28 days. Severe kidney damage, including glomerular shrinkage and epithelial alterations in the distal and proximal renal tubules, occurred in rats receiving 1000 mg kg^{-1} day^{-1} of ammonium molybdate (489 mg Mo kg^{-1} day^{-1}) in their diet for 20 days.

In sheep and cattle, a condition known as 'teart disease' occurs when these animals graze on plants containing abnormally high amounts of molybdenum. Dietary levels of ~10 ppm molybdenum and higher can cause teart disease. Symptoms that may occur within 24 h include weakness and diarrhea. Longer exposure can lead to decoloration of hair, skeletal deformities, sterility due to damage to testicular interstitial cells, poor conception and deficient lactation.

Rats exposed to molybdenum dust (19.7 mg Mo m^{-3}, 4 h daily for 4 months) exhibited inflammation of the uterine horns with necrotic foci and endometrial atrophy. Severe demyelinization of the central nervous system occurred in newborn lambs born to dams maintained on high-molybdenum diets during pregnancy. Seventy-five percent of male rats maintained on a diet containing 80 or 140 ppm Mo (as sodium molybdate dihydrate) from weaning until mating became sterile, and histological examination revealed seminiferous tubule degeneration. Female fertility, gestation, and litter size were not affected by these dietary levels of molybdenum; however, weaning weight of offspring was reduced, indicating deficiencies in lactation. Sterility due to damage to testicular interstitial cells, poor conception, and deficient lactation have also been reported in cattle ingesting large amounts of molybdenum. A three-generation study conducted on mice found that 10 ppm molybdenum in drinking water (1.9 mg Mo kg^{-1} day^{-1}) resulted in a significant increase in the number of dead offspring in the F_1 and F_3 generations compared to the controls; however, the total number of litters per generation and the average litter size per generation were not affected by the molybdenum treatment.

There are no published carcinogenicity studies on molybdenum, and it is not listed as a carcinogen by the (US) Environmental Protection Agency (EPA), the International Agency for Research on Cancer, the (US) National Institute of Environmental Health Sciences' National Toxicology Program (NTP), the (US) Occupational Safety and Health Administration (OSHA), and the American Conference of Governmental Industrial Hygienists (ACGIH). Animal data indicate that Mo may have an inhibitory effect on esophageal and mammary carcinogenesis. However, intraperitoneal injections of MoO_3 in mice produced a significant increase in the number of lung adenomas per mouse and an insignificant increase in the number of mice bearing tumors.

Human

There is no information available on the subchronic oral and inhalation toxicity of molybdenum in humans. In studies conducted in a region of Armenia where levels of molybdenum in the soil are high (77 mg Mo kg^{-1}), many of the adults examined were found to have elevated concentrations of uric acid in the blood and urine, increased blood xanthine oxidase activity, and gout-like symptoms such as arthralgia, articular deformities, erythema, and edema. The daily molybdenum intake was estimated to be 10–15 mg. An outbreak of genu valgum (knock-knees) in India was attributed to an increase in Mo levels in sorghum, the main staple food of the region (the estimated daily Mo intake was 1.5 mg).

An investigation of the incidence of gynecological diseases in female workers at an integrated copper–molybdenum mill in the former Soviet Union did not reveal any evidence of molybdenum toxicosis. Studies of workers chronically exposed to Mo indicate a high incidence of weakness, fatigue, headache, irritability, lack of appetite, epigastric pain, joint and muscle pain, weight loss, red and moist skin, tremor of the hands, sweating, and dizziness. Joint pains, backaches, headaches, and nonspecific hair and skin changes were also the most frequent complaints of male workers at a US molybdenum-roasting plant. The exposed workers, who had been employed for 0.5–20 years, had high levels of molybdenum in the plasma and urine and significantly higher levels of serum ceruloplasmin and uric acid when compared with values for a control group. Elevated blood uric acid levels, as well as symptoms of arthralgia occurred in most Russian workers at a copper–molybdenum plant. Russian studies have also suggested that exposure to molybdenum can result in increased serum bilirubin levels and decreased blood A/G globulin ratios due to a rise in α-immunoglobulins, with the latter interpreted as evidence of liver dysfunction. Pulmonary effects of chronic exposorted in a study in which some workers exposed to Mo and MoO_3 (1–19 mg m^{-3}) for 3–7 years were symptomatic and had X-ray findings indicative of

pneumoconiosis. Adverse reproductive or developmental effects have not been observed in molybdenum workers. Molybdenum is placed in the US EPA's group D, that is, it is not classifiable as to carcinogenicity in humans.

Clinical Management

Upon ocular exposure, the eyes should be generously washed with tap water. Molybdenum ingestion is treated using gastric lavage and saline catharsis.

Environmental Fate

Molybdenum occurs as iron molybdates in nature. Exposure occurs via weathering and release into rivers, from mining, and by the combustion of oil and coal. Terrestrial plants can contain enough Mo to be toxic to animals but the plants can still grow normally. Adding lime to soil increases Mo availability.

Ecotoxicology

A series of experiments were conducted to determine the physiological impact of acute sublethal molybdenum exposure to juvenile kokanee salmon (*Oncorhynchus nerka* Kennerlyi). Molybdenum was relatively nontoxic to juvenile kokanee as the 96 h LC_{50} was $>2000\,mg\,Mo\,l^{-1}$. Exposure to either 25 or $250\,mg\,Mo\,l^{-1}$ for 7 days was found to stimulate a significant dose-dependent increase in ventilation. Acute sublethal molybdenum exposure was found to have little or no impact on kokanee oxygen consumption at rest or immediately following about of forced activity, or on physiological indicators of stress such as plasma lactate, sodium, and cortisol. Despite these findings, prior exposure to 25 or $250\,mg\,Mo\,l^{-1}$ resulted in postexercise loss of equilibrium and exercise-induced delayed mortality. The findings suggest that despite the nontoxic nature of molybdenum, acute sublethal exposure to this metal has physiological consequences to fish exposed for only a brief period.

Exposure Standards and Guidelines

The ACGIH threshold limit value, 8 h time-weighted average (TWA), is $10\,mg\,m^{-3}$ (as Mo metal and as insoluble compound, inhalable fraction), and $3\,mg\,m^{-3}$ (as Mo metal and as insoluble compound, respirable fraction). The US OSHA permissible exposure limit, 8 h TWA, is $15\,mg\,m^{-3}$ (as Mo metal and as insoluble compound, inhalable fraction), limit. The US NIOSH immediately dangerous to life or health value is $5000\,mg\,m^{-3}$ (as Mo). The US EPA has set a federal drinking water guideline of $40\,\mu g\,l^{-1}$ and an oral reference dose of $0.005\,mg\,kg^{-1}\,day^{-1}$.

See also: Metals.

Further Reading

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Monoamine Oxidase Inhibitors

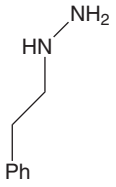
Rebeca Gracia

This article is a revision of the previous print edition article by Douglas J Borys, volume 2, pp. 344–345, © 1998, Elsevier Inc.

- Representative Chemicals: Phenelzine (Nardil, Stinerval, Monofen, Fenelzin, Kalgan, Nardelzine); Phenethylhydrazine hydrogen sulfate
- Chemical Abstracts Service Registry Number: CAS 51-71-8
- Synonyms:
  Monoamine oxidase inhibitors (MAOI)
  - Nialamide (Niamide): *N*′-(2-Benzylcarbamoylethyl)isonicotinohydrazide
  - Tranylcypromine (Parnate): (±)-*trans*-2-Phenylcyclopropylamine sulfate (2:1)
  - Iproniazid (Marsilid): 2′-Isopropylisonicotinohydrazide
  - Isocarboxazid (Marplan): 5-Methyl-3-isoxazolecarboxylic acid-2-benzylhydrazide or 2′-benzyl-5-methylisoxazole-3-carbohydrazide

- Moclobemide (Avrorix): 4-Chloro-*N*-(2-morpholinoethyl)benzamide
- Selegiline, l-Deprenyl (Eldepryl): (*R*)-(−)-*N*-2-dimethyl-*N*-2- propynylphenethylamine
- Others: Pargyline, clorgyline, pheniprazine, toloxatone, benmoxin, echinopsidine iodide, etryptamine, iproclozide, mebanazine, metfendrazine, phenoxypropazine, pivhydrazine

- Chemical/Pharmaceutical/Other Class: Antidepressants
- Chemical Structure: Phenelzine is the prototype monoamine oxidase inhibitor



Uses

Monoamine oxidase inhibitors are used to treat depression, atypical depression, bulimia, posttraumatic stress reactions, obsessive–compulsive disorder, panic attacks, narcolepsy, phobias, hypochondria, anxiety, and many other psychiatric disorders as well as night tremors, parkinsonism, postural hypotension, headache, and aphthous stomatitis.

Exposure Routes and Pathways

Monoamine oxidase inhibitors are available orally. Accidental or intentional ingestion are the most common routes of exposure.

Toxicokinetics

Monoamine oxidase inhibitors are rapidly and completely absorbed orally reaching peak blood levels within 2 h. Monoamine oxidase inhibitors are acetylated in the liver to many active and inactive metabolites. The volume of distribution is estimated to range from 1 to 4 l kg^{-1}. The inactive metabolites are excreted by the kidneys. The elimination half-lives of monoamine oxidase inhibitor parent compounds range from 15 min to 3.5 h. The biologic half-life often significantly exceeds the elimination half-life.

Mechanism of Toxicity

Monoamine oxidase is the enzyme principally responsible for degradation of amine neurotransmitters (norepinephrine, epinephrine, serotonin, and dopamine). In general, monoamine oxidase inhibitors irreversibly bind to monoamine oxidase leading to neurotransmitter accumulation. Moclobemide, the exception, binds reversibly. They do not have any effect on monoamine oxidase production. The enzyme then regenerates over many weeks. Monoamine oxidase inhibitors may also stimulate the release of norepinephrine from some nerve endings while having a sympatholytic effect at postganglionic terminals. In high doses, monoamine oxidase inhibitors also inhibit other enzymes, which may cause many toxic effects. Iproniazid, isocarboxazid, nialamide, phenelzine, and tranylcypromine are nonselective monoamine oxidase inhibitors. Clorgyline, moclobemide, and toloxatone are type A monoamine oxidase inhibitors whereas selegiline is a type B monoamine oxidase inhibitor. The type B receptors are less commonly found in the alimentary tract; therefore, selegiline does not result in as many drug–food interactions as the other monoamine oxidase inhibitors.

Acute and Short-Term Toxicity (or Exposure)

Animal

Monoamine oxidase inhibitors are not used therapeutically in animals. Toxicity would be expected to resemble that observed in humans.

Human

Any ingestion of 2 or 3 mg kg^{-1} is potentially toxic. At therapeutic levels, the most commonly reported adverse effects are dizziness, headache, nervousness, sleep disorders, drowsiness, ataxia, constipation, dry mouth, weight gain, postural hypotension, and edema. Monoamine oxidase inhibitors combined with sympathomimetic drugs or tyramine-containing foods, in either therapeutic amounts or in overdose, may result in a hypertensive crisis characterized by severe headache, tachycardia, diaphoresis, and hyperpyrexia. In very severe cases, subarachnoid hemorrhage and death have resulted. The clinical effects of monoamine oxidase inhibitor overdose have been categorized into four phases. Phase 1 is an asymptomatic period of 12–24 h. Sympathomimetic stimulation characterizes Phase 2. Symptoms in this phase include headache, agitation, mydriasis, tachycardia, drowsiness, hyperreflexia, flushing, and nausea. Symptoms may worsen to coma, muscle rigidity, hyperpyrexia, hypotension, seizures, and cardiac arrest. Phase 3 is cardiovascular or central nervous system (CNS) collapse. The fourth and last phase is marked by secondary complications that may include renal failure, pulmonary edema, and asystole.

Chronic Toxicity (or Exposure)

Animal

Procarbazine has been associated with increased development of tumors in several animal models compared to controls.

Human

Expected symptoms include drowsiness/CNS depression, and other CNS effects (e.g., psychosis, myoclonus, seizures, extrapyramidal effects).

Clinical Management

Basic and advanced life-support measures should be aggressively implemented. Gastric decontamination with activated charcoal should be performed in patients with recent ingestions. Hypertension should be managed with intravenous sodium nitroprusside or with phentolamine. Agitation, muscle rigidity, and seizures may be controlled with intravenous diazepam or other benzodiazepines. The hypotensive patient should be placed in Trendelenburg's position and given intravenous fluids and pressors such as norepinephrine or dopamine as needed. External cooling should be used in hyperpyrexic patients. Dantrolene has also been used in patients with hyperpyrexia. Hemodialysis and hemoperfusion have not been shown to lead to any improvement in clinical status.

Further Reading

Linden CH, Rumack BH, and Strehlke C (1984) Monoamine oxidase inhibitor overdose. *Annals of Emergency Medicine* 13: 1137–1144.

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Neuvonen PJ, Pohjola-Sintonen S, and Tacke U (1993) Five fatal cases of serotonin syndrome after moclobemide–citalopram or moclobemide–clomipramine overdoses (letter). *Lancet* 342: 1419.

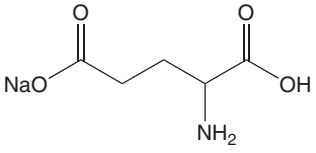
Monomethylhydrazine *See* Mushrooms, Monomethylhydrazine.

Monosodium Glutamate

Arezoo Campbell

This article is a revision of the previous print edition article by Linda Larsen, volume 2, pp. 345–346, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 142-47-2
- Synonyms: L-Glutamate; Sodium glutamate; Glutamic acid monosodium salt; L-Glutamic acid monosodium salt; MSG; Chinese seasoning
- Chemical/Pharmaceutical/Other Class: Acidic amino acid salt
- Chemical Formula: $C_5H_8NO_4Na$
- Chemical Structure:



Uses

Monosodium glutamate (MSG) is used as a food additive, mainly in Oriental cuisine, to enhance and impart a 'meaty' flavor.

Background Information

MSG is the sodium salt of glutamic acid, one of the most abundant nonessential amino acids found in nature. Virtually all foods including meat, vegetables, poultry, and fish contain glutamate. It can be synthesized *in vivo*. By weight, ~10–40% of proteins are composed of the amino acid.

In 1908, Professor Kikunae Ikeda of Tokyo Imperial University extracted crystals of glutamic acid from broth prepared from a type of seaweed. He recognized that the compound imparted a taste distinct from sweet, salty, bitter, and sour. He termed this new taste 'umami'(savoury) and decided to use the newly isolated glutamic acid as a seasoning which enhanced the original flavor of food. The use of MSG in Chinese cuisine has been connected to a complex of symptoms termed 'Chinese restaurant syndrome' (CRS). The most common symptoms are burning sensations in the mouth, facial pressure, chest pain, flushing, headache, tingling, numbness, and generalized weakness. It is thought that ~1–2% of the population is sensitive to MSG. However, two extensive scientific reviews, one in 1987 by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and another in 1995 by the Federation of

American Societies for Experimental Biology (FASEB), both concluded that concentrations of MSG in food are not hazardous to human health. The latter source does indicate, however, that there is a subset of individuals who are sensitive and may suffer transient adverse symptoms due to consumption of large amounts of MSG.

Exposure Routes and Pathways

Ingestion of food containing monosodium glutamate is the main route of exposure.

Toxicokinetics

Absorption of dietary glutamate occurs by an active amino acid transport system. The mucosal cells of the gastrointestinal tract then metabolize and utilize it as an energy source. Thus, the amount of glutamate that actually enters the portal blood supply is very low. However, if very large concentrations >5 g of MSG are ingested, the plasma levels can rise significantly. Ingestion of carbohydrates significantly attenuates this effect. The blood–brain barrier is very effective in blocking the passive transport of glutamate into the central nervous system even when the levels of glutamate are elevated in the plasma. The fetus is also protected from any adverse effects because the placenta is impermeable to glutamate. Studies of human infants show that they are capable of metabolizing glutamate similarly to adults.

Mechanism of Toxicity

Although several mechanisms have been proposed to be responsible for causing CRS, none has been extensively studied. One hypothesis has been that the effects are due to an immediate hypersensitivity reaction. Since no IgE-mediated reaction has been documented, there is no direct evidence that this is the case. Another hypothesis is that vitamin B_6 deficiency plays a role in the response because the symptoms were prevented by supplementing individuals with the vitamin. Since glutamate can be converted to acetylcholine by the tricarboxylic acid cycle, it has also been proposed that the effects are due to an increase in acetylcholine levels. It has been noted that after MSG ingestion, there is a decrease in cholinesterase levels. Due to inadequate investigations, it is not currently known if any or all of these mechanisms are responsible for CRS. The neurotoxicity of MSG, demonstrated after exposure to very large doses only in rodent species and rabbits, is attributed to excitotoxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

Large concentrations of MSG administered by gavage or intravenous injection causes focal lesions in the hypothalamus of rodents and rabbits. These occur only hours after exposure. The mouse appears to be the most sensitive species affected. Neonatal animals are more sensitive to MSG neurotoxicity. Neuronal damage occurs in neonatal mice when plasma levels reach between 100 and 300 $\mu mol\,dl^{-1}$. In adults, the plasma levels have to reach $>630\,\mu mol\,dl^{-1}$ before similar effects are noted. None of the primate studies were able to demonstrate hypothalamic lesions after exposure to MSG.

Human

The plasma levels of glutamate necessary to cause hypothalamic lesions in mice are never reached voluntarily in humans since the highest palatable dose is well below these concentrations. However, because of the animal studies, MSG is not recommended as an ingredient in baby formulas.

Double-blind controlled trials have failed to demonstrate an unequivocal link between exposure to MSG and CRS because many of the individuals who had a history of suffering from symptoms after ingestion of MSG also reacted positively to the placebo. Furthermore, upon second challenge, the symptoms were different than what they had experienced previously. Therefore, there are cases of individuals who are sensitive to MSG. However, the response is difficult to evaluate clinically. It has been suggested that MSG can trigger an asthma attack in sensitive individuals suffering from severe unstable asthma. However, the studies investigating this link have been inconclusive.

Chronic Toxicity (or Exposure)

There is no evidence of chronic toxicity in either animals or humans.

Clinical Management

The symptoms caused by MSG exposure are uncomfortable but not serious. Those who experience adverse effects should avoid foods containing MSG.

Exposure Standards and Guidelines

An acceptable daily intake has not been set for MSG because the levels typically found in foods do not pose a health threat. However, the FASEB review indicates that a subset of MSG-sensitive individuals will experience CRS after ingestion of >3 g bolus of MSG in the absence of food.

See also: Food Additives.

Further Reading

Walker R and Lupien JR (2000) The safety evaluation of monosodium glutamate. *Journal of Nutrition* 130(4S Suppl): 1049S–1052S.

Relevant Websites

http://www.fda.gov – FASEB MSG Report.
http://www.foodstandards.gov.au – Food Standards, Australia, New Zealand.

Monte Carlo Analysis

M A Jayjock, Paul Price, and Cristine F Chaisson

A Brief History and Introduction of Uncertainty Analysis in Risk Assessment

For much of its early and brief history, the field of human exposure/risk assessment has focused on characterizing the highest levels of exposure to substance that will occur to an individual or a population over time as the result of the interaction with a specific source. Examples of substances and their sources include residues in drinking water, chemicals present indoors in a factory or residence, contaminants at a waste disposal site, or a pesticide-containing product used by a consumer or worker. The approach that has been used is to characterize the upper bound of exposure using simple models of the doses received from exposure sources. These source-to-dose models are then evaluated with a series of conservative model inputs. This approach has great value for screening out exposures that are of little concern, and it has formed the basis for US Environmental Protection Agency (EPA) and other exposure and risk assessment guidance, for example, EPA's Risk Assessment Guidance for Superfund and the Office of Pesticide Programs' Residential Standard Operating Procedures (SOPs).

Some risk assessors and risk managers followed these initial efforts by beginning to seek analyses that are more sophisticated. Instead of just an upper bound worst-case overestimate, they wanted more information on the actual range of variation of the exposure, and some measure of the uncertainty associated with the estimate.

It has been stated that anytime we are absolutely certain of a fact, we are almost surely wrong. Indeed, one cannot measure any physical quantity without error, and any activity that aspires to gain and transmit knowledge, including exposure and risk assessment, requires a frank and explicit understanding and communication of inherent uncertainty associated with that practice.

Analyzing Uncertainty: Elements and a Simple Example

The following discussion centers on uncertainty related to the estimation of human exposure. Many of the general principles will be applicable to both exposure and toxicology, and the connection and context of this treatment of uncertainty associated with toxicological determinations will be made later in this discussion.

Uncertainty concerning the determinants of human exposure can be considered as coming from two sources or types

1. The natural variability of these predictors in any particular scenario of interest.
2. Our lack of knowledge about the basic nature of these variables (i.e., our fundamental ignorance of the reality and relationships within that reality that cause the exposure to occur).

We can describe type 1 uncertainty with sampling statistics. These, in turn, describe a tolerance of knowledge around the measurement or estimate.

The second source of uncertainty (type 2 from our lack of basic knowledge) is typically much more troublesome, and tends to dominate because it is often much larger than that posed by type 1. Thus, it is incumbent on the risk assessor to understand and describe this unavoidable subjectivity in as much detail as possible to facilitate the understanding of those who rely on the work, allowing them to comprehend and appreciate its boundaries and limitations.

Historically, the first and more conventional method to examine uncertainty in human exposure

assessment is to look at predictions of dose based on reasonable worst-case scenarios and the impact or sensitivity of the uncertainty for individual variables.

An example for this discussion is a simple indoor air model

$$C = \frac{G}{Q}$$

where C is the equilibrium airborne concentration of a toxicant ($mg\,m^{-3}$), G is the steady (unvarying) generation rate ($mg\,h^{-1}$), and Q is the steady (unvarying) ventilation rate ($m^3\,h^{-1}$).

A conventional way of addressing uncertainty in risk assessments is to estimate and assign 'reasonable' worst-case conditions for our evaluations or models. Thus, in this case one would typically pick the worst case (highest G and lowest Q) to estimate a worst case for C. Next, this could then be combined with 'best case' estimates (lowest G and highest Q) to provide a range for C. Finally, the impact or sensitivity of G or Q on either best or worst-case scenarios could be determined by calculating the results of varying these predictors from maximum to minimum individually in each.

Unfortunately, when a single or 'bright-line' prediction for exposure potential is required, often only the worst-case estimate is reported and used. This single worst-case value is the compounding of all the worst-case uncertainty in all of the predictors. Our example has only two variables, but in some cases with many predictors the estimate of exposure becomes compounded to a much higher order. Historically, the mention or note of the 'average case' or 'best case' is often omitted. Doing so essentially hides valuable information about the uncertainty since those viewing the results have no knowledge and thus no sense of the relative width of the error band around the prediction.

Using the example assume that one has data on the source rate (G) which indicates that between residence values are normally distributed with a mean of $50\,mg\,h^{-1}$ and a standard deviation of $5\,mg\,h^{-1}$ for the particular source of interest. (This is an example of uncertainty type 1 above – a known and measured quantity with natural variability.)

We might take a worst-case estimate of G to be the mean + 3 standard deviations. This is $50 + 15$ or $65\,mg\,h^{-1}$, which is a value greater than 99.8% of the values in this predicted set of values. Best case would be the mean -3 standard deviations or $35\,mg\,h^{-1}$. Thus

- Reasonable worst case, $G = 65\,mg\,h^{-1}$
- Average case, $G = 50\,mg\,h^{-1}$
- Reasonable best case, $G = 35\,mg\,h^{-1}$

However, for the ventilation rate (Q) in this case there is much less certain information or knowledge available. (This is an example of uncertainty type 2 – uncertainty from ignorance or a basic lack of knowledge.) Assume that this particular source will be used in large and small industrial settings almost invariably without benefit of local exhaust. Experience indicates that the general ventilation rate will not likely be less than 0.2 mixing air changes per hour and will most likely not be higher than 30 air changes per hour. (Note Q = (air change per hour)(-room volume).) However, the average or 'most likely' level of ventilation is essentially unknown. Venturing a guess that it is halfway in between 0.2 and 30 without data or confident knowledge would be unwise. So we have

- Worst Case air change per hour = 0.2
- Best Case air change per hour = 30

Using the traditional 'reasonable worst case' approach one could simply take a worst case estimate of G (the mean + 3 standard deviations $= 50 + 3(5) = 65$) and use the worst case estimate of ventilation as 0.2 air changes per hour. Assuming a relatively small room of $3\,m \times 3\,m \times 2\,m$:

$$C = \frac{65\ mg\ h^{-1}}{(0.2\ h^{-1})(18\ m^3)} = 18\ mg\ m^{-3}$$

Best case is

$$C = \frac{35\ mg\ h^{-1}}{(30\ h^{-1})(18\ m^3)} = 0.065\ mg\ m^{-3}$$

There is no average case since there is not enough confidence in any estimate of an average ventilation rate to use it.

The uncertainty range (prediction of exposure) in this analysis varies 277-fold from best to worst case.

Monte Carlo simulation modeling represents the next stage or advancement of uncertainty analysis. This computer-aided stochastic (i.e., random, involving chance) probability analysis technique allows one to more transparently and completely present information about the predictions of exposure and the uncertainty associated with these predictions. In this method the predictor variables, in this case G and Q are described as distributions rather than point estimates of best, worst or average.

Figure 1 shows the attributed distribution for the source rate (G) in the example where G = normal or Gaussian distribution with mean = 50 and standard deviation = 5.0.

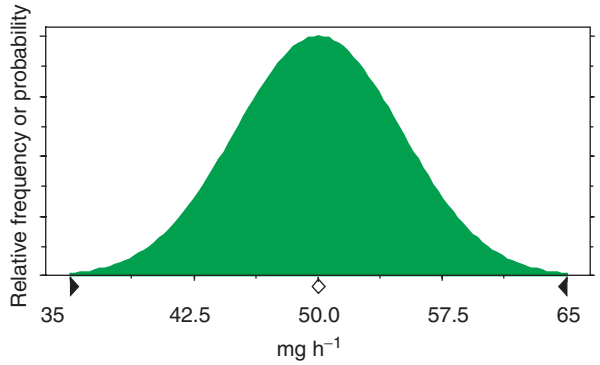


Figure 1 Measured/fitted probability distribution of source generation rate (*G*).

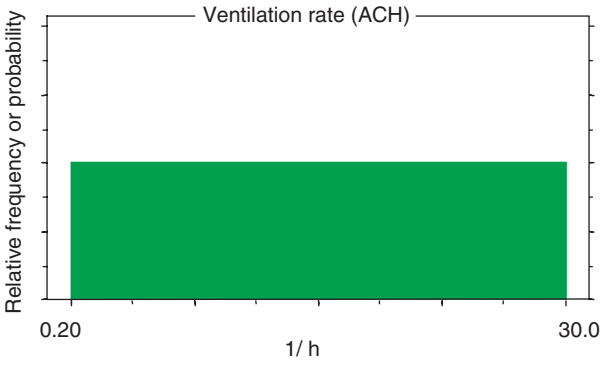


Figure 2 Ascribed probability distribution of ventilation rate (*Q*).

Figure 2 shows the attributed distribution for the ventilation rate expressed in air changes per hour (ACH) as a uniform (i.e., totally random) distribution from 0.2 to 30 air changes per hour. In a room 3 m × 3 m × 2 m (18 m^3) this is a uniform distribution of ventilation rate expressed in $m^3 h^{-1}$ of $Q = 3.6$ to 540 $m^3 h^{-1}$. That is, a distribution in which there is an equal probability of any values within this range occurring and a zero probability of any value occurring outside this range.

The distribution chosen for the air changes per hour is ascribed based on professional judgment; as such, it is a direct result of our lack of knowledge about it. It is important to note that this distribution is not reality, but instead is our best subjective description of our knowledge of reality. There is most likely some finite probability of air change rates being below 0.2 or above 30, and there is certainly some central tendency to this universe of values; however, this distribution represents the quantification of our best knowledge and professional judgment of this situation. Additional data could be used to refine this estimated distribution to be closer to the truth.

Thus, this analysis allows for a distribution (more accurately termed a probability density function or PDF) of values to be used for these two input variables (*G* and *Q*), and these PDFs reflect the quality of our understanding and data. Using a personal computer and readily available Monte Carlo modeling software, a large number (normally 10 000 or more) of independent 'samples' consisting of sets of values for each of the input variables are obtained, and the corresponding distribution of predicted airborne concentration is calculated. This is done by repeated computer runs through the concentration estimation algorithm using PDF selected values for the input parameters. These values are constrained by the known or inferred ranges, means and probability distributions of the individual input parameters. The resulting output is displayed as a forecast graph that shows the entire range of possible outcomes and the likelihood of achieving any of them. This includes a mean concentration and the probability for any concentration above and below the mean. It also provides the upper and lower limits as a measure of dispersion. **Figure 3** shows the output distribution for this example.

This distribution has the following properties

- Median = 0.19 mg m^{-3}
- Mean = 0.46 mg m^{-3}
- 5%tile = 0.09 mg m^{-3}
- 95%tile = 1.7 mg m^{-3}

It is interesting to note that our 'worst case' estimate of 18 mg m^{-3} was not reached in the 10 000 run simulation; the highest prediction in this run (i.e., the 100th percentile) was 14.5 mg m^{-3}. Similarly, the lowest value (i.e., the 0%tile) was 0.07 mg m^{-3}, which is relatively close to the 0.065 mg m^{-3} value as the absolute best case.

An added benefit of Monte Carlo analysis is that a common by-product of this computerized examination is a sensitivity analysis that shows how much each predictor variable contributed to the uncertainty or variability of the predictions. This, in turn, tells both the risk assessor and risk manager which portion of the variability is from natural fluctuation versus how much is caused by lack of knowledge. Given this information, decisions can be made as to where the most cost-effective allocation of resources may occur to refine the estimate of exposure and risk. In the example, the sensitivity analysis shown in **Figure 4** presents the apportionment of variance for the model.

In this analysis estimates of *G* contributed 2.4% of the variance of the predictions while estimates of *Q* contributed 97.6% of the variance of the predictions.

Clearly and not surprisingly the lack of knowledge about the ventilation (*Q*) in this scenario added most of the uncertainty of this analysis. Most important, the estimate of 95th percentile of concentration is

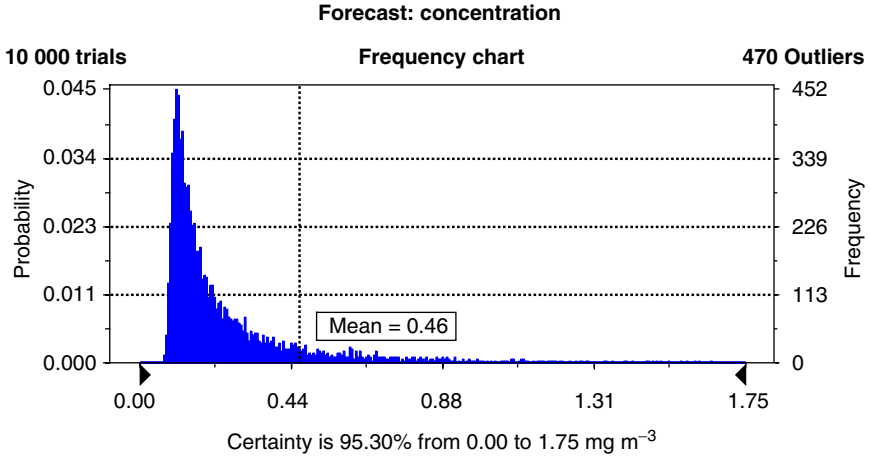


Figure 3 Forecast concentration distribution (C) in $mg\,m^{-3}$.

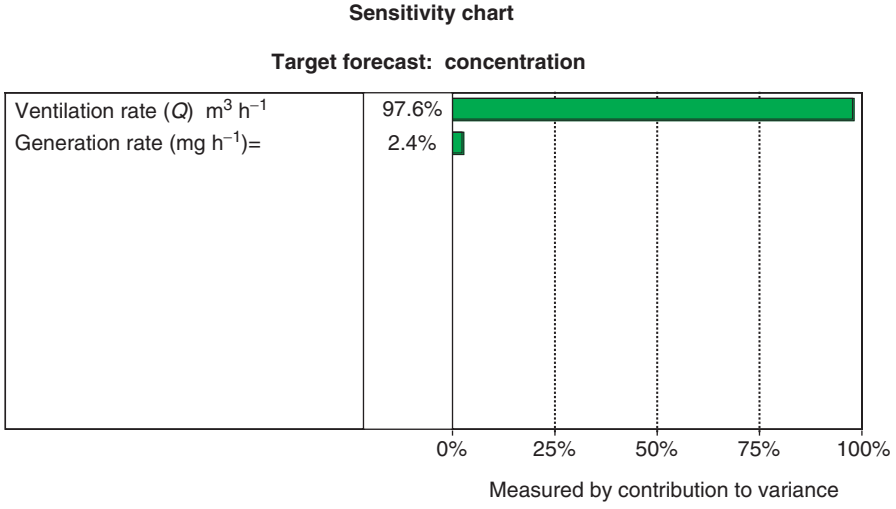


Figure 4 Sensitivity: relative contribution to model variance by each predictor variable.

significantly higher than it would have been with a more accurate description of the ventilation rates in these scenarios.

Relevance to Toxicology and Risk Assessment

The general equation for risk assessment is the product of exposure and toxic potential. The above discussion, focused on human health exposure assessment, is clearly applicable to the analysis of uncertainty in the determinants and predictions of toxic effect per unit exposure. Indeed the complete assessment of risk and its uncertainty comes from the integrated evaluation of uncertainty associated with both toxicity and exposure. It is simply an extension of the technique demonstrated above with the addition of algorithms for toxic effect yielding a forecasted distribution of predicted risk.

Direction of the Field and Related Topics

The next step in the fields of exposure and risk assessment is simulation modeling. Simulation models of exposure and risk have been developed in recent years by the US EPA, industry trade associations, private firms, nonprofit organizations, and academia to answer a need, the obligation to understand aggregate and cumulative exposure.

These simulation models use probabilistic approaches, but are much more complex than the simply Monte Carlo models described above that have been used in exposure assessments. Monte Carlo analysis has been applied to simple spreadsheet calculations of dose using 'add-in' software programs such as @Risk™ or Crystal Ball™. These analyses seek to understand the uncertainty and variation in the predictions of these simple dose models. In contrast, these new models are stand-alone computer

programs that are written in computer languages such as C++, Visual Basic, or SAS. This modeling is sufficiently complex that it can only be investigated using probabilistic techniques.

While the specific models vary in their details and in the sources of exposure that they consider, simulation models have a set of common characteristics. The primary characteristic is a focus on modeling people, not sources of pollution. The models seek to simulate people, their patterns of daily activities (e.g., what they eat, what they do, what consumer products they buy, the kinds of residences they live in, where they live geographically, and how long they live in a residence before moving to a new residence) and their physiological characteristics (e.g., how much they weigh, their breathing frequencies and breathing volumes, and the sizes of various organs of relevance to the assessment). These simulations seek to characterize both how one individual varies from another, and how each individual varies over time.

Each of the models defines individuals' exposures as a series of separate events. These events are defined in a way that allows the calculation of the dose received during the event. An event may consist of eating a specific food (an apple, a slice of pizza, or a fish from a contaminated river) or a specific act (mowing a lawn, applying a pesticide, or refueling a car).

The characteristics of these exposure events are allowed to change from one event to the next. The changes can reflect the day-to-day variation in the types and amounts of foods consumed, the levels of chemical residues in the foods, and in the activities and the characteristics of the person. These changes can reflect factors such as seasonal changes in diet or pesticide residues on foods, seasonal activities such as fishing or gardening applications, changes in activities on weekends versus weekdays, and physiological changes as a person grows from childhood to adult. The result is a set of time-varying doses that occur at specific times in an individual's life. Together the doses create an exposure history for a simulated individual.

These exposure histories can be used in a number of ways. The doses from all of the events in a day can be summed to give an estimate of the daily dose. The daily doses can be averaged to produce long-term estimates of dose (7 day, seasonal, annual, and, in some cases, lifetime). The models can also be used to look at doses when the simulated individual was a child or during certain seasons of the person's life.

By repeating this process for different types of individuals, the models create a picture of the exposure histories of a representative population of individuals. The variation of exposures in such a population can be used to estimate the variation in the US population or other population of interest.

The models provide a powerful tool for evaluating aggregate exposures (the simultaneous exposure to multiple sources of a single chemical) and cumulative exposures (simultaneous exposures to multiple chemicals). They provide the framework for the accurate portrayal of human exposure in the real world, including its uncertainty, while pointing the way to critical research needs in the realm of exposure assessment and toxicology. Indeed, the models have the potential to fully integrate exposure and toxicity models. By defining the person exposed and the temporal patterns of dose, the simulation models can serve as the starting point for pharmacokinetic and pharmacodynamic models of the occurrence of adverse effects. Such models may replace the current system of toxicological safety factors and policy-driven assumptions with actual predictions of risk to human from chemical exposure.

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Morning Glory

Christine Stork and Jeanna Marraffa

- SYNONYMS: *Ipomoea violacea*; *Ipomoea tricolor*; Pearly gates; Wedding bells; Heavenly blue; Blue star

Uses

Morning glory seeds are abused for their hallucinogenic effect.

Background Information

Morning glory is a climbing vine with blue, white, or red trumpet-shaped flowers that open in the morning and close in the afternoon. The leaves are green and heart shaped. A papery thin pod holds small black seeds.

Exposure Routes and Pathways

Exposure is via ingestion. The seeds must be pulverized (chewed) to induce toxicity because the intact seed coat prevents absorption.

Mechanism of Toxicity

Morning glory seeds contain the toxin lysergic acid hydroxyethylamide, which has one-tenth the potency of lysergic acid diethylamide (LSD). The toxic effect, at an equivalent dose, is similar to that of LSD. To avoid abuse of morning glory seeds, commercial seed producers treat the seeds with essential oils, which are irritants, which induce nausea, vomiting, and abdominal pain which precede the psychedelic effects.

LSD acts on several sites in the central nervous system. It is a nonselective serotonin, or 5-hydroxytryptamine (5-HT) agonist on both presynaptic and postsynaptic receptor sites. The 5-HT_{2A} receptor agonism is implicated in the modulation of hallucinations. In addition to the role of serotonin in causing hallucinations, other neurotransmitters, including glutamate and D_1 and D_2 dopamine receptors, are implicated; yet, their role remains elusive.

Acute and Short-Term Toxicity (or Exposure)

Human

Symptoms include nausea, disorientation, delusions, hallucinations, psychosis, panic reactions, paranoia, violent behavior, prolonged changes in perception and sensation, and suicidal ideations. Morbidity and mortality generally result from complications of hyperthermia including rhabdomyolysis, renal failure, and disseminated intravascular coagulopathy. Hyperthermia occurs secondary to autonomic instability and an increase in serotonergic activity. Hyperthermia ensues when there is excess muscle activity in the face of agitation. Sedation with benzodiazepines is usually sufficient to treat the excess agitation and muscle activity; however, occasionally hyperthermia may require more aggressive therapy with active cooling measures and muscle relaxants. Excessive physical restraint should be avoided to prevent further hyperthermia and excess muscle activity. A few morning glory seeds are unlikely to cause significant problems. Several packages of seeds must be eaten to produce toxic effects in adults; however, nausea and vomiting generally precede its psychedelic effects. The contents of the seeds are not absorbed unless chewed. Three hundred seeds have a potency equivalent to 200–300 g of LSD, an amount sufficient to produce an altered state of consciousness. Twenty to fifty seeds may result in increased sociability, restlessness, and alertness followed by a period of relaxation. About 100–150 seeds result in hallucinations, perceptual changes, and improved mood lasting up to 4 h. About 200–500 seeds will cause euphoria, hallucinations, and philosophical thought. Adverse side effects are likely at this dose and include nausea, vomiting, abdominal pain, fatigue peripheral temperature, and sensation changes.

Clinical Management

Standard decontamination methods are rarely needed due to the rapid absorption of the agent and the likelihood that their use will exacerbate the patient's emotionalism. The patient's environment should be managed to prevent self-harm and to promote calmness. Significant agitation, dysphoria, or autonomic instability can be effectively treated with benzodiazepines. Phenothiazines have been utilized but are associated with an increased risk of hallucinogen persisting perception disorder (flashbacks) as well as increased risk of hyperthermia and agitation secondary to its anticholinergic properties. Symptoms usually resolve in 8 h. Close psychiatric follow-up may be needed if symptoms persist.

See also: LSD (Lysergic Acid Diethylamide); Plants, Poisonous.

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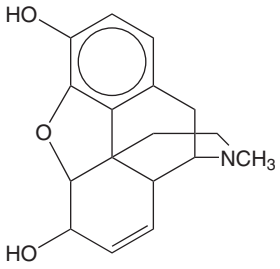
Morphine

Michael Hiotis

- Chemical Abstracts Service Registry Number: CAS 57-27-2
- Synonyms: 7,8-Didehydro-4, 5-epoxy-17- methyl-morphinan-3,6-diol morphine acetate; Morphine CHM; Morphine sulfate
- Chemical/Pharmaceutical/Other Class: Opiate analgesic; an alkaloid and phenanthrene derivative of opium
- Chemical Structure:



Uses

Morphine is used as an analgesic for acute and severe pain, as a sedative, as an antitussive, and for treatment of dyspnea in left ventricular failure and acute pulmonary edema. It is DEA Class II and has high drug abuse potential.

Exposure Routes and Pathways

Morphine is available for oral, rectal, and parenteral administration. Oral formulations include immediate and controlled release tablets, as well as oral solution regular strength and concentrate. Parenterally, it can be administered subcutaneously, intramuscularly, intravenously, or by continuous infusion. It is a drug of abuse and can be nasally insufflated.

Toxicokinetics

Morphine is rapidly absorbed from the gastrointestinal tract after oral administration and its bioavailability is ~40%, since it undergoes extensive first-pass metabolism in the liver and gut. Oral morphine is about one-sixth as potent as morphine administered parenterally. After parenteral injection, morphine is readily absorbed into the blood and peak effects occur within 30 min to 1 h. Morphine is metabolized in the liver by N-demethylation. The majority of a dose of morphine is conjugated with glucuronic acid to its major metabolite morphine-3-glucuronide (M3G) which is inactive, and the active metabolite morphine-6-glucuronide (M6G). Other active metabolites include normorphine, codeine, and morphine ethereal sulfate. Enterohepatic circulation of conjugated and intestinally deconjugated morphine has been reported. Morphine is distributed throughout the body, but mainly in the kidneys, liver, lungs, and spleen. The volume of distribution is 3 or $4\,l\,kg^{-1}$. Mean plasma elimination half-life is 1.7 h for morphine and 2.4–6.7 h for M3G. Up to 10% of a dose may eventually be excreted, as conjugates, through the bile into the feces. The remainder is excreted in the urine, mainly as conjugates.

Mechanism of Toxicity

Morphine is the prototype for the class of natural and synthetic opioid analgesics and its toxicity stems mainly from its extensive effect on the central nervous system (CNS), principally that of a descending depression. Opioids interact with stereospecific and saturable binding sites mostly located in the CNS. Interaction with the opioid receptors mimics the actions of endogenous enkephalins and endorphins. Morphine is a pure opiate agonist and exerts its activity primarily on the mu receptor. Activity also appears to involve an alteration in the release of neurotransmitters, such as the inhibition of acetylcholine, norepinepherine, and dopamine. These actions result in the therapeutic effects of analgesia, sedation, euphoria, and decreased gastrointestinal motility; however, in toxic amounts they can lead to

respiratory depression, coma, and cardiovascular collapse.

Acute and Short-Term Toxicity (or Exposure)

Animal

Dogs and monkeys act similarly to humans – symptoms may include drowsiness, ataxia, vomiting, respiratory depression, miosis (mydriasis in monkeys), coma, and hypotension. Opiates and their derivatives have an excitatory effect on the CNS of cats and horses. Naloxone may be used at $0.02\,mg\,kg^{-1}$ if needed. Morphine is used in veterinary practice for the treatment of pain and for cough suppression.

Human

Symptoms of toxicity may occur in varying degrees in nontolerant adults who receive greater than a therapeutic amount of morphine. The primary insult is respiratory depression from direct depression of the CNS. This state may then progress to apnea or respiratory arrest. Pulmonary edema is a common complication. Therapeutically, morphine results in analgesia; however, when toxic doses are used, CNS depression results in coma. Miosis is frequent, but in an acidotic or asphyxiated state, pupils may be dilated. From a cardiovascular perspective, morphine causes a decrease in systemic vascular resistance, which may result in a fall in systemic arterial pressure, thus leading to severe hypotension. A decrease in sympathetic tone can yield bradycardia. Hypothermia may also ensue with peripheral vasodilation. Laboratory analysis of morphine is useful only as confirmation of its presence; it does not dictate treatment. Semiquantitative and qualitative EMIT homogenous enzyme immunoassays are available for measurement of opiates in urine. Lastly, toxicity can result from therapeutic mistakes such as dispensing the wrong formulation of morphine, for example if solution concentrate is dispensed instead of the regular strength.

Chronic Toxicity (or Exposure)

Animal

As in humans, animals can become dependent on morphine after chronic use. Dependence has been produced with as few as two injections per day. Chronic dosing studies during pregnancy in rats have not produced teratogenic effects. However, growth retardation was observed in the intermediate dosing group ($35\,mg\,kg^{-1}\,day^{-1}$).

Human

Opiates have a high potential for abuse. Chronic users may develop tolerance, thus necessitating larger doses for the desired effect. Toxic effects in chronic abuse can result in decreased immunity. Abrupt cessation can cause withdrawal, yielding restlessness, agitation, yawning, vomiting, and diarrhea. It has been reported that patients with significant renal impairment may develop toxicity from accumulation of the active metabolite M6G.

In Vitro Toxicity Data

Drosophila studies of morphine have been either negative or inconclusive.

Clinical Management

Basic life-support measures should be instituted, as necessary, and initial management should include establishment of secure airway and support of ventilation and perfusion. Patients with mild to moderate toxicity may present with lethargy, miosis, hypotension, bradycardia, and muscle flaccidity and may require only supportive care. With more severe toxicity, respiratory depression, coma, noncardiogenic pulmonary edema, apnea, cardiovascular collapse, and death may occur. If taken orally, administration of activated charcoal is recommended, as soon as possible, to minimize absorption of morphine. Gastric lavage may be considered if time of ingestion is less than 1 h but should not delay administration of activated charcoal. Whole bowel irrigation may be considered for ingestions of controlled-release products. Emesis is contraindicated due to potential for significant CNS and respiratory depression. The specific antagonist naloxone is used to counteract respiratory depression and coma. A dose of 0.4–2.0 mg is given intravenously and can be repeated at intervals of 2 or 3 min. Naloxone can precipitate withdrawal syndrome in patients with physical dependence and caution is advised. The therapeutic effect of naloxone maybe of shorter duration than that of morphine activity; therefore, a naloxone continuous infusion may then be of benefit. Nalmefene and naltroxone are other opioid antagonists that are similar to naloxone but with longer duration of action and may be considered as an alternative to naloxone. These agents also have partial opiate agonist activity. Arterial blood gases, vital signs, and level of consciousness should be monitored continuously until cessation of symptoms.

See also: Poisoning Emergencies in Humans.

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Mothball *See* Naphthalene.

Mouse Lymphoma Assay

Robin C Guy

Background

The mouse lymphoma (MOLY) assay is an *in vitro* mammalian cell gene mutation test that can be used to detect gene mutations induced by chemical substances. The cell line used is the L5178Y MOLY cell. In these cell lines the most commonly used genetic end points measure mutation at the thymidine kinase (TK) locus on the mouse chromosome 11b.

In the early 1950s, while attempting to induce tumors in female DBA/2 mice by painting them with 3-methylcholanthrene, Dr Lloyd W. Law at the National Cancer Institute isolated the L5178Y cell line. Later, in 1958, Dr G. Fischer at Yale University was successful in getting the L5178Y cells to grow *in vitro*, using a semi-defined medium (Fischer's medium). In the early 1970s, D. Clive *et al*. developed the MOLY forward mutation assay.

TK is an enzyme that allows cells to take up thymidine from the surrounding medium for incorporation into the DNA. Specifically for this assay, the $TK^{+/-}$ phenotype is used. If a thymidine analog were added to suspension or soft agar, it would eventually be incorporated into the DNA, thereby resulting in the death of the cell. Cells deficient in thymidine kinase ($TK^{-/-}$) due to the mutation ($TK^{+/-} \rightarrow TK^{-/-}$) are resistant to the cytotoxic effects of the pyrimidine analogue trifluorothymidine (TFT). TK-proficient cells are sensitive to TFT, which causes the inhibition of cellular metabolism and halts further cell division. Thus mutant cells are able to proliferate in the presence of TFT, whereas normal cells, which contain thymidine kinase, are not.

When TFT is used as the selective agent and the ($TK^{+/-} \rightarrow TK^{-/-}$) mutation occurs, a possibility of two colony sizes of mutants may be observed. Large colonies of $TK^{-/-}$ mutants would have cytogenically normal 11b chromosomes while smaller colonies of $TK^{-/-}$ mutants would have cytogenically damaged 11b chromosomes. Therefore, increased numbers of the smaller mutant colonies are generally considered to be the result of a clastogen. This assay is unique in that it can detect chromosomal damage because the TK is functionally heterozygous. Therefore, if a deleted essential function is supplied by the homologous portion of the homologous chromosome, the cells will survive, but the colony may be slower growing and hence, smaller. Other mammalian mutagenesis assays do not pick up the chromosomal damage.

The preferred selective agent, 5-trifluorothymidine (TFT), may be used in this study as a thymidine analog. 5-Bromo 2′-deoxyuridine (BUdR) has also been successful in selecting mutants; however, BUdR is mutagenic in mammalian cells and it requires several cell divisions before triggering cell death, thereby giving a hazy background lawn, and is presently not used very often. Cells exposed to TFT do not need to go through the prolonged cell division, and therefore, treating with TFT creates a clearer background lawn.

Many compounds that are positive in the MOLY assay are mammalian carcinogens; however, there is not an exact correlation between the MOLY assay and carcinogenicity. Correlation may be dependent on chemical class. There is a possibility that pseudodiploid transformed cells may affect the response and thymidine analogs are not recommended for testing with this assay. Care should be taken to avoid conditions that would lead to results not reflecting authentic mutagenicity. Positive results that do not reflect authentic mutagenicity may arise from changes in pH, osmolality (including very high

concentrations of test article), extended exposure to S9 (a rat liver homogenate prepared from the livers of rodents treated with enzyme-inducing agents such as Aroclor 1254), or high levels of cytotoxicity.

The MOLY assay is recommended by ICH guidelines as part of a standard genetic toxicology battery. The other assays include the Ames (bacterial reverse mutation) and micronucleus tests.

Cultures of established cell lines or cell strains should be used. These should be determined to be mycoplasm free and should be karyotyped. To reduce the spontaneous frequency of the $TK^{-/-}$ mutants, the cells should be cleansed, that is, exposed to conditions that inhibit this phenotype, then returned to normal growth media for a few days before the start of the study. The cells used are selected on the basis of growth ability in culture and stability of the spontaneous mutation frequency.

Tests conducted *in vitro* generally require the use of an exogenous source of metabolic activation. This metabolic activation system simulates the metabolic characteristics of a mammal under *in vivo* conditions. Therefore, a typical assay should determine the chemical's mutagenic potential in the absence and presence of a metabolic activation system (S9). For both of the metabolic situations, a negative (solvent) and the appropriate positive control should be tested concurrently.

The MOLY assay consists of a preliminary dose range-finding phase and the final mutagenicity phase. For the dose range-finding phase, cells in suspension or monolayer culture are exposed to the test substance, both with and without metabolic activation, for about 4 h or any other suitable period of time. Nine to 10 concentrations should be used. They are then subcultured to determine cytotoxicity and to allow phenotypic expression prior to mutant selection. The vehicle used may include sterile water, dimethylsulfoxide, ethanol, or acetone. Cytotoxicity is usually determined after 24–48 h by measuring the relative cloning efficiency (survival) or the reduction in relative total growth of the cultures after the treatment period as compared to the negative controls.

Concentrations for the mutagenicity phase are then selected by determining at least four, but up to 10 concentrations that cover a range of 0–50% to 80–90% reduction in growth. Test and control cultures are prepared with freshly cleansed cells. The cells, with and without S9, should then be exposed to the test article, as appropriate. The cells are treated for 4 h, then are washed and placed in growth medium. The cells are counted and diluted twice in the next 2 days. After the second dilution, cultures are cloned; a specific number of cells are added to a flask containing cloning medium. Some of those cells are then exposed to TFT, and some of the cells are allowed to grow for the determination of cloning efficiency of the cells. Cells are incubated for a sufficient period of time to allow for phenotypic expression of induced mutations (10–12 more days). After this incubation time, colonies are counted. Mutant frequency is determined by seeding known numbers of cells in medium containing the selective agent to detect mutant cells, and in medium without selective agent (nonselective) to determine the cloning efficiency (viability). The mutant frequency is the number of mutant cells observed divided by the number of viable cells.

A typical MOLY study utilizes 10 ml of test article solution. A newer screening study only requires 2 ml of solution, thereby reducing the amount of test article needed for exposure to the cells.

Negative (solvent) and positive controls must be utilized for a valid study. A historical database must be maintained for these results. Examples of positive control substances that detect both large and small colonies include:

- Absence of exogenous metabolic activation:
  - Methylmethanesulfonate (CAS 66-27-3)
- Presence of exogenous metabolic activation:
  - cyclophosphamide (monohydrate) (CAS 50-18-0 (6055-19-2));
  - benzo(*a*)pyrene (CAS 50-32-8); and
  - 3-methylcholanthrene (CAS 56-49-5).

To ensure that the results of an assay are valid, specific criteria have been determined. The mutation frequency of the positive control group should be twice that of the solvent control group. The solvent controls should have a spontaneous mutation frequency between 20 and 100 per 10^6 surviving cells. In addition, the plating efficiency of the solvent controls must be $>0\%$.

Once the data are available for analyses, evaluation of the results follows. A positive result is one from which the test article, at two or more concentrations that produce $>10\%$ growth, produces a concentration-related increase in mutant frequency that is twofold greater than the background level. An equivocal result is one from which the test article, at a concentration that produces $>10\%$ growth, produces a mutant frequency that is twofold greater than the background level. In this case, the study should be repeated and if the results were repeatable, the study is positive. A negative result is one from concentrations that produced $>10\%$ growth; there were no concentration-related increase in mutant

frequency and no twofold greater increase in the background level.

See also: Ames Test; Federal Insecticide, Fungicide, and Rodenticide Act, US; Food and Drug Administration, US; Genetic Toxicology; Good Laboratory Practices (GLP); International Conference on Harmonisation; Micronucleus Assay; Redbook; Toxicity Testing, Mutagenicity.

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Relevant Websites

http://www.epa.gov – US Environmental Protection Agency.
http://www.fda.gov – US Food and Drug Administration Website.

Mouthwash

Nancy Linde

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- SYNONYMS: Cepacol; Gel-kam (Rx); Hexetidine; Listerine; Listermint; Plax; Prevention Mouth Rinse; Scope; Signal; Therasol; Viadent

Use

Mouthwash is used to improve oral hygiene through the reduction and/or prevention of dental plaque and gingivitis.

Background Information

Over-the-counter (OTC) mouthwashes may contain ethanol in concentrations of 14–27% (v/v), water, flavor, sweetener, preservative, color, and an astringent. Ethanol is a universal diluent that is mildly polar, able to easily cross cell membranes, and considered the toxic constituent in mouthwashes. Active ingredients are generally considered safe and may include combinations of eucalyptol (0.02–0.1%), menthol (0.04–2.0%), methyl salicylate (0.06–0.4%), and thymol (0.06%), as well as cetylpyridinium chloride, stannous fluoride, hydrogen peroxide, and povidone iodine.

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposures to mouthwash. Ocular exposure is also a possible route of exposure.

Toxicokinetics

Ethanol absorption through the stomach wall is minimal. Since rapid absorption occurs in the small intestine, factors that delay or enhance gastric emptying will influence the rate of absorption of ethanol into the blood. The enzymatic oxidation of ethanol occurs primarily in the liver, first to acetaldehyde by the enzyme alcohol dehydrogenase and then conversion to acetic acid by the enzyme aldehyde dehydrogenase. Acetic acid is available for the formation of acetyl coenzyme A, which enters the Krebs cycle and is eventually metabolized to carbon dioxide and water. Ethanol is uniformly distributed throughout all tissues and body fluids. The volume of distribution approximates 0.47–0.61 kg^{-1}. Approximately 2–10% is eliminated by the kidneys and lungs. Ethanol follows Michaelis–Menton kinetics. Therefore, half-life determination is not meaningful. An average adult decreases blood ethanol levels by 15–20 mg dl^{-1} h^{-1}.

Mechanism of Toxicity

The toxic component of commercial mouthwash products is ethanol. Ethanol is a central nervous system depressant that selectively depresses the reticular

activating system, resulting in disruption of the motor and thought processes. Preferential suppression of inhibitory neurons most likely causes the excitation seen at low ethanol concentrations.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animal toxicity corresponds to ethanol toxicity in humans.

Human

Since common commercially available mouthwashes contain only moderate concentrations of ethanol, casual ingestions will produce no toxicity. Significant ingestions, which result in blood alcohol levels of $\geqslant 100\,\mathrm{mg\,dl^{-1}}$ may result in ataxia, slurred speech, decreased motor skills, diplopia, and decreased attention. Unpredictable hypoglycemia may occur in children. Extreme ingestions, resulting in blood alcohol concentrations of $\geqslant 300\,\mathrm{mg\,dl^{-1}}$, may result in vision impairment, stupor, or respiratory failure. Mouthwashes may be irritating to the eyes on contact.

Chronic Toxicity (or Exposure)

Animal

Animal toxicity corresponds to ethanol toxicity in humans.

Human

The complications of ethanol abuse are many. Ethanol-containing mouthwash products may be used by a chronic abuser as an alcohol substitute.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Gastrointestinal decontamination procedures should be used as deemed appropriate to the patient's level of consciousness and the history of the ingestion. Casual ingestions of mouthwash do not necessitate treatment.

Ocular exposures require immediate flushing of the affected eye(s) with a steady stream of tepid water for a minimum of 15 min. If ocular irritation persists, an ophthalmology consultation is required.

Treatment of chronic alcohol toxicity involves replacement of nutritional deficiencies such as thiamine, pyridoxine, and vitamins K and C. Correction of dehydration, electrolyte imbalance, and acid–base imbalances is of paramount importance. Chronic abuse may be associated with dependence liability.

Environmental Fate

Normal uses of mouthwash do not present environmental concerns. The major ingredient, ethanol is water soluble and readily biodegrades.

Ecotoxicology

Normal uses of mouthwash do not present ecotoxicological concerns. The major ingredient, ethanol, is water soluble and does not bioaccumulate.

Exposure Standards and Guidelines

There are no exposure standards for mouthwash. Formulations, doses, and labeling are regulated by the Center for Drug Evaluation and Research of the US Food and Drug Administration (FDA). The US FDA also has requirements for manufacturing products according to Good Manufacturing Processes, and requires that all manufacturers, distributors, and mouthwash products be formally registered.

See also: Ethanol; Eye Irritancy Testing; Food and Drug Administration, US.

Further Reading

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Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Mouthwash.

Multiple Chemical Sensitivities

Kathleen Rodgers

Introduction

The constellation of symptoms that has come to be known as multiple chemical sensitivities (MCS) or idiopathic environmental intolerance (IEI) is increasingly recognized, although the definition of the phenomenon is elusive and its pathogenesis as a distinct entity is unconfirmed. Reports of patients with MCS are increasing, but information on its natural history is lacking. Individuals diagnosed with MCS do not exhibit a specific symptom pattern; a wide range of individual symptoms have been described with the most prominent symptoms being cardiorespiratory (nasal congestions, sore throat, and breathing difficulties), constitutional (fatigue, headache, joint aches, back pain, muscle aches, and weakness), and neuropsychologic (memory loss, forgetfulness, and mood or personality changes). These symptoms are similar to those seen in other syndromes such as chronic fatigue syndrome. In MCS, however, the symptoms have been attributed to chemical exposure. The diagnostic label of MCS is primarily used by a group of physicians who are called clinical ecologists. A description or diagnostic categorization of MCS, which would more readily allow animal modeling and clinical testing, has been difficult due to the involvement of multiple organ systems, lack of consistent symptomatology, and/or the absence of objective and measurable end points (either physical or laboratory findings). No pathophysiologic mechanism for these symptoms has been established although several speculative theories have been proposed: (1) MCS is a purely biologic or psychobiologic response to exposure to low levels of chemicals, (2) MCS is a misdiagnosis of physical or psychologic illness, (3) MCS is a illness belief system shaped by the patient's culture and is possibly iatrogenic, or (4) MCS is a somatoform disorder with genetic factors.

Four hypotheses with regard to the first theory have been invoked to explain the symptoms of MCS: (1) altered immune regulation, (2) neurogenic inflammation, (3) neurologic sensitization of the limbic system by odor, and (4) variations in an individual's biochemical makeup. Experimental models to test these hypotheses have not been established, thereby slowing research into mechanisms.

Definition

The first definition of MCS was created by Theron Randolph in 1962. Randolph described MCS as a condition that (1) is acquired; (2) includes physical and mental symptoms that can be triggered by chemical exposure; (3) has a specific adaptation syndrome (i.e., adaptation to chemicals is followed by chronic illness, withdrawal symptoms upon removal, and shock upon re-exposure); (4) is characterized by a spreading phenomena (i.e., an intolerance to an increasing number of environmental chemicals); and (5) may be resolved by avoidance of chemicals.

Another definition was put forth by ME Cullen and his clinical definition is most commonly used. Although no definition is widely accepted, the Cullen definition allows physicians to distinguish MCS from other collections of similar, commonly experienced symptoms. There are four important characteristics of this definition:

(1) MCS is acquired in relation to some documentable environmental exposure that may initially have produced a demonstrable toxic effect. This aspect excludes patients with long-standing health problems who later attribute certain symptoms to chemical exposure. (2) Symptoms involve more than one organ system, and recur and abate in response to predictable environmental stimuli. (3) Symptoms are elicited by exposures to chemicals that are demonstrable but very low. The exposures eliciting symptoms may be several standard deviations below the average exposures known to cause toxic or irritant health effects in humans and typically involve chemicals of widely varied structural classes and different mechanisms of toxicologic action. (4) The manifestations of MCS are subjective. No widely available test of organ system function can explain symptoms, and there is no objective evidence of organ system damage or dysfunction.

This definition is derived from Cullen (1987) and quoted exactly due to the need to precisely define the contribution of environmental exposure and to distinguish MCS from objectively defined illnesses such as asthma.

One problem with these definitions is that the relationship between the symptoms and the exposure is solely dependent on the patient's report. Further, patients with MCS are generally polysymptomatic and report sensitivities to multiple unrelated substances. In addition, cross-sectional studies indicate that persons diagnosed with MCS have impaired social and occupational adjustment and exhibit other characteristics

of disability. A recent definition by Ashford and Miller is an operational definition; that is, if symptoms disappear on removal from the agent and recur with specific challenges, then one can infer a causal association. In this definition, the rechallenge must be done under strictly controlled environmental conditions.

Theories of Etiology of MCS

Biologic and Psychobiologic Mechanisms

MCS, as defined by clinical ecologists, results from chemical exposure; however, the mechanisms that have been proposed include altered immune function, neurogenic inflammation, neurologic sensitization, and conditioned reflexes. To date, no mechanism has been established.

Various immunologic mechanisms have been postulated based on case reports. Alterations in various measures of antibody and cell-mediated immunity have been measured in patients with MCS, but no consistent pattern of abnormalities has been observed. Several factors, however, confound these studies: lack of standardization of protocols, wide variability (day to day and person to person) with most tests, lack of control for variables known to modulate the immune system (e.g., stress and smoking), and lack of concordance in reports of immune function response. In addition, despite some similarities in symptomatology, MCS is distinctly different from traditional allergy. Patients with allergy generally have well-defined, clinical reactions to allergens and symptoms of rhinitis, asthma, urticaria, or gastrointestinal symptoms occurring shortly after exposure. In addition, if a substance acts as an allergen, a specific cell- or antibody-mediated response develops so the body will only recognize the precise antigen or one with the same structure. It is difficult to explain how structurally different chemicals could result in such diverse symptomatology and organ involvement due to an adverse effect on the immune system.

Another postulated mechanism is an altered function of the central nervous and respiratory systems through an amplification of a nonspecific inflammatory response to low-level irritants (neurogenic inflammation hypothesis). This suggests that MCS may be initiated by the interaction of chemical irritants with sensory nerves or C-fiber neurons, a nonspecific response pathway. It is proposed that inhaled chemicals stimulate irritation receptors which activate sensory nerves to release mediators producing vasodilation, edema, and other manifestations of inflammation, leading to neurogenic inflammation. There is some evidence in animals for this theory, though similar studies in humans do not generally support this theory. In animals, nasal irritation activates systemic reflexes, producing increased blood pressure and bradycardia.

Another hypothesis is that environmental chemicals gain access to the central nervous system via the olfactory and limbic pathways. The absence of a blood–brain barrier in the olfactory system could permit direct access of environmental chemicals through the nasal mucosa to the olfactory bulb. The olfactory and limbic systems are anatomically linked and participate directly and indirectly in the regulation of cognitive, endocrine, and immune functions. In this hypothesis, chemical exposure could induce lasting changes in limbic and neuronal activity and alter a broad spectrum of behavioral and physiological functions.

Animal studies show the olfactory and limbic pathways are particularly susceptible to kindling, the ability of a stimulus previously unable to induce a seizure to later induce one. Animal studies also show that acute administration of a high dose or intermittent repeated low-dose exposures to chemicals cause limbic 'kindling', and that this response is amplified depending on the time between stimuli. Kindling without a seizure has been shown to cause affective behavior changes in animals. Kindling could amplify reactivity and lower the threshold response to low levels of chemicals.

Similar to the hypothesis of kindling is neural sensitization with progressive host amplification of polysymptomatic responses elicited by chemical exposures following an initiating event. In this theory, repeated exposures to the same or cross-sensitizing stimuli elicit enhanced responses. Within this framework, there may be genetic components that are associated with greater susceptibility to sensitization. These include female gender, alcohol-preferring parents, and preferences for carbohydrates. It is suggested that sensitization acts as an adaptive, sentinel function that allows adaptation to threatening environments. The hypothesis further suggests that individual response specificity rather than toxicant properties may determine the central, automic, and/or peripheral nervous system dysfunctions that are manifest in the disorder.

Additionally, cacosmia, a subjective sense of altered olfactory function and feeling ill on exposure to chemical odors, which is experienced by many MCS patients, might be associated with neurocognitive dysfunction. However, MCS patients do not demonstrate a consistent or specific pattern of neurocognitive deficits and disturbances of memory and attention, which may be a result of depression and anxiety. Some have suggested that cascosmia as

well as MCS may be a manifestation of a stress response. The 'precipitating event' leads to stress and may lead to heightened sensitivity to chemical odors or irritants. Animal studies suggest that exposure to a stressor produces a long-lasting sensitization to some drugs. In surveys of students, individuals reporting extreme cascosmia also had higher incidences of anxiety and depression.

Biochemical mechanisms have also been suggested to explain the symptoms associated with MCS. One hypothesis states that individuals who have genetically or nutritionally defective enzyme detoxification systems might be more susceptible to exposure to low levels of chemicals. Another hypothesis states that chemicals may cause blood vessel constriction, inflammation, or leaking in multiple organ systems which would produce various combinations of symptoms.

Arguing against biologic alterations by chemicals as a cause of MCS symptoms is the observation the patients attribute their symptoms to levels of chemicals much lower than those to which others are occupationally exposed with no adverse effects. Therefore, the relationship of MCS symptoms to chemical exposure does not meet accepted toxicologic principles. In addition, the current evidence and models do not meet the criteria set forth in toxicology to establish a causal relationship between chemicals and MCS, such as strength of association, consistency, specificity, temporality, biologic gradient, and plausibility.

Misdiagnosis

As stated, some physicians believe MCS symptoms are not due to chemical exposure but may be the result of misdiagnosed physical or psychologic illness. Many investigators have concluded that MCS patients are not significantly different from psychiatric patients who do not project their disorder onto the environment. Studies show that patients with MCS meet criteria for depression, anxiety, somatization, and obsessive–compulsive and personality disorders. Psychiatric evaluations have shown that MCS may be a new variant of a 'somatoform disorder'. In this disorder, the patients have a psychological tendency to somatocize or misconstrue normal physical sensation. In addition, studies show that psychologic mechanisms are important in the manifestation, if not the etiology, of MCS. Since it is theoretically possible that MCS produces the psychiatric symptoms, through chemical exposure, lifestyle limitations, or nonbelief of family members, studies have been conducted to attempt to evaluate the presence of psychiatric illness in patients that predated the onset of MCS symptoms. While these studies are ongoing and there is difficulty in discerning the temporal pattern of disease, most of them indicate that the prevalence of somatization symptom pattern among MCS patients prior to onset of symptoms attributed to chemical exposure was significantly greater than that in matched controls. In a group of patients followed over 9 years, 83% met DSM-IV criteria for lifetime mood disorder, 56% for lifetime anxiety disorder, and 56% for lifetime somatoform disorder. These studies also show that there was no apparent difference in the prevalence of preexisting anxiety or depression. However, there are a small number of persons diagnosed with MCS who do not have histories of psychiatric disorders who should be more closely examined for possible mechanisms.

MCS as a Belief System

Others suggest that MCS is a belief system that is supported and reinforced by the clinical ecology subculture. Physicians within this subculture claim special expertise in the diagnosis and treatment of MCS, perpetuating the belief that this disorder is widespread and is responsible for substantial suffering and disability. Within this model, the cause of the illness is believed to be outside the control of the patients and leads to the role of a victim with adversarial interactions with those who do not share the belief system, such as conventional health care providers. MCS shares many features, such as pain, fatigue, and headache, with several syndromes, such as chronic fatigue syndrome, fibromyalgia, and neurasthenia, with few or no objective findings of pathology which encompass patients with functional disability. A factor that may contribute to this culturally shaped illness belief system is the increasing concern of the public regarding health effects of chemical exposure. It is unlikely that the majority of MCS patients are simulating their symptoms or that the symptoms result from suggestion. However, it is likely that the attribution of the symptoms to chemical exposure is due to suggestion in some cases. It is also likely that a patient's beliefs regarding illness modify the expression of symptoms even when resulting from a direct toxic effect of a chemical.

Conclusions

To understand the phenomenon of MCS will require interaction among many disciplines to allow for the examination of the influence of the mind on the body and the influence of physical disease on the psyche. Illness should not be regarded as less 'real' because psychogenic mechanisms may play a major role in

causation, and this should not prevent treatment of the symptoms. In addition, patients diagnosed with MCS are heterogenous and there may be more than one causal mechanism in each person. Since none of the hypotheses described have substantial scientific support, dogmatic adherence to any one theory is unwise, particularly by the physician.

See also: Immune System; Neurotoxicity; Occupational Toxicology; Psychological Indices of Toxicity.

Further Reading

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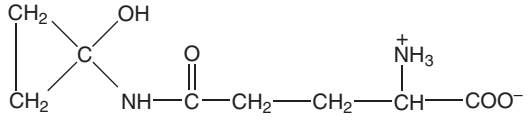
Winder C (2002) Mechanisms of multiple chemical sensitivity. *Toxicology Letters* 128(1–3): 85–97.

Muscarine *See* Mushrooms, Muscarine.

Mushrooms, Coprine

Anthony S Manoguerra

- Chemical Names: *Coprinus atramentarius; Coprinus insignis; Coprinus variegatus* (also known as *C. quadrifidus*); possibly other *Coprinus* species
- Synonym: Inky-caps
- Chemical Structure:



Uses

There are no uses for mushrooms in this group. They are usually consumed when mistaken for edible mushrooms.

Exposure Routes and Pathways

Ingestion is the route of exposure.

Toxicokinetics

Symptoms occur within 20 min to 2 h after the ingestion of this mushroom and ethanol. The effect of coprine may persist for up to 5 days after ingestion of the mushroom.

Mechanism of Toxicity

Poisoning with mushrooms in this group occurs when ethanol is consumed shortly before or within 5 days after eating the mushrooms. Coprine (*N*(5)-(1-hydroxy cyclopropyl)-L-glutamine) is the active constituent in these mushrooms and has been shown to inhibit liver aldehyde dehydrogenase. The active metabolite, cyclopropanone hydrate, has also been shown to possess similar activity. This inhibition of ethanol metabolism at the point of aldehyde dehydrogenase results in accumulation of acetaldehyde. In the absence of concurrent ethanol consumption, these mushrooms are edible.

Acute and Short-Term Toxicity (or Exposure)

Animal

The consumption of mushrooms and ethanol concurrently is a typically human occurrence; therefore, poisoning in animals is unlikely.

Human

When these mushrooms and ethanol are consumed within the appropriate time frame, symptoms typically develop within 20 min to 2 h. The reaction resembles a disulfiram–ethanol reaction. Symptoms commonly include nausea, vomiting, facial flushing, throbbing headache, weakness, and paresthesias. Less frequently, chest pain, hypotension, and shortness of breath have been seen. No laboratory methods are available for determining the presence of coprine in biologic fluids.

Clinical Management

Because the syndrome is self-limiting and recovery is complete, symptomatic care is often all that is required. Emesis is not indicated because often many hours have elapsed since the ingestion of the mushroom. Also, vomiting is a prominent feature of this type of poisoning and induced emesis may worsen fluid and electrolyte losses. There is no evidence that activated charcoal and/or cathartics provide any benefit in this syndrome. Experimentally, 4-methylpyrazole inhibits the production of acetaldehyde by blocking alcohol dehydrogenase. The clinical usefulness in this type of treatment in mushroom poisoning has not been demonstrated.

See also: Disulfiram; Ethanol; Plants, Poisonous.

Further Reading

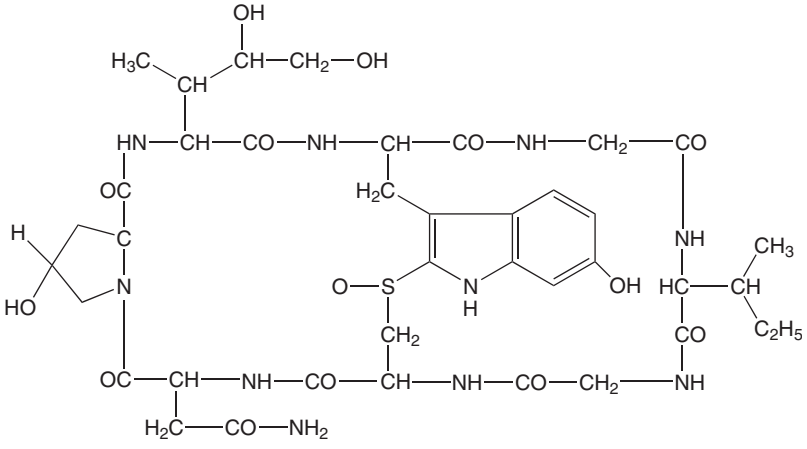
Buck RW (1961) Mushroom toxins – A brief review of the literature. *New England Journal of Medicine* 265: 681–686.

Reynolds WA and Lowe FH (1965) Mushrooms and a toxic reaction to alcohol: A report of 4 cases. *New England Journal of Medicine* 272: 630–631.

Mushrooms, Cyclopeptide

Anthony S Manoguerra

- Chemical Names: Several genera of mushrooms contain toxic cyclopeptides; these mushrooms include but are not limited to *Amanita bisporigera*; *Amanita hygroscopica*; *Amanita ocreata*; *Amanita phalloides*; *Amanita subalbiacea*; *Amanita tenuifolia*; *Amanita verna*; *Amanita virosa*; *Galerina autumnalis*; *Galerina fasciculata*; *Galerina marginata*; *Galerina sulcipes*; *Galerina venenata*; *Lepiota castanea*; *Lepiota helveola*; *Lepiota subincarnata*; *Lepiota josserandii*; *Conocybe filiaris*
- Synonyms: Destroying angel (*Amanita virosa*); Death cap (*Amanita phalloides*)
- Chemical Structure: α-Amanitin



Uses

There are no uses for mushrooms in this group. They are usually consumed when mistaken for edible mushrooms.

Exposure Routes and Pathways

Ingestion is the route of exposure.

Toxicokinetics

Amatoxins are absorbed rapidly from the gastrointestinal tract. These toxins may be detected in the urine as early as 90–120 min after ingestion of the mushrooms. Radiolabeled amatoxins given to a dog model showed a volume of distribution equal to the volume of extracellular water (160–290 ml kg^{-1}). Amatoxins

disappear rapidly from the blood because they are taken into cells rapidly. No biotransformation of amatoxins occurs. Amatoxins are found in the urine shortly after ingestion of the mushrooms and continue to be detectable for up to 96 h after ingestion. They have also been detected in diarrhea fluid and bile.

Mechanism of Toxicity

Cyclopeptide mushrooms contain both amatoxins and phallotoxins. Studies in animals have shown that, although the phallotoxins are highly toxic when given parenterally, they are not absorbed from the gastrointestinal tract and do not produce toxicity when given orally. The toxicity of cyclopeptide mushrooms is believed to be due to the amatoxins. At least six amatoxins have been identified that differ according to amino acid substitutions on the peptide ring. The α- and β-amanitins are felt to be the predominant cyclopeptides producing systemic toxicity. The phallotoxins may contribute to gastrointestinal symptoms but this is unclear. Amatoxins interfere with RNA and DNA transcription by interfering with RNA polymerase II. Cells with the highest rate of turnover are affected most severely.

Acute and Short-Term Toxicity (or Exposure)

Animal

Ingestion of these mushrooms in dogs have taken place both accidentally and experimentally. The toxic effects mimic those seen in humans.

Human

There is typically a delay of 6–24 h between the ingestion of the mushrooms and the onset of symptoms. The first symptoms are nausea, severe vomiting, and diarrhea, which may result in severe dehydration and electrolyte imbalances. Gastrointestinal symptoms may persist for several days. Over the course of the next 24–36 h, evidence of hepatic injury becomes evident both clinically and by laboratory measurements. Patients may progress to hepatic failure with coma, hemorrhage, and renal failure or begin to recover depending on the degree of injury. In fatal poisonings, death usually occurs after 5 or 6 days. Mortality ranges up to 50% in untreated patients and appears to be $<5\%$ in patients who receive modern intensive supportive care. The severity of poisoning also seems to correlate with the amount of toxin ingested on a body weight basis. The mortality in children appears to be greater probably because of the larger amount of toxin ingested in relation to their body size.

Clinical Management

Immediate and vigorous fluid and electrolyte replacement must be carried out. Oral activated charcoal may be given if the ingestion occurred within the previous 24 h and severe vomiting has not yet begun. No specific antidotal therapy exists for the treatment of this ingestion, although many substances have been tried. The mainstay of therapy is meticulous supportive care.

Some authors have advocated the use of multiple dose activated charcoal or gastroduodenal drainage, but evidence for the effectiveness of these procedures is lacking. Treatment of hepatic failure follows the routine supportive procedures standard for this process. Liver transplantation has been used successfully in patients who appeared to have developed irreversible hepatic failure. Experimental studies in animals have suggested the use of high-dose penicillin G and silibinin. These substances may inhibit the uptake of amatoxins into the liver and are often given in combination in Europe. Silibinin is not available in the United States. The efficacy of these treatments in humans is unknown. Thioctic acid has also been advocated; its efficacy is unclear. It does not appear to provide any protection in experimental animal studies. It also is not available in the United States. High-dose cimetidine, high-dose vitamin C, and *N*-acetylcysteine have been studied in animals and do not provide protection against toxicity. Based on the fact that large amounts of amatoxins are found in the urine during the first 24 h after ingestion, it has been suggested that forced diuresis may increase the excretion of the toxin. Proof of efficacy is lacking. Hemodialysis or hemoperfusion have not been shown to enhance survival and hemodialysis is only indicated as a supportive measure in patients who develop renal failure.

Further Reading

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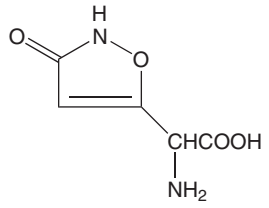
Hanrahan JP and Gordon MA (1984) Mushroom poisoning. *Journal of the American Medical Association* 251: 1057–1061.

Mushrooms, Ibotenic Acid

Anthony S Manoguerra

- Chemical Names: *Amanita muscaria*; *Amanita pantherina*; *Amanita corthurnata*
- Synonyms: Fly-agaric; Panther
- Chemical Structure:



Uses

There are no uses for this mushroom. It is typically consumed for its psychopharmacologic effects.

Exposure Routes and Pathways

Ingestion is the route of exposure.

Toxicokinetics

The symptoms of intoxication seen after ingestion of this mushroom appear ~1 h after ingestion. Ibotenic acid is converted to muscimol by decarboxylation. Ibotenic acid and muscimol can both be detected unchanged in the urine. Other metabolites found in the urine include pantherin, tricholomic acid, and solitaric acid. Intoxication with this mushroom peaks at ~5 h after ingestion and lasts for up to 10 h with a hangover effect the next day.

Mechanism of Toxicity

Ibotenic acid is structurally similar to glutamic acid, whereas muscimol closely resembles gamma-aminobutyric acid (GABA). Muscimol has an affinity for GABA receptors in the central nervous system, functioning as a false neurotransmitter, and appears to mimic the effects of GABA.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animal ingestions of these mushrooms are rare and, therefore, experience is limited. Of nine puppies that supposedly had ingested *Amanita pantherina*, three died and the others developed seizures and the inability to walk. All survivors fully recovered by 17 h after ingestion.

Human

Following the ingestion of a single mushroom, symptoms of intoxication typically appear within an hour. Nausea and vomiting are common. Users describe a typical experience where they can "view themselves from outside their own bodies along with a sense of being freed from gravity." Small objects appear large. The appearance of the user during this time may resemble someone with ethanol intoxication. On occasion, users appear to have the desire to carry out extreme physical activity followed by a deep, 'death-like' sleep from which arousal is difficult. Upon wakening, users describe vivid visions during this dream period. Severe poisoning is rare, but seizures have been reported to occur in children. Deaths are said to have occurred from these mushrooms but the documentation of these occurrences is poor and the deaths may have been related to medical problems that were exacerbated by the intoxication.

Clinical Management

Most patients who ingest these mushrooms require no treatment other than observation. In recent, accidental ingestions, activated charcoal may be administered, although the efficacy of this treatment is unknown. In severe cases, when seizures occur, benzodiazepine therapy may be required. Long-term anticonvulsant therapy should not be required because the effects of the mushroom are short-lived. All other treatment is supportive and symptomatic in nature. Atropine should not be administered because these mushrooms, despite their name, contain only trace to no amounts of muscarine or other cholinergic substances.

Further Reading

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Carter CA, Wojciechowski NH, and Skoutakis VA (1983) Management of mushroom poisoning. *Clinical Toxicology Consultant* 5: 103–118.

Mushrooms, Monomethylhydrazine

Anthony S Manoguerra

- Chemical Names: *Gyromitra esculenta; Gyromitra fastigiata; Gyromitra infula; Gyromitra ambigua; Gyromitra brunnea; Gyromitra californica; Gyromitra korfii; Gyromitra sphaerospora; Gyromitra giga*. All other species of Gyromitra should be considered toxic unless proven edible. Gyromitra sp. mushrooms are nongilled mushrooms in the class Ascomycetes
- Synonyms: False morel; Beefsteak mushroom; Elephant ears mushroom
- Chemical Structure: Monomethylhydrazine $CH_3–NH–NH_2$

Uses

There are no uses for mushrooms in this group. They are usually consumed when mistaken for edible mushrooms.

Exposure Routes and Pathways

Ingestion is the most likely route of exposure; however, it has been stated (without good documentation) that poisoning can occur from inhaling monomethylhydrazine vapors that come off in the steam during boiling of the mushrooms.

Toxicokinetics

The absorption rate is not known. Symptoms typically occur after a latent period of 6–24 h after ingestion of the mushroom. Gyromitrin is converted to methylformylhydrazine (MFH) and then to monomethylhydrazine (MMH). Some MFH is also converted to nitrosamide, which causes liver tumors in experimental animals.

Mechanism of Toxicity

Gyromitra sp. mushrooms contain ~0.12–0.16% of the toxin *Gyromitrin* (*n*-methyl-*N*-formylhydrazone). This compound is very unstable and undergoes hydrolysis at low cooking temperatures to the toxic compound MMH. In some species of *Gyromitra*, MMH may be found in its free form and, therefore, cooking may not be required for toxicity to occur. Because MMH is highly volatile, it has been suggested that boiling of the mushroom and discarding the water may yield an edible mushroom, although cases exist in the European literature in which poisoning occurred even after the mushroom was boiled and the water discarded. MMH is thought to act similar to other naturally occurring and synthetic hydrazines by acting as a pyridoxine antagonist. Because many enzyme systems require pyridoxine as a cofactor, hydrazines are capable of affecting numerous metabolic pathways. For example, hydrazines interfere with pyridoxine utilization by both glutamic acid decarboxylase and γ-aminobutyric acid (GABA) transaminase leading to decreased concentrations of the inhibitory neurotransmitter, GABA, in the brain and producing the resultant seizures. Hepatotoxicity is thought to result from a direct toxic action of a metabolite similar to that which occurs with isoniazid. MMH has also been shown to inhibit glycolysis; blood glucose levels fall markedly in experimental animals after exposure to MMH. MMH may also cause methemoglobinemia and hemolysis of red blood cells.

Acute and Short-Term Toxicity (or Exposure)

Animal

The same pattern of toxicity that occurs in humans has been induced experimentally in small animals. The treatment is similar.

Human

Symptoms typically appear following a 6–8 h latent period; however, symptoms have been reported to occur as early as 2 h after ingestion or as long as 24 h after ingestion of the mushrooms. The initial symptoms are vomiting, fatigue, dizziness, and headache. In some cases, but not consistently, a watery diarrhea may also occur. In mild cases, recovery then occurs over a period of 2–5 days. In severe cases, the poisoning may progress to coma, seizures, and hepatic injury leading to hepatic coma and death. Hemolysis with a resultant anuria has also been reported.

Chronic Toxicity (or Exposure)

Animal

MMH and its precursor metabolite nitrosamide are low-grade carcinogens in experimental animals. How this relates to chronic, subacute consumption of this substance in mushrooms is unknown.

Clinical Management

As most patients do not present for care until symptoms develop, emesis or gastric lavage are unlikely to provide much benefit. Activated charcoal administration may be considered to reduce absorption of any remaining material in the gastrointestinal tract, but proof of efficacy is lacking. Based on the resemblance to isoniazid and other hydrazine toxicity, pyridoxine has been suggested, although experience is limited. The recommended dose is $25\,mg\,kg^{-1}$ given as an intravenous infusion over 15–30 min. It can be repeated to treat neurologic symptoms (seizures and coma) as needed, up to a maximum of $15–20\,g\,day^{-1}$. Seizures may also be controlled with benzodiazepines or barbiturates. Severe hemolysis may require blood transfusions and anuria may require short-term hemodialysis until renal function recovers. Hepatic injury leading to hepatic coma is treated with supportive care.

See also: Hydrazine; Plants, Poisonous; Pyridoxine.

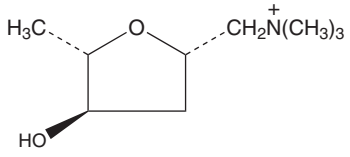
Further Reading

Braun R, Greeff U, and Netter KJ (1979) Liver injury by the false morel poison gyromitrin. *Toxicology* 12: 155–163.
Giusti G and Carnevale A (1974) A case of fatal poisoning by *Gyromitra esculenta*. *Archives of Toxicology* 33: 49–54.

Mushrooms, Muscarine

Anthony S Manoguerra

- Chemical Names: Muscarine and muscarine-like compounds are present in varying quantities in many different species of mushrooms but reach clinically significant quantities only in certain species of *Clitocybe*, *Inocybe*, and *Omphalotus* mushrooms. These species include but are not limited to *Clitocybe dealbata*, *Clitocybe rivulosa*, *Clitocybe dilatata*, *Inocybe agglutinata*, *Inocybe cincinnata*, *Inocybe entheles*, *Inocybe fastigata*, *Inocybe geophylla*, *Inocybe godeyi*, *Inocybe griseolilacina*, *Inocybe lacera*, *Inocybe lilacina*, *Inocybe mixtilis*, *Inocybe napipes*, *Inocybe obscuroides*, *Inocybe pallidipes*, *Inocybe patouillardi*, *Inocybe pudica*, *Inocybe purica*, *Inocybe rimosus*, *Inocybe sororia*, *Inocybe subdestricta*, *Inocybe umbrina*, *Omphalotus illudens*
- Synonym: 'Jack-O-Lantern' mushroom (*Omphalotus illudens*)
- Chemical Structure:



Uses

There are no uses for mushrooms in this group. They are usually consumed when mistaken for edible mushrooms.

Exposure Routes and Pathways

Ingestion is the route of exposure.

Toxicokinetics

Symptoms occur typically within 15–120 min following ingestion of the mushroom.

Mechanism of Toxicity

Mushrooms in these genera may contain varying amounts of muscarine isomers and muscarine-like compounds. L(+)Muscarine is the most potent of the muscarine isomers found. The varying amounts of these isomers in different mushrooms account for the diversity of reports of the severity of symptoms produced by muscarine-containing mushrooms.

Muscarine binds to the so-called 'muscarinic' receptors in the parasympathetic nervous system. These are primarily postganglionic cholinergic receptors in smooth muscle and glands. Muscarine does not act on so-called 'nicotinic' receptors, which are found in ganglionic synapses and at the neuromuscular junction. Muscarine is a tertiary amine structure and, therefore, does not diffuse into the central nervous system to an appreciable extent. Symptoms are, therefore, limited to the peripheral nervous system.

Acute and Short-Term Toxicity (or Exposure)

Animal

Toxic reactions similar to those found in humans can be induced in experimental animals and are, therefore,

expected to occur following ingestion of the mushrooms. Treatment is similar to that for humans with the use of atropine.

Human

Stimulation of nicotinic receptors produces small pupils, blurred vision, excessive perspiration, salivation and lacrimation, bradycardia, increased intestinal peristalsis, increased pulmonary secretions, and decreased blood pressure. Acute asthma exacerbations may occur in patients with reactive airway disease. The onset is typically within 15–30 min after ingesting the mushrooms but may be delayed by up to 120 min. Nausea and vomiting are often the first symptoms to occur. The rare deaths reported appear to occur from cardiovascular collapse and respiratory failure.

Clinical Management

Atropine is a competitive antagonist of muscarine at the cholinergic receptors and is, therefore, 'antidotal' in these types of poisonings. Doses sufficient to reverse the effects of excessive pulmonary secretions and bradycardia should be administered. Activated charcoal may be useful in recent ingestions, but because most patients have vomiting and diarrhea, its usefulness is limited and unproved.

See also: Atropine; Neurotoxicity.

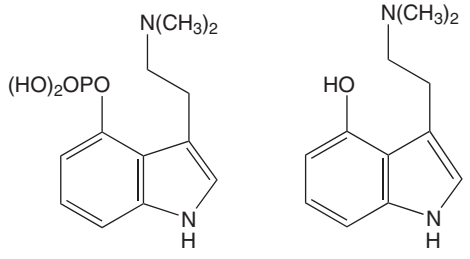
Further Reading

Benjamin DR (1995) *Mushrooms – Poisons and Panaceas*. New York: WH Freeman & Co.

Mushrooms, Psilocybin

Anthony S Manoguerra

- Chemical Names: Psilocybin and psilocin have been found in the following mushroom species (as well as in others): *Psilocybe aztecorum; Psilocybe baeocystis; Psilocybe bonetti; Psilocybe caerulescens; Psilocybe caerulipes; Psilocybe candidipes; Psilocybe cambodginiensis; Psilocybe coprinifacies; Psilocybe cubensis; Psilocybe cyanescens; Psilocybe fimetaria; Psilocybe mexicana; Psilocybe pelliculosa; Psilocybe quebecensis; Psilocybe semilanceata; Psilocybe sempervira; Psilocybe serbica; Psilocybe strictipes; Psilocybe stuntzii; Psilocybe subaeruginosa; Psilocybe zapatecorum; Conocybe cyanopus; Conocybe smithii; Gymnopilus aeruginosa; Gymnopilus validipes; Panaeolus africanus; Panaeolus ater; Panaeolus cambodginiensis; Panaeolus fimicola; Panaeolus foenisecii; Panaeolus subalteatis; Panaeolus tropicalis*
- Synonyms: Hallucinogenic mushrooms; Magic mushrooms; 'Blue legs'; 'Liberty caps'
- Chemical Structures: Psilocybin and Psilocin



Psilocybin Psilocin

Uses

There are no uses for mushrooms in this group. These mushrooms are intentionally ingested for their hallucinogenic activity.

Exposure Routes and Pathways

Ingestion is the route of exposure.

Toxicokinetics

The onset of symptoms typically begins 20–60 min following ingestion of the mushroom, although it has been reported to be delayed up to 3 h. The typical hallucinogenic experience lasts ~3 or 4 h.

Mechanism of Toxicity

These mushrooms contain the psychoactive compound psilocybin and, in some cases, also the lesser active substance psilocin. Psilocybin is highly stable and is not destroyed by cooking or drying. Psilocin is rapidly destroyed by oxidation. Psilocybin can be extracted from the mushroom by boiling the mushroom in water. The exact mechanism of action of psilocybin has not been determined but as an indoleamine it is thought to act similarly to LSD, as an agonist at 5-hydroxytryptamine receptors in the central nervous system.

Acute and Short-Term Toxicity (or Exposure)

Animal

Horses and dogs have been reported to have eaten these mushrooms with predictable effects on their behavior. Treatment has included sedation or placement of the animal in a darkened, quiet environment. In severe cases seizures and hyperthermia may occur.

Human

These hallucinogenic mushrooms are small and, therefore, consumption of more than a single mushroom is required for the effects to occur. For example, two or three mushrooms of *P. cubensis* will produce the desired hallucinogenic experience, while 20–40 *P. cyanescens* are required for an equivalent experience. In addition, the concentrations of psilocybin vary significantly between species of mushrooms. Following the ingestion of an effective number of mushrooms, dizziness, giddiness, muscle twitching, restlessness, and anxiety begin within 30 min. Approximately 20% of users will also experience some nausea and vomiting. In 30–60 min, the user will begin to experience visual hallucinations and perceptual distortions. The user may experience a 'good' or 'bad' 'trip' depending on mood, environment, and prior hallucinogenic experience. Mild tachycardia and hypertension are common during this time period. The effects of psilocybin are short lived and recovery is typically complete in 4–6 h. The experience usually ends with drowsiness progressing to sleep. Rare cases of flashbacks occurring 2 weeks to several months after the initial experience have been reported. Severe or life-threatening toxicity is not expected except in children or in adults who have ingested large amounts. These cases are reported to have had seizures and hyperthermia.

Clinical Management

No specific treatment is indicated for the ingestion of these mushrooms. Patients having a bad trip may require isolation in a dark, quiet environment, and calm reassurance until the effects of the drug have worn off. In severe cases, sedation may be indicated. In severely symptomatic cases, seizures can be controlled with benzodiazepines and hyperthermia treated with external cooling.

See also: Drugs of Abuse; LSD (Lysergic Acid Diethylamide); Plants, Poisonous.

Further Reading

Peden NR, Bissett AF, and Macaulay KE (1981) Clinical toxicology of 'magic mushroom' ingestion. *Postgraduate Medical Journal* 57: 543–545.

Southcott RV (1974) Notes on some poisonings and other clinical effects following ingestion of Australian fungi. *South Australian Clinics* 6: 441–478.

Mustard Gas

Harry Salem*

Published by Elsevier Inc.

- Chemical Name: bis(2-Chloroethyl) sulfide
- Chemical Abstracts Service Registry Number: CAS 505-60-2
- Synonyms: Sulfur mustard; 1,1-Thiobis(2-chloroethane); 1-Chloro-2-(β-chloroethylthio)ethane; 2,2′-Dichlorodiethyl sulfide; Distilled mustard; S mustard; S-lost; Schwefel-lost; Yellow cross liquid; Yperite; Kampstoff lost; HD; HT; H
- Chemical/Pharmaceutical/Other Class: Thioether
- Chemical Formula: $C_4H_8Cl_2S$
- Chemical Structure:

$$Cl–CH_2–CH_2–S–CH_2–CH_2–Cl$$

*The views of the author do not purport to reflect the position of the US Department of Defense. The use of trade names does not constitute official endorsement or approval of the use of such commercial products.

Uses

Mustard gas is a chemical warfare agent belonging to the blister agent/vesicant class. It is a cytotoxic alkylating compound similar to the other type of vesicants or blister agents such as nitrogen mustard, Lewisite, and phosgene oxide. Mustard causes blistering of the skin and mucous membranes.

Exposure Routes and Pathways

Actually, mustard gas is not a gas, but a dense, yellow to brown oily liquid with a low vapor pressure and relatively high melting point. Mustard can also be released in the air as a vapor and thus exposure of

skin, eyes, and respiratory tract can be to vapor or liquid. If sulfur mustard is released into water supplies, exposure can occur from drinking contaminated water or getting it on the skin. Although it currently has no medical use, it was available for use as a treatment for psoriasis. Mustard may smell like garlic, onions, mustard, or have no odor.

Toxicokinetics

Mustard gas is lipophilic and will accumulate in brain and fatty tissue. Mustard gas is soluble in water to less than 0.1% and hydrolyzes in water with a half-life of 3–5 min. It is freely soluble in organic solvents. It has been detected in the blood after dermal or inhalation exposure. Although mustard dissolves slowly in aqueous solution, it must first dissolve in sweat or extracellular fluid to be absorbed. Following dissolution, mustard molecules rapidly rearrange to form extremely reactive cyclic ethylene sulfonium ions that immediately bind to intracellular and extracellular enzymes, proteins, and other cellular components. Mustard binds irreversibly to tissues within several minutes after contact. If decontamination is not done immediately after exposure, injury cannot be prevented. However, later decontamination might prevent a more severe lesion.

The biological half-life of the chemical has not been published, but products are excreted in the urine for several days after acute exposure. Traces of the agent are exhaled and excreted in the feces. Several hours can pass before symptoms become manifest but this is attributed to the mechanism of action and not to direct effects of residual levels of the agent. Mustard gas is a greater threat in hot and humid climates.

Mechanism of Toxicity

Although the exact mechanism by which mustard produces tissue injury is not known, the mechanism of action has been suggested to be its ability to directly alkylate DNA. This DNA alkylation and cross-linking in rapidly dividing cells such as basal keratinocytes, mucosal epithelium, and bone marrow precursor cells leads to cellular death and inflammatory reactions. Systemic effects with extensive exposures include bone marrow inhibition, with a drop in the white blood cell count, and gastrointestinal tract damage.

Human Toxicity

Mustard gas is a powerful irritant and vesicant. Dermal effects range from itching to erythema, blistering, corrosion, and necrosis. Dermal blistering is delayed in onset and slow to heal. Ocular irritation, conjunctivitis, and blindness can occur. Respiratory symptoms include a harsh and painful cough, bronchitis, sneezing, rhinorrhea, and sore throat. Mustard gas is not acutely lethal; 2–3% of soldiers exposed to mustard gas during World War I died of its direct effects. Death is generally due to respiratory collapse, shock, and secondary infections.

The estimated human incapacitating and lethal dosages by different routes of exposure are listed below:

	ICt_{50} ($mg\,min\,m^{-3}$)	LCt_{50} ($mg\,min\,m^{-3}$)	LD_{50} ($mg\,kg^{-1}$)
Eyes	200	–	–
Inhalation	–	1 500	–
Skin	2000	10 000	9
Oral	–	–	0.7

The time-weighted average exposure limit for the workplace is estimated at $0.003\,mg\,m^{-3}$, while for the general population it is estimated to be $0.0001\,mg\,m^{-3}$. The inhalation minimal risk level (MRL) for acute exposure for 14 days or less is $0.0007\,mg\,m^{-3}$ and for 15–364 days it is $0.00002\,mg\,m^{-3}$. The oral MRL is $0.0005\,mg\,kg^{-1}\,day^{-1}$ for 14 days or less, and $0.00007\,mg\,kg^{-1}\,day^{-1}$ for 15–364 days.

Survivors may be susceptible to bronchitis and pneumonia, and may be at an increased risk to develop tumors of the respiratory tract. Mustard gas produces tumors in animal studies.

The US National Advisory Committee has developed acute exposure guidelines (AEGLs) to protect people from harmful effects of short-term exposures (8 h or less) to sulfur mustard. The AEGL-1 for a 10 min exposure is $0.40\,mg\,m^{-3}$ and it is $0.008\,mg\,m^{-3}$ for an 8 h exposure. Exposure to higher concentration may result in eye irritation. The AEGL-2 for 10 min is $0.60\,mg\,m^{-3}$ and for 8 h it is $0.013\,mg\,m^{-3}$. Exposure to higher concentration may result in swelling of the eyes, sensitivity to light, and eye irritation. The AEGL-3 is $3.9\,mg\,m^{-3}$ for 10 min and for 8 h it is $0.27\,mg\,m^{-3}$. Exposure to higher concentrations may result in death.

Sulfur mustards are vesicants and alkylating agents; however, the biochemical mechanisms of action are not clearly understood. They are highly reactive and combine rapidly with proteins, DNA, or other molecules. Therefore, within minutes following exposure, intact mustard or its reactive metabolites are not found in tissue or biological fluids. Sulfur mustards also have cholinergic activity, stimulating both muscarinic and nicotinic receptors. The onset of clinical symptoms and their time of onset depend on the severity of exposure. The death rate

from exposure to sulfur mustard is low (2–3% during World War I). Death usually occurs between the fifth and tenth day due to pulmonary insufficiency complicated by infection due to immune system compromise.

The eye is the most sensitive tissue to sulfur mustard effects. Sulfur mustard vapor or liquid may cause intense conjunctival and scleral pain, swelling, lacrimation, blepharospasm, and photophobia; however, these effects do not appear for an hour or more. Miosis due to cholinergic effects may occur. High concentrations of vapor or liquid can cause corneal edema, perforation, blindness, and later scarring.

Direct skin exposure to sulfur mustards causes erythema and blistering. Generally, a pruritic rash will develop within 4–8 h followed by blistering 2–18 h later. Contact with the vapor may result in first- and second-degree burns, while contact with the liquid typically produces second- and third-degree chemical burns. An area of burn covering 25% or more of the body surface area may be fatal.

Dose-dependent inflammatory reactions in the upper and lower airway begin to develop several hours after exposure and progress over several days. Burning nasal pain, epistaxis, sinus pain, laryngitis, loss of taste and smell, cough, wheezing, and dyspnea may occur. Necrosis of respiratory epithelium can cause pseudomembrane formation and local airway obstruction.

Ingestion may cause chemical burns of the gastrointestinal tract and cholinergic stimulation. Nausea and vomiting may occur following ingestion or inhalation. Early nausea and vomiting is usually transient and not severe. Nausea, vomiting, and diarrhea occurring several days after exposure indicate damage to the gastrointestinal tract and is thus a poor prognostic sign.

High doses of sulfur mustards can cause hyperexcitability, convulsions, and insomnia. Systemic absorption of sulfur mustard may induce bone marrow suppression and an increased risk for fatal complicating infections, hemorrhage, and anemia.

Relapsing keratitis or keratopathy may develop years after apparent healing of severe eye lesions. Persistent eye conditions, loss of taste and smell, and chronic respiratory illness including asthmatic bronchitis, recurrent respiratory infections, and lung fibrosis may persist following exposure to sulfur mustards. Prolonged or repeated acute exposure to sulfur mustards may cause cutaneous sensitization and chronic respiratory disease. Repeated exposures result in cumulative effects because mustards are not naturally detoxified by the body.

The International Agency for Research on Cancer has classified sulfur mustard as carcinogenic to humans (Group I). Epidemiological evidence indicates that repeated exposures to sulfur mustard may lead to cancers of the upper airways. There is limited evidence that repeated exposures to sulfur mustards may cause defective spermatogenesis years after exposure. Sulfur mustard has been implicated as a potential developmental toxicant because of its similarity to nitrogen mustard; however, data are inconclusive.

Chronic Toxicity (or Exposure)

Production workers exposed to mustard gas had increased rates of bronchitis and pneumonia. Increased incidence of tumors of the respiratory tract, lung cancer, bladder cancer, and leukemia were also found. Mustard gas is classified as a human carcinogen.

Clinical Management

It is important that the agent be washed from the skin with soap and water as soon after exposure as possible. The eyes should be thoroughly flushed. The onset of symptoms typically is delayed and an absence of immediate effect does not rule out toxicity. Although ingestion is unlikely, due to the sources of mustard gas, an emetic should not be administered because of the extreme caustic nature of the chemical. If the patient is not comatose, dilution of stomach contents with milk or water, prior to gastric lavage, may be attempted. Application of a solution of sodium thiosulfate to the skin and inhalation of a nebulizing mist of sodium thiosulfate may speed inactivation of mustard gas. Animal studies have shown that administration of corticosteroids (e.g., dexamethasone) and antihistamines (e.g., promethazine) may prove beneficial.

Animal Toxicity

The irritant, vesicant, and respiratory effects of mustard gas are the same in animals and humans, although the hair coat and lack of extensive sweat glands may somewhat protect the animal from the dermal effects of the agent. The grooming habits of animals may result in some exposure by the oral route.

The estimated animal toxicity is listed below:

Species	*Percutaneous LD_{50} ($mg\,kg^{-1}$)*	*Inhalation LCt ($mg\,min\,m^{-3}$)*
Rats	18	800
Rabbits	100	900
Dogs	–	600
Goats	–	900

Other H-Series Blister Agents

Sulfur mustard is a component of the H-series blister agents including undistilled sulfur mustard (H; sulfur mustard with 20–30% impurities, also known as Levinstein mustard), distilled sulfur mustard (HD or HS; ~96% pure), a mustard–lewisite mixture (HL), and HD/agent T mixture (HT; a mixture of HD and nonvolatile agent T), and an HD/agent Q mixture (HQ; a mixture of HD and nonvolatile Agent Q (Agent Q is also known as sesquimustard).

Sulfur Mustards

- 2-Chloroethyl chloromethylsulfide: CAS 2625-76-5
- Bis(2-chloroethylthio)methane: CAS 63869-13-6

Sesquimustard

- 1,2-Bis(2-chloroethylthio)ethane: CAS 3563-36-8
- 1,3-Bis(2-chloroethylthio)-*n*-propane: CAS 63905-10-2
- 1,4-Bis(2-chloroethylthio)-*n*-butane: CAS 142868-93-7
- 1,5-Bis(2-chloroethylthio)-*n*-pentane: CAS 142868-94-8
- Bis(2-chloroethylthiomethyl)ether: CAS 63918-90-1

See also: Bio Warfare and Terrorism: Toxins and Other Mid-spectrum Agents; Blister Agents/Vesicants; Chemical Warfare Delivery Systems; Nerve Agents; Nitrogen Mustard.

Relevant Websites

http://sis.nlm.nih.gov – US National Library of Medicine, Specialized Information Services, Chemical Warfare Agents.

http://www.bt.cdc.gov – US Centers for Disease Control and Prevention, Agency for Toxic Substances and Disease Registry, Chemical Agents.

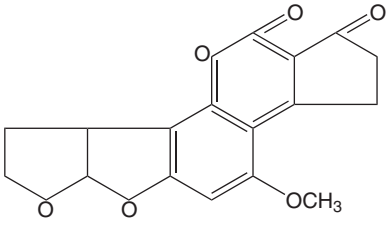
Mutagenicity Tests *See* Sister Chromatid Exchanges; Ames Test.

Mutagenicity Toxicity Testing *See* Toxicity Testing, Mutagenicity.

Mycotoxins

Samantha E Gad and Shayne C Gad

- Representative Compounds: Aflatoxins; Citrinin; Ergot alkaloids; Fumonisins; Ochratoxin A; Patulin; Trichothecenes; Zearalenone; Stachybotrys toxin
- Chemical Formula: $C_{17}H_{12}O_6$ (Aflatoxin B_1)
- Chemical Structure: Aflatoxin B_1



Uses

While most of the mycotoxins do not have beneficial uses, some mycotoxin derivatives have been used as antibiotics, growth promoters, and other kinds of drugs. In addition, some mycotoxins have been implicated as chemical warfare agents.

Background Information

Mycotoxins are metabolites of fungi that are generally produced during the growth or storage of plant products (e.g., cereal grains, nuts, corn, sorghum, rice, dried beans, and apples). However, recently mycotoxins have been found in the indoor environment where they are produced by molds growing in damp environments. Mycotoxins represent a very diverse group of chemicals that differ from each other structurally and in their ability to cause adverse effects. They may be classified in a variety of ways; for example, by the type of toxicity they cause, by the organism that produces them, or by their chemical structures. While from 300 to 400 compounds have been identified as mycotoxins, only a limited number have been studied in detail. Most of these are not of significant human health concern; rather they have

more impact on food animals. There has been recent concern about possible adverse effects in humans from toxins produced by molds in the indoor environment but this has not been fully researched. Based on extensive data, the mycotoxin that has the greatest public health impact is one of the aflatoxins, aflatoxin B_1, and this will be the focus of the remainder of this entry.

Exposure Routes and Pathways

The main route of human exposure is ingestion of foods contaminated with mycotoxins. Skin exposure to and inhalation of moldy materials may also be significant sources of exposure in some populations.

Toxicokinetics

Aflatoxin B_1 is absorbed into the blood, excreted mainly through bile, and can cross the placenta. In urine, aflatoxin B_1 is excreted as aflatoxin M_1. The liver is the primary site of metabolism, with P450 CYP 3A4 and 1A2 being primarily involved in the activation to highly reactive species. Half lives in humans are short (8 h or less).

Mechanism of Toxicity

Aflatoxin B_1 is transformed in the liver into a highly reactive epoxide that forms covalent bonds with DNA, RNA, and proteins. Toxicity and carcinogenicity are attributed to interaction with nucleic acid and proteins resulting in the inhibition of nucleic acid synthesis.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} of Aflatoxin B_1 is $9\,mg\,kg^{-1}$ in the mouse, $4.8\,mg\,kg^{-1}$ in the rat, and $10\,mg\,kg^{-1}$ in the hamster. Acute exposures to Aflatoxin B_1 have been shown to cause liver damage in rodents. Dermal effects are apparent at $2–12\,mg\,kg^{-1}$.

Human

Aflatoxin B_1 can cause a rare condition known as acute aflatoxicosis, which may result in death in some cases. At lower exposures, this mycotoxin has been linked to liver damage.

Chronic Toxicity (or Exposure)

Animal

In 1960–63, the death of turkeys in England (referred to as turkey X disease) was associated with the consumption of peanut meal feeds containing aflatoxins. Death usually occurs from hepatoxicity. Aflatoxin B_1 is carcinogenic to a wide variety of animal species; rats are particularly sensitive to this effect. It is also mutagenic and teratogenic in rodents.

Human

Long-term exposure to low levels of Aflatoxin B_1 can cause liver damage and this mycotoxin is considered to be the most potent natural carcinogen known. It is associated with liver cancer, especially in individuals who have been exposed to hepatitis B. Aflatoxin B_1 and hepatitis B appear to be synergistic in the induction of liver cancer. This mycotoxin has also been linked to cancers in other organs, particularly the lungs. Aflatoxin B_1 is classified as carcinogenic to humans by International Agency for Research on Cancer.

Clinical Management

Reducing the opportunity for exposure is the first line of defense. After high-dose exposure watch for signs of pulmonary insufficiency and provide ventilation if needed. Monitor for shock and treat if necessary. For eye contamination, flush eyes immediately with water and then irrigate with saline. For ingestion exposure, rinse mouth and use water for dilution if the patient can swallow. Do not use emetics.

Ecotoxicology

The LD_{50} (oral) of Aflatoxin B_1 in a day-old duckling was $18.2\,\mu g\,50\,g^{-1}$ body weight. Chronic low dose ($0–1.5\,\mu g\,kg^{-1}\,day^{-1}$) dietary exposure of rainbow trout to Aflatoxin B_1 resulted in liver cancer.

See also: Kidney; Mold; Veterinary Toxicology.

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N

Nails (of the Fingers and Toes)

Pertti J Hakkinen

The nails of the fingers and toes have received little attention by toxicologists, and by exposure and risk assessors. However, chemicals coming in contact with the outer surface of the nails can be taken up into the nails. Further, nails have been shown to be useful in some biomonitoring studies and in the development of some pharmaceuticals. The structure of nails includes strongly linked keratinocytes surrounded by phospholipid layers. The factors that affect drug and chemical uptake and permeation through the nail plate include solute molecular size, hydrophilicity/hydrophobicity charge, and the nature of the vehicle. Further, research has found ways of enhancing drug transport into and through the nail plate, and diseased or hydrated nails can have altered penetration characteristics.

The possible roles of nails (fingers and toes) in exposure to chemicals include direct contact with the cuticle and nail, inhalation from volatilization of a chemical applied to the nails, and oral intake via nail biting and finger sucking. Consumer products of relevance include nail lacquers ('polishes') and nail lacquer removers applied via various means (applicator, cotton ball, etc.). Also, handwashing, dishwashing, shampoo, hard surface cleaning, etc., products would involve nail contact, as would contact with residential water and soil, and paints and paint removers, and petrol. Nail lacquers can include toluene, 1,1,1-trichloroethane, and phthalates, while nail lacquer remover can include ethyl acetate.

Nails have been used in the biomonitoring of various elements, for example, arsenic, fluoride, mercury, nickel, and thallium. The advantages of analyzing nail samples include the easy and noninvasive collection of the samples, the small sample size required for analysis, and their easy storage at room temperature. The great toenail, which reflects body exposure in the previous 12 months, has been stated to be the nail best utilized for biomonitoring because it is less exposed to external contamination.

Nail clippings have also been used in the monitoring of creatinine during the diagnosis and treatment of renal failure, and as noted above, can be used in the monitoring of fluoride exposure. The absorption of drugs into nails following topical application to the nail plate has been shown to be useful for treating nail disorders, such as onychomycosis (fungal infections of the nail), and is known as ungual drug delivery. Finally, the fingernail clippings of victims have been utilized in looking for the DNA of aggressors in cases where the victims struggled to defend themselves.

See also: Biomarkers, Human Health; Biomonitoring; Exposure; Exposure Assessment.

Further Reading

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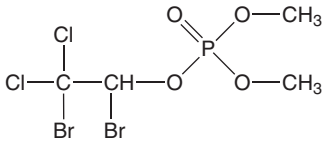
Naled

Danny Villalobos

- Chemical Name: (*RS*)-1,2-Dibromo-2,2-dichloroethyl dimethyl phosphate
- Chemical Abstracts Service Registry Number: CAS 300-76-5
- Synonyms: Dibrom; Bromchlophos; BRP; Bromex; Fly Killer-D; Lucanel
- Chemical/Pharmaceutical/Other Class: Organophosphorus insecticide and acaricide
- Chemical Formula: $C_4H_7Br_2Cl_2O_4P$
- Chemical Structure:



Uses

Naled is a fast acting, nonsystemic contact and stomach organophosphorus insecticide used to control aphids, mites, mosquitoes, and flies on crops and in greenhouses, mushroom houses, animal and poultry houses, kennels, food processing plants, and aquaria. Naled is also used in outdoor mosquito control. Liquid formulations can be applied to greenhouse heating pipes to kill insects by vapor action. It has been used by veterinarians to kill parasitic worms (other than tapeworms) in dogs. Naled is available in dust, emulsion concentrate, liquid, and ultra-low volume (ULV) formulations.

Exposure Routes and Pathways

Inhalation, dermal, and gastrointestinal exposures are possible.

Toxicokinetics

Naled is readily absorbed by inhalation, through intact skin, and through the gastrointestinal tract. Naled is rapidly hydrolyzed to give a number of metabolites which include dichlorvos, dichlorobromoacetaldehyde, dimethyl phosphate, and complex amino acid conjugates. When 25 mg kg^{-1} of ^{32}P naled was given to a cow by oral intubation, 9% was recovered in urine and 34% in feces up to 1 week after dosing. Rats given 1/10 of the LD_{50} daily for 9 weeks showed moderate inhibition of blood and brain cholinesterase.

- *Inhalation:* No toxic effects were observed in rats and guinea pigs exposed to vapor at a concentration of 19 μg l^{-1} for 6 h per day, 5 days per week for 5 weeks.
- *Cumulation of compound:* Naled is not cumulative in body tissues.
- *Cumulation of effect:* Repeated exposure to naled may have a cumulative effect on cholinesterase levels.

Mechanism of Toxicity

Naled is an inhibitor of acetylcholinesterase, and can lead to typical signs and symptoms of cholinergic crisis through elevation of tissue acetylcholine levels.

Acute and Short-Term Toxicity (or Exposure)

Animal

Naled is moderately to highly toxic via the oral route, with oral LD_{50} values of 91–430 mg kg^{-1} in rats and mice. Dermal LD_{50} values were 1100 mg kg^{-1} in rabbits and 800 mg kg^{-1} in rats. Naled can cause dermatitis and sensitization, and can be corrosive to skin and eyes. Effects due to naled exposure are similar to those caused by other organophosphorus pesticides.

Human

Symptoms of acute exposure to organophosphate or cholinesterase-inhibiting compounds may include the following: numbness, tingling sensations, incoordination, headache, dizziness, tremor, nausea, abdominal cramps, sweating, blurred vision, difficulty breathing or respiratory depression, and slow heartbeat. Very high doses may result in unconsciousness, incontinence, and convulsions or fatality.

The most commonly reported symptoms from acute naled exposures have been limited to contact dermatitis, erythematous and maculopapular rashes, which may be followed by edematous vesiculating blisters, which become dry, itchy, and flake off.

The acute toxicity data, inhalation data, and experience in the early reports indicated that naled is not a highly toxic organophosphate.

Effects due to naled exposure will be similar to those caused by other organophosphate pesticides, including inhibition of cholinesterase and neurological and neuromuscular effects.

Chronic Toxicity (or Exposure)

Animal

Chronic exposure may lead to nervous system toxicity due to cumulative acetylcholinesterase inhibition. There are little data on the carcinogenic or teratogenic effects of naled. Dietary administration of 1, 5, and 25 mg naled kg^{-1} diet to three generations of albino rats had no effect on mating and fertility indices, incidence of pregnancy, parturition or gestation times, lactation indices, offspring and their survival. Rats treated with 28 mg kg^{-1} day^{-1} naled for 9 weeks showed cholinesterase inhibition but no overt signs of neurotoxicity.

Human

Little information is available on effects of naled in humans. Repeated exposure to naled may have a cumulative effect on cholinesterase levels. Chronic exposure to organophosphates may cause the neurological and neuromuscular effects associated with cholinesterase inhibition.

In Vitro Toxicity Data

Naled did not influence DNA repair in *Proteus mirabilis*e, but did increase the frequency of mutations in the Ames assay.

Clinical Management

Therapy for acute naled poisoning: (1) Respiration should be supported. The airways should be kept clear, and artificial respiration with oxygen should be used if cyanosis is indicated. Death from pesticide poisoning is usually due to respiratory failure. (2) Decontamination should be done as indicated. Contaminated clothing should be removed. Skin, hair, and fingernails should be washed with soap and water, and eyes should be rinsed. Dermal exposures should be treated symptomatically as indicated. If ingested, gastric lavage may be indicated as may the administration of activated charcoal, 1–2 g kg^{-1} body weight. First-aid and medical personnel should be protected. (3) Five milliliters of heparinized blood should be drawn for cholinesterase determination. First urine and first/early vomitus samples should be saved for possible laboratory analysis. (4) Insecticide label should be consulted under 'active ingredients' for specific chemicals involved. (5) When mixtures of organophosphates and chlorinated hydrocarbons are involved, specific treatment should be given for organophosphates first and indicated support therapy and decontamination.

Adults: After cyanosis is overcome, atropine sulfate should be used, 2–4 mg i.v. Doses should be repeated at 5–10 min intervals until signs of atropinization appear. This should be maintained for 24 h or longer if necessary. 2-PAM (pralidoxime chloride) should be given. Adult dose: 1 g, slowly, intravenously. Contraindicated are morphine, aminophylline, theophylline, phenothiazine tranquilizers, and barbiturates.

Children: Atropine sulfate in proportion to body weight: ~ 0.05 mg kg^{-1}. 2-PAM, 0.25 g, should be given slowly, intravenously.

Environmental Fate

Naled is practically nonpersistent in soil, with half-life of less than 1 day. It degrades in sunlight to dichlorvos (DDVP). Naled does not bind strongly to soils, but is not highly soluble in water. It is moderately volatile. Soil microorganisms break down most of the naled in the soil. Naled is rapidly broken down in water, with a half-life of ~ 2 days. Plants reductively eliminate bromine from naled to form DDVP, which may evaporate or be further modified.

Chemical hydrolysis and biodegradation are the major processes involved in the transformation of naled. Volatilization from soils and/or from water is the major mode of transport for degraded naled and its bioactive degradate DDVP, as opposed to leaching to ground water.

A major route of contamination of surface waters by naled is spray drift and direct application for mosquito abatement. There are no data on the fate and transport of degradates containing only the organophosphate group, which form by cleavage of the P–O bond in naled and/or DDVP.

Ecotoxicology

Based on acute toxicity data, naled is moderately to highly toxic to birds. Acute oral LD values in birds ranged from 37 to 65 mg kg^{-1}. On a subacute dietary basis, naled is slightly toxic to birds. Naled is highly toxic to honey bees.

Naled is moderately to highly toxic in freshwater fish, with 96 h LC_{50} values ranging from 87 ppb to 3 ppm. Growth in fathead minnow was impaired at concentrations of greater than 6.9 ppb. Naled was very highly toxic to Daphnia: length was affected at concentrations > 0.045 ppb.

Exposure Standards and Guidelines

US Environmental Protection Agency (EPA) toxicity class I.

- Threshold limit value (TLV): 3 mg m^{-3} A4 (skin) (American Conference of Governmental Industrial Hygienists, ACGIH 1996).
- Acceptable daily intake (ADI): Not available
- Maximum contaminant level (MCL): Not available
- Reference dose (RfD): 0.002 mg kg^{-1} day^{-1}
- Permissible exposure limit (PEL): 3 mg m^{-3} (8 h)
- Health advisory (HA): Not available

See also: Cholinesterase Inhibition; Dichlorvos; Organophosphates.

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http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Naled.
http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.

Nanotechnology

David B Warheit

Nanotechnology is an emerging multidisciplinary science that deals with the creation and use of molecules a few billionths of meter in size. Assessing the potential hazards of this technology's nanoparticles, and the nanomaterials manufactured using the nanoparticles is an emerging area in toxicology and health risk assessment. The development of toxicity data sets and exposure assessments for various nanoparticles and nanomaterials is ongoing as new particles and materials are developed. A related issue in toxicology and risk assessment is the extent to which nanoparticle toxicity can be extrapolated from existing toxicology databases for particles and fibers. Other information needs being addressed include the environmental and biological fate, transport, persistence, and transformation of manufactured nanoparticles, and the recyclability and overall sustainability of manufactured nanomaterials.

Ultrafine or nanoparticles are generally defined as particles in the size range <100 nm. A nanometer (nm) is roughly the width of 10 hydrogen atoms. The terms 'ultrafine' and 'nano' can be used interchangeably, with the latter being the current nomenclature. Having been called 'the next big thing' in science and the foundation for the 'next industrial revolution', governments and companies around the world are providing billions of dollars into nanotechnology research. As other emerging technologies have over the years, nanotechnology has attracted the attention of the public and others. This has led to concerns about potential effects of materials made using nanotechnology on human health and environmental impact.

In theory, nanoparticles can be produced from nearly any chemical. Most of the current nanoparticles are made from transition metals, silicon, carbon, and metal oxides. Manufactured nanoparticles display physicochemical characteristics and coatings that provide highly desirable electrical, thermal, mechanical, and imaging properties for various technology applications.

The field of nanotechnology is currently undergoing a large amount of research and development. Nanotechnology can be used to manufacture materials, devices or systems less than 1000th the width of a human hair. Materials made using nanotechnology are already used in hundreds of products, including sunscreens and cosmetics to make them clear (e.g., the sunscreens have ultraviolet-absorbing nanoparticles so small they cannot reflect light, making them transparent), in textiles using nanofibers to make them stain-resistant; and in power machinery to add durability. Further examples are tennis rackets and airplane bodies made using nanomaterials with carefully arranged atoms to make the materials especially strong.

In addition, nanotechnology is expected to become very important in various biomedical applications such as in drug delivery, molecular imaging, biomarkers, and biosensors. Target-specific drug therapy and methods for early diagnosis and therapy of

diseases are among the priority research areas for application of nanotechnology. Further, nanotechnology could play a very important role in environmental science, for example, via the development and usage of nanospheres to trap polychlorinated biphenyls and toxic metals, and via nanopore materials that can filter out bacteria, viruses, and toxins from water.

Given their extremely small size, a key area of toxicology for nanotechnology thus far has been the evaluation of pulmonary toxicity. Pulmonary toxicology studies in rats demonstrate that nanoparticles administered to the lung produce an enhanced pulmonary inflammatory response when compared to larger particles of identical chemistry at equivalent mass concentrations. Particle surface area and particle number appear to play important roles in the mechanisms of nanoparticle toxicity. Contributing to the effects of inflammation-promoting effects of nanoparticles is their very high size-specific deposition when inhaled as singlet particles rather than as aggregated particles. Some evidence suggests that inhaled nanoparticles, after deposition in the lung, largely escape alveolar macrophage surveillance and transmigrate through epithelial cells to the pulmonary interstitium, generally considered a vulnerable anatomical compartment of the respiratory system.

It is important to note that most of the early published lung toxicity studies with nanoparticles have been conducted in laboratory animals at very high particle concentrations, which significantly exceed workplace or ambient exposures. These hazard-based toxicity studies are designed to assess pulmonary effects caused by particles at high concentrations and can result in the induction of lung tumors in rats following 2 year exposures. Specifically, chronic inhalation studies with nano- and fine-sized titanium dioxide (TiO_2) particles (average primary particle sizes $\sim$20 and $\sim$270 nm, respectively) have shown that ultrafine particles are greater than 10 times more potent than fine particles in producing pulmonary fibrosis and consequent lung tumors in rats.

Additional studies have been conducted using intratracheal instillation exposures to aggregates of ultrafine and fine carbon black, as well as to TiO_2 particles in rats. The results have demonstrated a significantly enhanced lung inflammatory potency of the ultrafine particles when compared to fine-sized particulates of similar composition. However, when the instilled doses were expressed in terms of particle surface area, the responses of the ultrafine and fine TiO_2 particles fell on the same dose–response curve. This is because a given mass of ultrafine particles has a much greater surface area (and particle number) than the same mass of fine, yet respirable (3 μm) particles and therefore is more likely to cause particle overload in the lung. Thus, from a risk assessment and regulatory viewpoint, it will be important to delineate the pulmonary toxicity effects of ultrafine particles in rats at overload versus nonoverload conditions.

It may be surprising to note that the total lung toxicity database for systematic comparisons of the effects of ultrafine/nanoparticles versus fine-sized particles in rats consists of studies on only three particle types: namely titanium dioxide, carbon black, and diesel exhaust particles. Moreover, as stated above, the rat model, for which most if not all of the nano versus fine-size comparisons have been reported, is known to be an extremely sensitive species for developing adverse lung responses to particles, particularly at overload concentrations. As a consequence, long-term (2 year), high-dose, inhalation studies in rats with poorly soluble, low-toxicity dusts can ultimately produce pulmonary fibrosis and lung tumors via an 'overload' mechanism. The tumor-related effects are unique to rats and have not been reported in other particle-exposed, rodent species such as mice or hamsters, under similar chronic conditions. For the mechanistic connection, it has been postulated that the particle-overload effects in rats result in the development of 'exaggerated' lung responses, characterized by increased and persistent levels of pulmonary inflammation, cellular proliferation, and inflammatory-derived mutagenesis in the rat, and this ultimately results in the development of lung tumors following high dose, long-term exposures to a variety of particulate-types.

In contrast to the response in rats, evidence from numerous studies demonstrate that particle-exposed mice and hamsters do not develop sustained inflammation, mesenchymal cell alterations, and consequent lung tumors following high-dose, long-term exposures to low-toxicity dusts. Therefore, species differences in lung responses to inhaled particles are important considerations for assessing the health risks to nanoparticles.

To complicate further our perceptions of nanoparticle toxicity, some recent evidence suggests that, on a mass basis, not all nanoparticle-types are more toxic than fine-sized particles of similar chemical composition. As mentioned previously, the limited numbers of studies that have been reported suggest that ultrafine TiO_2 particles produced greater pulmonary inflammation when compared with fine-sized TiO_2 particles. However, in contrast to the conclusions of the earlier findings, the results of recent preliminary studies comparing the effects of nano- versus

fine-sized particles, have indicated that pulmonary exposures in rats to uncoated TiO_2 nanorods (200 nm lengths $\times$ 30 nm diameters) and TiO_2 nanodots (particle size <30 nm) did not produce enhanced lung inflammation in rats when compared to fine-sized TiO_2 particle exposures (particle size ~ 270 nm).

Other lung bioassay studies have compared the toxicity effects in rats of uncoated nanoscale quartz particles (50 nm) versus fine-sized quartz particles (particle size ~ 1.6 μm). In pulmonary instillation studies, at equivalent mass doses, the nanoquartz particles produced less intensive and sustained pulmonary inflammatory and cytotoxic responses when compared to the effects produced by the Min-U-Sil quartz particles. This result is intriguing since crystalline quartz silica particles are classified as a Category 1 human carcinogen by the International Agency for Research on Cancer. In summary, the preliminary findings from these two studies suggest that particle size is only one factor in determining pulmonary toxicity.

In addition to the issues of particle size and species differences as discussed above, several additional variables are likely to play important roles in modifying the pulmonary toxicity of nanoparticles. For example, the surface coatings on particles may play an important role in influencing pulmonary effects. In this respect, a pulmonary bioassay toxicity methodology was used to assess the pulmonary toxicity of a number of commercial formulations of fine-sized TiO_2 particles in rats, each formulation with different surface coatings/treatments. The results demonstrated that one of the formulations containing enhanced amounts of amorphous silica and alumina surface coatings on the TiO_2 particle produced greater pulmonary inflammation and cytotoxic effects when compared to the other formulations containing different surface treatments.

The degree to which engineered nanoparticles aggregate in the ambient aerosol and subsequently disaggregate following inhalation will strongly influence particle deposition patterns and interactions with lung cells. If the ultrafine particles disaggregate upon interaction with alveolar lung fluids, then they could behave as discrete individual nanoparticles and may stimulate enhanced inflammatory cell recruitment and/or the particles could preferentially translocate to more vulnerable compartments of the lung.

An additional factor which may modify the lung toxicity and corresponding risk following exposures to engineered nanoparticulates is the electrostatic attraction/aggregation or agglomeration potential of some nanoscale materials, such as single wall carbon nanotubes (SWCNT). The dimensions of individual SWCNTs have been reported as 1 nm (diameter dimension) by >1 μm (length dimension). SWCNTs, however, rarely exist as discrete individual particles, and due to their strong electrostatic characteristics, form agglomerates of 'nanoropes' or 'nanomats', consisting of agglomerates of 10–200 individual SWCNTs.

Two recently reported pulmonary bioassay studies with SWCNTs have been reported in mice and in rats. In one study, groups of rats were exposed by intratracheal instillation with multiple doses of SWCNTs, quartz particles (positive control), or carbonyl iron particles (negative control). Exposures to high-dose ($5\ mg\ kg^{-1}$) SWCNTs resulted in mortality in about 15% of the instilled rats within 24 h postinstillation exposure. This mortality was not due to inherent toxicity of the nanotubes, but resulted from mechanical blockage of the large airways by the instilled agglomerated SWCNT nanoropes. Exposures to quartz particles produced significant increases versus controls in lung inflammation responses, cytotoxicity, and lung parenchymal cell proliferation indices, while exposures to SWCNTs produced transient lung inflammation. Histopathological observations revealed that exposures to quartz particles produced inflammation, foamy alveolar macrophage accumulation, and tissue thickening (fibrosis). In contrast, pulmonary exposures to SWCNTs produced a non dose-dependent series of multifocal granulomas. Contained within the granulomas were agglomerated carbon nanotubes surrounded by monocytic cell-types. Similar findings in SWCNT-exposed mice have been observed.

It is noteworthy that, unlike the results with quartz particles, the finding of unusual pulmonary lesions (i.e., multifocal granulomas) in rats was not consistent with indices of lung cellular injury and sustained lung inflammation. In addition, the results of two recent independent exposure assessment studies have reported very low respirable aerosol SWCNT concentration exposures at the workplace. Thus, the physiological relevance of these pulmonary bioassay findings remains to be determined and can only be reconciled by conducting an inhalation toxicity study in rats with aerosols of SWCNTs. Moreover, it must be noted that single wall carbon nanotubes, due to their unique electrostatic and agglomerative characteristics, are not likely to be representative of other nanoscale particulates.

At this point in time, no generalized conclusions can be drawn regarding the human health effects of inhaled engineered nanoparticulates. This is due, in large part, to the following:

- A paucity of data exists on the pulmonary toxicity of nanoparticles.

- On a mass basis, nano or ultrafine particles (i.e., <100 nm) are considered to produce greater pulmonary toxicity when compared to fine-sized particles (i.e., size range from 100 nm to >3 μm) of identical composition. This conclusion has been derived based only on comparisons of two or three particle types.
- Some recently reported findings indicated that, even on a mass basis, some nanoparticle-types are not more inflammogenic and cytotoxic than fine-sized particulates of similar or identical chemical composition.
- It seems likely that in addition to particle size, other factors such as surface coatings, aggregation/disaggregation potential, origin and method of particle synthesis/composition (e.g., gas phase (fumed) versus liquid phase (colloidal/precipitated)), and surface charge will have a significant impact on modifying potential toxicity of inhaled engineered nanoparticles.
- The impact of surface coatings on biological effects will predominate, particularly on smaller nanosized particles (i.e., particles <25 nm), wherein surfaces will comprise 25–50% of the particle composition.
- It is expected that much additional safety and mechanistic toxicology data on nanoparticulates will be generated by about the year 2010.
- As a consequence, no general conclusions regarding nanoparticle toxicity can be made. Thus, it is important that assessments of safety and health risks of newly developed engineered nanoparticles should be made following relevant testing on a case-by-case basis for each nanoparticle type.

See also: Respiratory Tract.

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Relevant Websites

http://www.nano.org.uk – The Institute of Nanotechnology (IoN) was one of the world's first nanotechnology information providers, and leads the European network of networks, Nanoforum (www.nanoforum.org) as well as working closely with governments, universities, researchers and companies worldwide on micro and nanotechnology.

http://www.nano.gov – The National Nanotechnology Initiative (NNI) is a US program established to coordinate the efforts of eighteen federal agencies in nanoscale science, engineering, and technology.

Naphthalene

Heriberto Robles

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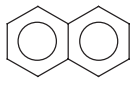
- Chemical Abstracts Service Registry Number: CAS 91-20-3
- Synonyms: Naftalen; Naphthene; NCI-C52904; Albocarbon; Dezodorator; Camphor tar; Mothballs; Moth flakes; Tar camphor; Naftaleno
- Chemical/Pharmaceutical/Other Class: Polynuclear aromatic hydrocarbon
- Chemical Formula: $C_{10}H_8$
- Chemical Structure:



Uses

Naphthalene is commonly used in the manufacture of dyes, resins, and mothballs and may also be found in association with coal tar production and in wood preservatives. It is also used as a chemical intermediate in the synthesis of pharmaceuticals, insect repellents, and pesticides.

Background Information

Naphthalene is a component of crude oil and is found in petroleum-derived fuels and consumer products. The most common use of naphthalene in consumer products is in the production of mothballs. The two active ingredients in mothballs are naphthalene and paradichlorobenzene.

Exposure Routes and Pathways

The primary exposure route for naphthalene is via inhalation, although it may also be absorbed into blood from the gastrointestinal tract and the skin. However, percutaneous absorption is too limited to produce acute systemic reactions except in newborns.

Toxicokinetics

Naphthalene ingestion can result in acute as well as delayed toxicity. The primary target organs of toxicity are the blood and eyes. Individuals deficient in glucose-6-phosphatase dehydrogenase are especially sensitive to the hemolytic effects of naphthalene. Normal individuals may also develop hemolysis when exposed to high doses.

Mechanism of Toxicity

Systemic absorption of naphthalene vapor may result in cataracts. The biochemical basis for naphthalene cataract has been investigated. Naphthalene is metabolized in the liver to 1,2-dihydro-1,2-dihydoxynaphthalene. Lenticular catechol reductase biotransforms 1,2-dihydro-1,2-dihydroxylnaphthalene to 1,2-dihydroxynaphthalene, which in turn is autooxidized in air at neutral pH to 1,2-naphthaoquinone and hydrogen peroxide. Ascorbic acid reverses the latter reaction and forms dehydroascorbic acid, which diffuses out of the lens very slowly. Dehydroascorbic acid has been shown to accumulate in the lens of rabbits fed naphthalene and lens incubated *in vitro* with 1,2-dihydro-1,2-dihydroxynaphthalene. The sequence of reactions involves reduction of ascorbic acid by 1,2-naphthoquinone in the aqueous humor to dehydroascorbic acid, which rapidly penetrates the lens and is reduced by glutathione. Oxidized glutathione and 1,2-naphthoquinone may compete for enzyme glutathione reductase, which normally maintains high reticular levels of reduced glutathione. A reduction in the concentration of these coupled with the removal of oxygen from the aqueous humor due to the autooxidation of 1,2-dihydroxynaphthalene may make the lens sensitive to naphthalene toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute ingestion of naphthalene by rabbits produced effects similar to those observed in humans. Rabbits fed acute doses of naphthalene presented browning of the lenses and eye humors and developed cataracts. In contrast to humans, where the major target organ of toxicity is the blood and eyes, for rats and mice the major target organ of toxicity is the lung.

The maximum air concentration of naphthalene that can be generated (78 ppm) has not been shown to be lethal to rats. Animal studies report that the oral LD_{50} for naphthalene ranges from 354 mg kg^{-1} in mice to 2400 mg kg^{-1} in rats. In extreme circumstances, death may also occur as a result of dermal exposure.

Human

Skin or eye surface contact may result in naphthalene cataracts, ocular irritation, skin irritation, and in the case of a sensitized person, severe dermatitis. Topical lesions will clear spontaneously, as soon as the exposure is terminated.

Inhalation of vapor may result in headache, confusion, excitation, nausea, and sometimes vomiting and extensive sweating. Dysuria, hematuria, and acute hemolytic reaction can also be seen.

Ingestion may cause abdominal cramps with nausea, vomiting, diarrhea, headache, profuse perspiration, listlessness, confusion, and coma with or without convulsions in a case of severe poisoning. It also may cause irritation of the urinary bladder, dysuria, the passage of brown or black urine with or without albumin and casts, and acute intravascular hemolysis.

Adverse neurologic effects have also been reported in humans who ingested naphthalene. Signs and symptoms reported include vertigo, lethargy, muscle twitching, decreased response to painful stimulus, and coma. It has been speculated that the neurological symptoms may have resulted from cerebral edema produced by acute hemolysis and not from a direct toxicological effect of naphthalene.

There are no reported cases of death due to inhalation of naphthalene. Naphthalene-induced deaths are usually related to mothball ingestion during suicide attempts. Based on suicide data, the lethal dose in humans is estimated to range from 319 to 574 mg kg^{-1}. The most prominent effect of high-dose naphthalene exposure in humans is hemolytic anemia. There is a report of an infant who died of acute hemolytic anemia after being exposed to

mothball-treated diapers. Another infant reportedly experienced skin rashes, systemic poisoning, and then death apparently due to naphthalene exposure from mothballs used with clothes or blankets that had been stored in or near the infant's room.

Reported effects of naphthalene overexposure in humans include hemolytic crisis, characterized by increased bilirubin levels, and the appearance of Heinz bodies and fragmented red blood cells. Other effects that have been associated with ingestion of high doses include gastrointestinal distress, vomiting, nausea, jaundice, proteinurea, hemoglobinuria, hemoglobinuria, methemoglobinemia, kernicterus, and coma.

A 69-year-old woman exposed to naphthalene and *p*-dichlorobenzene developed aplastic anemia 2 months after exposure. A 36-year-old pharmacist was given 5 g of unpurified naphthalene in an emulsion of castor oil in divided doses over 13 h. On awakening 8 or 9 h later, he had severe pain in the bladder and found that he was nearly blind, although he had had no eye problems. After 1 year, an examination showed that his vision was reduced to the ability to count fingers at 1.5 m and his visual fields were constricted 30–50°. The condition was unimproved by glasses.

Chronic Toxicity (or Exposure)

Animal

Naphthalene does not appear to be teratogenic. Mice exposed to $300\,mg\,kg^{-1}\,day^{-1}$ produced normal offspring, although a decrease in litter size was reported.

Oral administration of $1\,g\,kg^{-1}\,day^{-1}$ in rabbits leads to lenticular changes, initially observed as swelling in the peripheral portion of the lens. Within 2 weeks, the whole lens is affected with mature cataract. The biochemical basis for cataract has been shown to be related to a liver metabolite of naphthalene, 1,2-dihydro-1,2-dihydoxynaphthalene.

Selective lung damage and necrosis occurred in Clara cells of mice that were administered naphthalene. It produced selective depression of pulmonary monooxygenase activities without accompanying changes in hepatic monooxygenease. A dose-dependent alteration of Clara cells (bronchiolar epithelial cells) was noted.

Mice exposed to naphthalene vapor at concentrations as high as 30 ppm in air for 6 h a day, 5 day a week for 104 weeks developed nose and lung lesions. These lesions were described as nose inflammation accompanied by metaplasia and hyperplasia of the olfactory and respiratory epitheliums.

Carcinogenicity studies conducted in rats and mice prior to 1992 reported either negative or non-conclusive results. Continued efforts to determine the potential carcinogenicity of naphthalene has resulted in improved design studies. Some of these studies have reported evidence of carcinogenic activity for naphthalene. For example, rats of both sexes exposed to naphthalene by inhalation presented a dose-dependent increased incidence of respiratory epithelial adenoma and olfactory epithelial neuroblastoma.

Human

Reports of adverse effects following chronic naphthalene exposure include the development of cataracts and retinal hemorrhage in a 44-year-old man occupationally exposed to powdered naphthalene. Unilateral chorioretinitis was reported for a coworker and cataracts developed in 8 of 21 workers exposed to naphthalene fumes or dust for ~ 5 years in an industrial setting. Chronic exposure to powdered naphthalene in the workplace has been associated with an increased incidence of cataracts. However, few of these effects have been confirmed in animal studies.

Carcinogenicity studies conducted in the late 1990s and early 2000s have found some evidence that naphthalene may be carcinogenic to rats. These findings have prompted the International Agency for Research on Cancer to classify naphthalene as a chemical that is possibly carcinogenic to humans (group 2B classification).

In Vitro Toxicity Data

Several *in vitro* genotoxicity studies have been conducted for naphthalene. Naphthalene has not been found to be genotoxic in the *Salmonella* reverse mutation assay. However, naphthalene has been reported to have genotoxic effects in nonmammalian assay studies. In various *in vitro* studies, naphthalene has been shown to have the potential to induce chromosomal damage in mammalian cells.

Clinical Management

There is no specific antidote for naphthalene toxicity. Treatment is symptomatic and supportive. Gastric decontamination should be considered with emesis or lavage, followed by activated charcoal. Hemolysis may require urinary alkalinization and transfusion. Methemoglobinemia may be treated with methylene blue. Emesis is more useful for mothballs because of size. Lavage may be useful for ingestion of flakes. Information on activated charcoal is scant, but adsorption is thought to occur. Mothballs dissolve slowly; gastric decontamination should be performed

even in patients presenting late after ingestion. Emesis may be indicated in recent substantial ingestion unless the patient is or could rapidly become obtunded, comatose, or convulsant. It is most effective if initiated within 30 min of ingestion. The recommended dose of ipecac syrup is 30 ml for an adult and 15 ml for a child.

Gastric lavage may be indicated if performed soon after ingestion or in patients who are comatose or at risk of convulsing. The airway should be protected by placement in Trendelenburg and left lateral decubitus position or by cuffed endotracheal intubation.

Environmental Fate

Naphthalene may be released to the environment as a wood and fossil fuel combustion product or from unintentional, accidental release of petroleum fuels. It is relatively volatile at room temperature and tends to evaporate readily. In air, naphthalene will react with hydroxyl and nitrate radicals. This compound has a relatively short half-life in soil and water due to its volatility and rapid degradation. The half-life of naphthalene in soil ranges from 2 to 18 days.

Exposure Standards and Guidelines

- US Environmental Protection Agency (EPA) drinking water standard for naphthalene is $20\,\mu g\,l^{-1}$.
- State regulated drinking water standards for naphthalene range from $6.8\,\mu g\,l^{-1}$ in Florida to $300\,\mu g\,l^{-1}$ in Minnesota.
- The US EPA 10-day Health Advisory (HA) for a 10 kg child is $\sim 0.5\,mg\,l^{-1}$.
- The US EPA long-term HA for a 10 kg child is $\sim 0.4\,mg\,l^{-1}$.
- The US EPA long-term HA for a 70 kg adult is $\sim 1\,mg\,l^{-1}$.
- The US EPA lifetime HA for a 70 kg adult is $\sim 0.02\,mg\,l^{-1}$.

See also: Blood; Carcinogenesis; Polycyclic Aromatic Hydrocarbons (PAHs); Sensory Organs.

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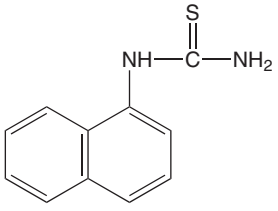
Naphthyl Thiourea, α-

Swarupa G Kulkarni and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 86-88-4
- Preferred Name: ANTU
- Synonyms: α-Naphthyl urea; 1-(1-Naphthyl)-2-thiourea; α-Naphthyl thiocarbamide
- Chemical Formula: $C_{11}H_{10}N_2S$
- Chemical Structure:



Uses

ANTU is a single-dose rodenticide used as bait and tracking powder and is specifically used against Norway rats. It is ineffective against all species of field rodents. It is used in baits in concentrations of 1–3%. Because of its specificity to Norway rats and its tendency to cause resistance, this poison rapidly lost popularity and is no longer manufactured. It is not produced commercially in the United States.

Background Information

ANTU is a gray crystalline odorless powder with a bitter taste. It has a molecular weight of 202.7. It has a melting point of 198°C and does not ignite readily. On contact with strong oxidizers it may cause fire

and explosions. Fire may produce irritating or poisonous gas. Hazardous decomposition products include sulfur dioxide, oxides of nitrogen, and carbon monoxide.

Exposure Routes and Pathways

Inhalation, ingestion, and dermal contact are possible routes of exposure.

Toxicokinetics

Limited data on the toxicokinetics of ANTU are available. However, absorption does occur following oral administration. ANTU toxicity in the rat is thought to depend on metabolic activation via the hepatic and lung microsomal enzymes to form a hydrosulfide and α-naphthyl urea.

Mechanism of Toxicity

ANTU toxicity in the rat is thought to depend on metabolic activation via the hepatic and lung microsomal enzymes to form a hydrosulfide and α-naphthyl urea. The metabolites are covalently bound to lung macromolecules. However, it is not known if these metabolites are produced in humans. ANTU presumably acts on some enzyme system involving the sulfhydryl group. Analogous pulmonary edema is produced by sulfhydryl inhibitors, such as alloxan, iodoacetamide, and oxophenarsine. Production of oxygen free radicals via the cyclooxygenase pathway has been implicated in mediating ANTU-induced lung damage. Following exposure to ANTU, there are a number of biochemical events, such as alteration in carbohydrate metabolism, adrenal stimulation, and interaction of the chemical with sulfhydryl groups, but none of these appear to bear any relationship to the observed signs of toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

Toxicity of ANTU is strikingly higher for wild Norway rats than for other species. Mice and dogs rank next in susceptibility. Young animals are less resistant. Rats that survive sublethal doses develop a high degree of tolerance owing partly to refusal to eat freely. Symptoms in rats appear within 12–25 min with a sharp fall of body temperature, huge pleural and intraalveolar edema, anuria, dyspnea, and death. Blood sugar rises to nearly three times the normal level within 2.5 h with a severe fall of liver glycogen and failure to deposit liver glycogen. Observations in experimental animals indicate that the principal organ affected is the lung; pulmonary edema and pleural effusion develop due to the action of ANTU on pulmonary capillaries causing marked edema of the subepithelial spaces of the alveolar walls; pericardial effusion is less marked.

Dogs are quite susceptible to toxicity but may be protected by prompt vomiting. Pulmonary effusion in dogs showed an increase in albumin globulin ratio. Hemorrhagic glomerular nephritis has been seen after acute exposure in rats. Hyperglycemia has been reported in experimental animals. LD_{50} (dog) is $16\,mg\,kg^{-1}$ ip and $380\,\mu g\,kg^{-1}$ po.

Human

The estimated mean lethal dose in humans is 25 g/70 kg. ANTU is classified as being moderately toxic. No human fatalities have been reported. It is probably not toxic to humans except in large amounts. Although it appears that humans are resistant to ANTU intoxication, probably because insufficient quantities are ingested, poisonings have occurred, with tracheobronchial hypersecretion of a white, nonmucous froth containing little protein, pulmonary edema, and respiratory difficulty. Ingestion may cause vomiting, shortness of breath, and bluish discoloration of the skin. Inhalation of ANTU powder may result in dyspnea, rales, cyanosis, and pulmonary edema or effusion. A case of contact eczema due to handling a rat poison containing ANTU as a base has been reported.

Chronic Toxicity (or Exposure)

Animal

Chronic exposure in rats results in stunted growth, thinning and coarsening of hair, deformities of the legs and feet, hyperplasia of the thyroid and splenic pulp, hyaline changes in the hepatic cells, decreased thickness of the adrenal cortex, and calcified tubular casts. Continued administration to cats produces fatal intrahepatic obstructive jaundice without pulmonary lesion. Available data were inadequate to evaluate the carcinogenicity of ANTU in experimental animals.

Human

Chronic exposure to ANTU led to the investigation of two cases of bladder cancer in two rodent operators. Therefore, the use of ANTU was restricted to professional operators. Available data were inadequate to evaluate carcinogenicity in humans. Chronic sublethal exposure may result in antithyroid activity and hyperglycemia.

Clinical Management

For ingestion, emesis is indicated unless the patient becomes comatose or shows convulsions. Emesis is most effective if initiated with 30 min of ingestion. Syrup of ipecac can be used for inducing emesis. Charcoal slurry, aqueous or mixed with saline cathartic, or sorbitol may be used. Treatment would be by liberal gastric lavage, the substance being only slightly soluble. Ventilation and oxygenation with close arterial blood gas monitoring should be maintained in case of pulmonary edema. In case of an inhalation exposure, the patient should be moved to fresh air and monitored for respiratory distress. The person should also be evaluated for respiratory tract irritation, bronchitis, or pneumonitis. Humidified supplemental oxygen (100%) with assisted ventilation may be administered as required. Exposed eyes should be irrigated with copious amounts of tepid water. For dermal exposure, the affected skin should be washed with soap and water. No antidotes are established.

Since ANTU is a sulfhydryl blocking agent, cysteine has been tried in rats and was effective in some cases. There is no human experience with cysteine and its use is not recommended.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value time-weighted average (TWA) and the Occupational Safety and Health Administration permissible exposure limit – TWA are both $0.3\,mg\,m^{-3}$.

See also: Pesticides.

Further Reading

Hardwick SJ, Skamarauskas JT, Smith LL, Upshall DG, and Cohen GM (1991) Protection of rats against the effects of alpha-naphthyl thiourea (ANTU) by elevation of non-protein sulphydryl levels. *Biochemical Pharmacology* 42: 1203–1208.

Martin D, Korthuis RJ, Perry M, Townsley MI, and Taylor AE (1986) Oxygen radical-mediated lung damage associated with alpha-naphthyl thiourea. *Acta Physiologica Scandinavica Supplementum* 548: 119–125.

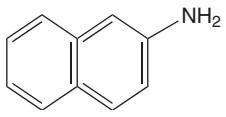
Mason CM, Guery BP, Summer WR, and Nelson S (1996) Keratinocyte growth factor attenuates lung leak induced by alpha-naphthyl thiourea in rats. *Critical Care in Medicine* 24: 925–931.

Scott SM, Powell GM, Upshall DG, and Curtis CG (1990) Pulmonary toxicity of thioureas in the rat. *Environmental Health Perspectives* 85: 43–50.

Naphthylamine, 2-

Glenn Talaska

- Chemical Abstracts Service Registry Number: CAS 91-59-8
- Synonyms: 2-Aminonaphthalene; *β*-Naphthylamine; 2NA; BNA; Fast Scarlet Base B
- Chemical/Pharmaceutical/Other Class: Aromatic amine
- Chemical Formula: $C_{10}H_7NH_2$
- Chemical Structure:



Uses

2-Naphthylamine (2NA) was used as an intermediate in the dye industry and as an antioxidant in the rubber industry (e.g., the last company to manufacture it in the United States supposedly ceased production in 1972). However, it probably still presented an exposure hazard for at least some time afterward as a contaminant of dye stocks such as Broenner's acid and replacement antioxidants that retain the 2NA nucleus; for example, Nonox S. In addition, antioxidants in the rubber industry such as *N*-phenyl-2-naphthylamine and *N*,*N*′-di-2-naphthyl-*p*-phenylenediamine have been shown to be metabolized to 2NA following absorption. 2NA and other aromatic amines such as 4-aminobiphenyl are also produced during the burning of tobacco, especially low temperature burning, and when cooking fats and oils are heated. Trace amounts of 2NA have been found in dye-containing products such as children's finger paints.

Exposure Routes and Pathways

2NA is well absorbed through the skin, as well as via the gastrointestinal and respiratory tracts. With this and other aromatic amines, the skin appears to be a significant, if not the major occupational exposure pathway. Workers tolerate skin contamination since the acute effects of exposure are minimal. Inhalation

is the major route of exposure for tobacco smokers. There have been reports that passive burning of tobacco (environmental tobacco smoke) produces larger amounts of 2NA and other aromatic amines on a per cigarette basis than is seen with active smoking. Thus, there is some concern that passive smoke exposure may contribute to the burden of urinary bladder cancer in the nonsmoking population.

Occupational exposure to compounds containing a 2NA nucleus can result in 2NA exposure if metabolic enzymes can degrade the material. For example, workers inhaling ~ 30 mg *N*-phenyl-2-naphthylamine in 1 day excreted 3–4 μg 2NA in their urine over the next 24 h. This is the 2NA exposure equivalent of smoking ~ 5 packs of cigarettes.

Toxicokinetics

Aromatic amines are well absorbed from the skin, the gut, and the respiratory tract. Aromatic amines like 2NA are metabolized rapidly and several systems compete for these agents as substrate. For example, the majority of 2NA is excreted in the urine as the glucuronide that is deconjugated prior to analysis. Ring oxidation and *N*-acetylation are considered detoxification reactions and this is evidenced by the finding that persons with the slow *N*-acetyltranferase 2 phenotype and exposed to many aromatic amines are at elevated risk of urinary bladder cancer in comparison to their fast acetylating cohorts. *N*-Oxidation by cytochrome P450 enzymes is considered activating for bladder carcinogenesis.

Mechanism of Toxicity

The acute toxicity of 2NA is low and due to the formation of methemoglobin. However, this is greatly overshadowed by the urinary bladder carcinogenicity of this compound. The proposed mechanism for this affect includes *N*-oxidation and excretion of the amine into the blood in an unconjugated form. Then the *N*-hydroxy-2-naphthylamine is co-oxidized to the corresponding nitroso form while hemoglobin is oxidized to methemoglobin. 2-Nitrosonaphthalene is then capable of covalent binding with sulfhydral groups on hemoglobin, forming stable hemoglobin adducts.

The mechanism of chronic toxicity is related, but not identical. 2NA and other aromatic amines including benzidine and 4-aminobiphenyl are potent human urinary bladder carcinogens. Apparently the *N*-hydroxy-2-naphthylamine is *N*-glucuronide and the product is transported from the liver to the urinary bladder, where the glucuronide can be hydrolyzed liberating *N*-hydroxy aromatic amine. This material is capable of binding with DNA in the urothelium of the exposed persons. While this pathway has not been shown specifically using 2NA as a substrate in humans, it is consistent with the animal data.

The low acute toxicity of 2NA masks its extreme carcinogenicity as exposed persons experience no or very slight ill effects and assume that the material is not toxic. The latency period for 2NA urinary bladder cancer is estimated from 16 to 30 years after initial exposure.

Acute and Short-Term Toxicity (or Exposure)

Animal

Dogs and cats have been shown to be sensitive to methemoglobinemia following exposure to 2NA. A dose of 200 mg kg^{-1} was found to produce this effect 'reliably'.

Human

Methemoglobin formation was considered the most serious health outcome following exposure to 2NA until the increased rate of urinary bladder tumors were confirmed in the workers. Methemoglobinemia is symptom-less until acutely toxic. In one report workers exposed to 2NA were required to pass through the medical department following their shift so that any cyanosis could be ascertained.

Chronic Toxicity (or Exposure)

Human

Humans exposed to 2NA during the production of this compound are at dramatically increased risk of urinary bladder cancer. According to one report all 15 workers involved with distilling the product fell victim to the disease. In other studies the relative risk of urinary bladder cancer ranged from 30 to 60 times higher than expected; it not being uncommon that 50% of the exposed workforce was prevalent cases. As noted above the estimates of the so-called 'latency' (time between first exposure and disease) period ranged for 16–30 + years for 2NA-exposed workers.

Clinical Management

Fortunately, urinary bladder cancer is amendable to effective treatment if detected early. A full spectrum of biomarkers and early diagnostic screens are available to alert the health professional when exposure to 2NA has occurred and when changes consistent with early neoplasia are occurring.

The US Occupational Safety and Health Administration (OSHA) standard for β-naphthylamine, 29 CFR 1910.1009, contains regulations covering periodic medical surveillance, examinations, and medical records for current employees who may have been exposed to 2NA. However, it should be noted that these regulations do not apply to former employees and that medical surveillance or treatment of former employees is not regulated or required by OSHA.

Exposure Standards and Guidelines

As indicated above, 2NA is one of the carcinogens covered under a specific OSHA regulation, 29 CFR 1910.1009. The American Conference of Governmental Industrial Hygienists indicates that exposure by all routes to 2NA should be controlled to levels as low as possible.

Miscellaneous

The molecular weight of β-naphthylamine is 143.2. It has a negligible vapor pressure until heated; at 200°C the vapor pressure is 1 mmHg. The concentration of 2NA in the air can be determined using National Institute for Occupational Safety and Health method 5518 and it oxidizes in air. Biological monitoring has been done for this material using urinary analysis of metabolites by high-performance liquid chromatography, hemoglobin adducts using gas chromatography–mass spectrometry, and DNA adducts in lymphocytes and exfoliated urothelial cells using ^{32}P-postlabeling.

See also: Dyes; Tobacco Smoke.

Further Reading

Felknor SA, Delclos GL, Lerner SP, *et al.* (2003) Bladder cancer screening program for a petrochemical cohort with potential exposure to beta-napthylamine. *Journal of Occupational and Environmental Medicine/American College of Occupational and Environmental Medicine* 45: 289–294.

IARC (1983) *Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Rubber Manufacture*. Lyon, France: International Agency for Research on Cancer.

Talaska G and Al-Zoughool M (2003) Aromatic amines and biomarkers of human exposure. *Journal of Environmental Science and Health* 21: 133–164.

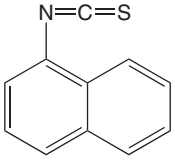
Relevant Website

http://www.osha.gov – OSHA website for analytical method #93, 4-aminobiphenyl, 1-naphthylamine, and 2-naphthylamine.

Naphthylisothiocyanate

Samantha E Gad

- Chemical Abstracts Service Registry Number: CAS 551-06-4
- Synonyms: α-Naphthylisothiocyanate; ANIT; 1-Isothiocyanato-naphthalene
- Chemical Formula: $C_{11}H_7NS$
- Chemical Structure:



Uses

1-Naphthylisothiocyanate is used as an ingredient in insecticides, and as a laboratory agent for inhibiting microsomal based metabolism. It is also found in cyanamide, which is used in many industrial applications.

Exposure Routes and Pathways

Inhalation, ingestion, and dermal contact are all possible routes of exposure.

Mechanism of Toxicity

1-Naphthylisothiocyanate causes separation of extracellular tight junctions that seal bile canaliculi, impairing bile formation. 1-Naphthylisothiocyanate inhibits microsomal drug-metabolizing activity. It has also been suggested that ANIT depletes hepatocytes of glutathione through a reversible process.

Acute and Short-Term Toxicity (or Exposure)

Animal

A single dose can induce intrahepatic cholestatis (reduction in bile flow) in rats, producing hyperbilirubinemia. In China, several herbal formulations have been shown to reduce the liver damage caused by naphthylisothiocyanate in rats. The oral LD_{50} in rats is 200 mg kg^{-1}.

Chronic Toxicity (or Exposure)

Animal

1-Naphthylisothiocyanate is a potent hepatotoxin and mutagen in animals.

Human

1-Naphthylisocyanate can cause liver and kidney damage as well as dermatitis, ocular irritation, and corrosion.

Clinical Management

After ocular exposure, eyes should be immediately flushed with water for at least 15 min. Following skin exposure, the skin should be flushed with water for at least 15 min. If ingested, vomiting should not be induced. If conscious, the individual should ingest two to four cupfuls of milk or water. After inhalation exposure, the individual should be removed to fresh air immediately and provided breathing support if necessary. Mouth-to-mouth resuscitation should not be used.

See also: Kidney; Liver; Pesticides.

Further Reading

Bingham E, Cohrssen B, and Powell CH (eds.) (2001) *Patty's Toxicology*, 5th edn. New York: Wiley.

Hill DA and Roth RA (1998) Alpha-naphthylisothiocyanate causes neutrophils to release factors that are cytotoxic to hepatocytes. *Toxicology and Applied Pharmacology* 148(1): 169–175.

Roth RA and Dahm LJ (1997) Neutrophil- and glutathione-mediated hepatotoxicity of alpha-naphthylisothiocyanate. *Drug Metabolism Reviews* 29(1–2): 153–165.

National Environmental Policy Act, US

Samantha E Gad and Shayne C Gad

- Agency: US Council on Environmental Quality
- Year Passed: 1969
- Groups Regulated: US government agencies

Synopsis of Law

The National Environmental Policy Act (NEPA) was signed into law in 1970, and established a national policy to protect the environment, created a Council on Environmental Quality (CEQ), and required that environmental impact statements be prepared for major federal actions having a significant effect on the environment. The CEQ's efforts laid the groundwork for almost all current US environmental legislation, except for Superfund and asbestos control legislation. The CEQ also developed guidelines for the environmental impact statement process. The NEPA process resulted in a major change in the way governments deal with environmental issues, and this model has been replicated in whole or in part in 23 states.

See also: Clean Air Act (CAA), US; Clean Water Act (CWA), US; Ethanol; Resource Conservation and Recovery Act, US; Toxic Substances Control Act, US

Relevant Websites

http://www4.law.cornell.edu – National Environmental Policy (from the US Code).

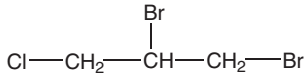
http://ceq.eh.doe.gov – NEPANet.

http://www.epa.gov – US Environmental Protection Agency (EPA) website on 'NEPA: Past, Present, and Future'.

Nematocides

Samantha E Gad

- Representative Compound: Nemagon (1,2-dibromo-3-chloropropane)
- Chemical Abstracts Service Registry Number: CAS 96-12-8 (Nemagon)
- Chemical/Pharmaceutical/Other Class: Nema-tak; Halogenated hydrocarbons
- Chemical Formula: $C_3H_5Br_2Cl$
- Chemical Structure:



Uses

Nematocides are pesticides that kill parasitic worms such as roundworms or threadworms. Early nematocides were used as soil fumigants. Since the 1960s, a completely new group of 'nonfumigant' nematocides has been developed. All are organophosphorus or carbamate pesticides with marked (acute oral and dermal) toxicity to humans.

Exposure Routes and Pathways

Dermal contact, inhalation, and ingestion are possible exposure pathways.

Toxicokinetics

In rats, 98% of nemagon is absorbed into the stomach. Within 3 days, 90% of the compound is excreted. Within the first 24 h period, 49% is excreted through urine, 14% through feces, and 16.5% through expired air.

Mechanism of Toxicity

Defatting creates cell necrosis. Nematocides also reduce cell P450 content. Sulphyril, but not glutathione, mediates toxic effects. Biotransformation (hydrolysis and oxidation) is via the mercapturic acid route, producing α-chlorohydrin and α-bromohydrin, two antifertility agents. Further oxidation of these substances may produce oxalic acid, which causes liver and kidney damage.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} of nemagon is 100 mg kg^{-1} in rabbits and 260 mg kg^{-1} in mice.

Human

Acute exposure to high concentrations produces dyspnea, gasping, and coughing.

Chronic Toxicity (or Exposure)

Animal

Chronic exposure results in eye damage, kidney degeneration, and central nervous system (CNS) effects. It is a carcinogen to nasal passages, pharynx, and respiratory tract. A dose–response relationship exists between exposure and damage to the reproductive system. Age or stage of sexual development also mediated damage.

Human

Chronic exposure affects the liver, kidneys, and heart. Other symptoms include CNS depression and pulmonary congestion. Nemagon is a reproductive toxin resulting in reduced sperm count. Adverse effects are presumed to be reversible. It is a possible carcinogen. Most human exposure to nematocides is as trace residues in meat.

Clinical Management

The victim should be moved from exposure site and given respiratory therapy. Treatment should be symptomatic.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration permissible exposure limit is 1 ppb per 8 h.

See also: Federal Insecticide, Fungicide, and Rodenticide Act, US; Pesticides.

Further Reading

Krieger R (2001) *Handbook of Pesticide Toxicology*. San Diego, CA: Academic Press.

Neon

Lynda M Ewers

Published by Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 7440-01-9
- Chemical/Pharmaceutical/Other Class: Neon is an element in the family referred to as the inert, rare, or noble gases. Members of this family, in increasing order by atomic number, are helium, neon, argon, krypton, xenon, and radon. All of these gases are colorless, odorless, and nonflammable. The noble gases are thought to originate primarily under conditions of high pressure and temperature such as existed at the origin of the universe and continues to exist in some stars. Neon

is highly inert and does not form chemical compounds under normal environmental conditions.

Uses

Neon is primarily used in luminous tubes (vacuum electric discharge tubes), airplane beacons, helium–neon lasers, high-voltage indicators, cryogenic refrigerant, and laboratory experiments.

Background Information

In 1898, a Scottish scientist and a British scientist, Sir William Ramsay and Morris M Travers, respectively, discovered neon as a condensation product in liquefied air, a process similar to that used to collect neon today. Neon's use in lighting evolved from the discoveries that gases under low pressure will conduct electricity. When some of the flowing electrons collide with residual gas in an evacuated glass tube, the resulting ions emit light as they return to their nonexcited state. The color of the light produced depends on the residual gas; neon gas produces a red color and argon, another inert gas often used in tubes (which are frequently and incorrectly called "neon" lights), produces a blue color. These two basic colors are often modified into many different hues by the addition of such elements as mercury and cadmium. The neon found on the earth is considered to be primordial in origin. Most of the neon is sequestered in the earth's rocks or dissolved in water, with small amounts escaping into the atmosphere during geologic weathering. The escaped gas is slowly lost into space faster than it is replenished. Consequently, neon constitutes only a small part (0.0018%) of the earth's atmosphere, although this element is estimated to be the fourth most abundant in the universe.

Exposure Routes and Pathways

The most important route of exposure is inhalation, occurrs when neon escapes from natural sources (rocks and water) or neon-containing products (see Uses). Skin exposure and ingestion can also occur; however, only the inhalation route is considered to be important from a toxicological standpoint, because of the way inhalation of excessive concentrations of this inert gas can still potentially produce harmful effects (see Mechanism of Toxicity).

Mechanism of Toxicity

Neon is a simple asphyxiant. It displaces the oxygen necessary to support life. When normal levels of oxygen are not present in the body, then all tissues, organs, and organ systems eventually malfunction. Tissues with particularly high oxygen and energy requirements, including the brain and heart, are particularly susceptible to harmful effects resulting from reduced levels of oxygen in the body.

Acute and Short-Term Toxicity (or Exposure)

The primary adverse health effect attributed to neon exposures is simple asphyxiation due to the displacement of oxygen necessary for life. No animal or *in vitro* studies were found in scientific literature, but it is known that humans and other animals requiring oxygen can die by asphyxiation, if exposed to high concentrations of neon.

Chronic Toxicity (or Exposure)

The typically small quantities of the gas in the environment and amounts used in manufacturing consumer products result in very low levels of neon in workplace and ambient environments, and thus cause negligible health risks to workers and the general public who may experience chronic exposures.

Clinical Management

Oxygen should be provided to the affected individual.

Ecotoxicology

No known reports on ecotoxicology of neon could be found. Neon is very inert and does not deplete ozone.

Other Hazards

Neon is not explosive or flammable. Hazards related to neon include use of cryogenic neon-gas tanks, emission of eye-damaging light from lasers, or escape of mercury, cadmium, or lead from luminous tubes. The manufacture of neon-type advertising signs are of special concern because such signs are often produced by small business artisans who may have limited knowledge or resources to deal with hazardous material, such as mercury and lead, which have been documented to contaminate these workplaces.

Exposure Standards or Guidelines

No standards or guidelines are available for neon.

See also: Cadmium; Lead; Mercury.

Further Reading

Ewers L, Page E, and Mortimer V (2003) Hazards associated with the manufacture and repair of neon lights. *Applied Occupational and Environmental Hygiene* 18(1): 1–9.

Neonicotinoids

Josef Seifert

History of Neonicotinoid Development

Neonicotinoids are a new class of synthetic insecticides that became commercially available in the 1990s. Currently there are only a few neonicotinoid insecticides on the market but those are being increasingly used with a good prognosis for their further development. These new-generation pesticides have potential as replacements for some of the more toxic organophosphorus and methylcarbamate insecticides.

Nicotine (**Figure 1**) isolated from tobacco plants (*Nicotiana tabacum*) has been used as a systemic insecticide against sucking insects for centuries. The nicotinic acetylcholine receptor-Na^+/K^+ ionophore of the insect central nervous system is the target site, and the consequences of the altered cholinergic neurotransmission provides the mechanism of insecticidal action. Since nicotine is equally or more toxic to mammals than to insects, the major objective in developing new insecticides modeled on nicotine has been to change this unfavorable feature and synthesize compounds with greater selectively and toxicity to insects. Preservation of the nicotinic acetylcholine receptor as a target for these novel insecticides is important in order to address the development of insect resistance to other insecticides. Nicotine and its analogs, such as nornicotine or anabasine (**Figure 1**), are grouped together as nicotinoids. Neonicotinoids are synthetic, newly developed insecticides with the nicotinic acetylcholine receptors as their target but, in contrast to nicotinoids, have a high degree of selectivity toward insects.

Nithiazine (**Figure 2**) was the first neonicotinoid developed by Shell (Modesto, USA) in the 1970s. Nithiazine is a 2-nitromethylene tetrahydro-1, 3-thiazine selected from a series of nitroalkyl heterocyclic compounds, the molecular models being distinct from nicotine but acting on the nicotinic acetylcholine receptors like nicotine. Nithiazine is selectively toxic to insects but its field application is limited because of its low photostability.

Nithiazine was the lead compound in syntheses of the first commercially successful neonicotinoids that surpassed the parent compound in both insecticidal properties and environmental stability. A 6-chloro-3-methylpyridine moiety and a pharmacophore of varying structures (**Figure 2**) are the two components of a neonicotinoid molecule. Insecticides of the first generation of neonicotinoids are best represented by imidacloprid (Nihon Bayer Agrochem, Japan) (**Figure 2**) and are also called chloronicotinyls or chloropyridyls.

The most recent efforts in the development of neonicotinoids focused on the search for heterocycles and pharmacophores that would further improve insecticidal properties of the current compounds. This search must be a compromise between the requirements for the optimal electron distribution in the pharmacophore needed for insecticide binding to the receptor subsites and the need for hydrophobicity of a neonicotinoid for efficient penetration through the protective lipoid shield that surrounds the insect central neural system. The synthesis of thiamethoxam (compound CGA 293 343; Novartis, Switzerland) from a heterocycle 2-chloro-5-methylthiazine and a pharmacophore 4-nitroimino-N^5-methyl-1,3,5-oxadiazinane was the first success in the development of the second generation of neonicotinoids also called thianicotinyls. Examples of the second generation of neonicotinoids that have been or are being introduced on the market are shown in **Figure 3**. Most recently, the third generation of neonicotinoids, furanicotinyl compounds (e.g., dinotefuran, **Figure 3**), has been developed.

Mechanism of Neonicotinoid Action

The nicotinic acetylcholine receptors of the neural excitatory cholinergic system are the targets for both nicotine and neonicotinoids in mammals and insects.

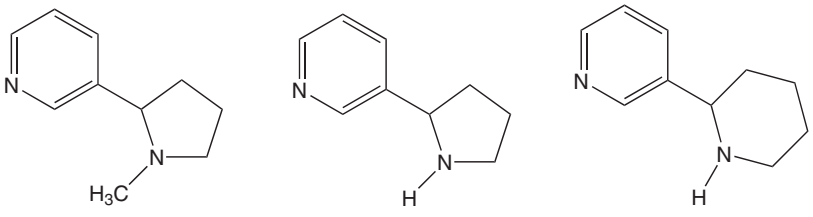


Figure 1 Nicotinoids.

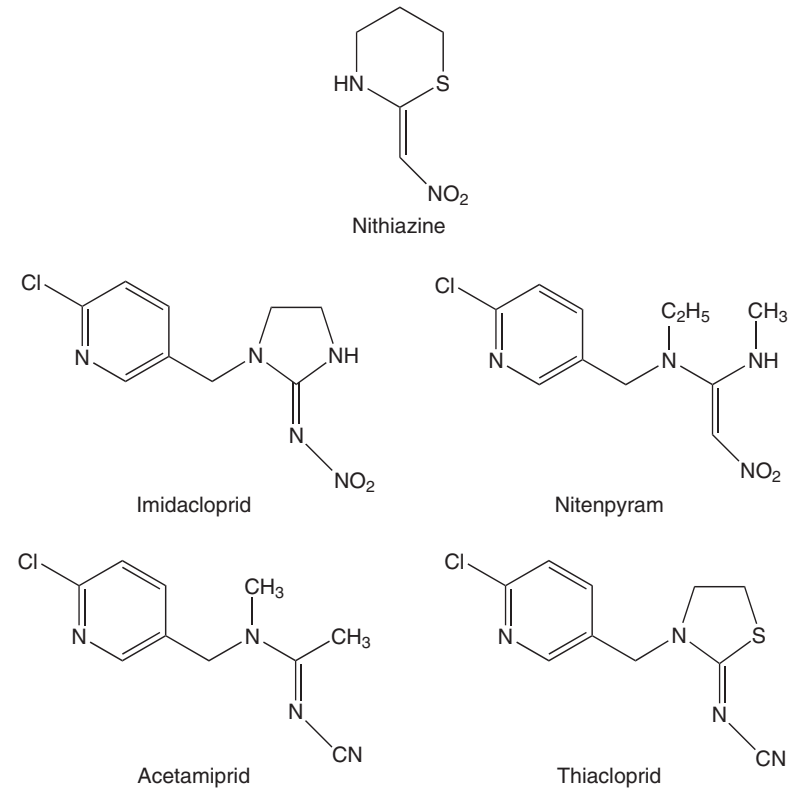


Figure 2 Nithiazine and the first generation of neonicotinoids.

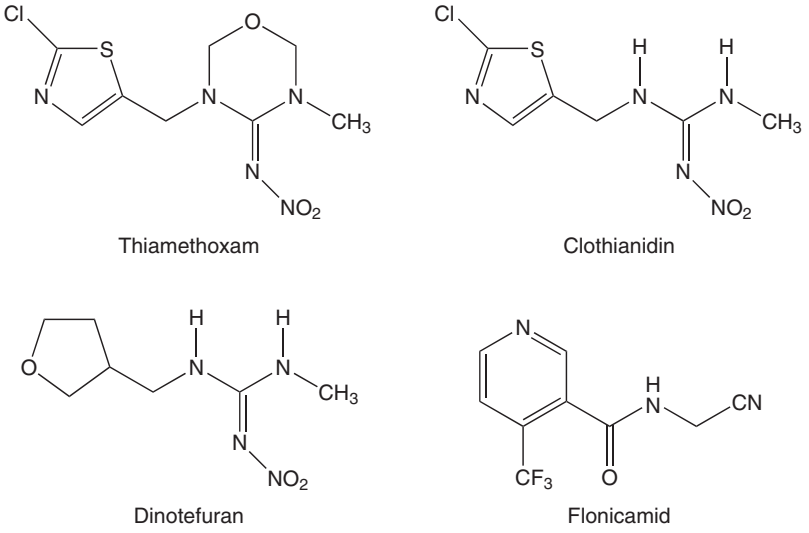


Figure 3 The second and third generations of neonicotinoids.

Nicotinic acetylcholine receptors regulate the flow of Na^+ and K^+ through the channels in the neural postsynaptic membranes. Opening and closing of the channels by acetylcholine maintains the dynamic ratio of the intracellular to extracellular concentrations of Na^+ and K^+ which is needed for the initiation of the electric signal in the postsynaptic neuron. Nicotine and neonicotinoids are agonists, both of which act at the nicotinic acetylcholine receptor – Na^+/K^+ ionophore. The structural differences between the insect and mammalian receptors define the selectivity of neonicotinoid toxicity to insects and nicotine toxicity to vertebrates. The proposed concept of the neonicotinoid electronegative pharmacophore

model (**Figure 4**) considers the presence of a positively charged site unique for the insect receptor that interacts specifically with the negatively charged tip of neonicotinoid pharmacophores. On the other hand, protonation of the nicotinoid nitrogen at physiological pH is the determining factor for their strong binding to the vertebrate receptors. Ionization of nicotinoids also negatively affects their penetration into the insect central nervous system, in contrast to the nonionized and more hydrophobic neonicotinoids.

Neonicotinoid Toxicity to Nontarget Species

Acute Toxicity

Unlike nicotine, neonicotinoids are only moderately toxic to mammals mainly because of their lower affinity for the mammalian neural and muscle nicotinic acetylcholine receptors. In laboratory animals, high neonicotinoid doses that are near the LD_{50}s cause tremor, gait incoordination and hypothermia appearing 2–6 h after oral administration. The signs generally cease within 24 h following treatment. LD_{50}s of the currently used neonicotinoids are in the range of 170–2000 mg kg^{-1} for oral administration and $\geqslant$2000 mg kg^{-1} with dermal administration, dependent on animal species and the type of neonicotinoid. Death from oral neonicotinoid overdose occurs within 3–7 h.

Subchronic and Chronic Toxicity

Tests of neonicotinoids for neurotoxicity, reproductive toxicity, teratogenesis and mutagenesis in a variety of laboratory animals, generally conducted for the purpose of insecticide registration, were negative. Neither of the neonicotinoids induced growth of malignant tumors in laboratory animals. Based on the current knowledge, neonicotinoids can be considered safe for both humans and farm animals or pets.

Environmental Toxicity

Some toxic consequences of neonicotinoids for nontarget beneficial aquatic and terrestrial arthropods such as bees can be expected since these creatures have nicotinic acetylcholine receptors as functional components of the cholinergic system similar to those of insect pests. Surprisingly, neonicotinoid toxicity to numerous nontarget insect species and wildlife marker vertebrates, for example, rainbow trout, is lower than expected. In general, the environmental safety of neonicotinoids surpasses that of other insecticides.

Neonicotinoid Stability

Physical–Chemical Factors

Neonicotinoids, products with medium to high water solubility, are relatively stable in water, buffers or physiological media in pH range 5–7. Their stability decreases with an increasing pH (e.g., $t_{1/2}$ for thiamethoxam at pH 5–7 is $\geqslant$1 year while only a few days at pH 9). Photostability of neonicotinoids with a nitromethylene group ($=CH-NO_2$) is low since this group absorbs strongly sunlight in the range of 290–400 nm. For instance, degradation of

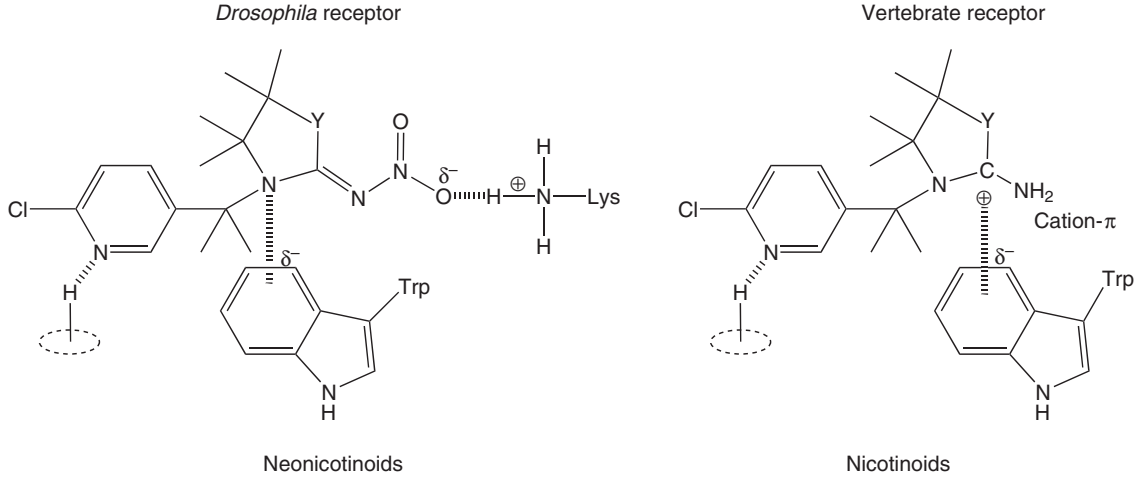


Figure 4 Molecular models of binding subsites in the *Drosophila* or vertebrate nicotinic acetylcholine-regulated receptors. (Reprinted with permission from Tomizawa *et al.* (2003) The neonicotinoid electronegative pharmacophore plays the crucial role in the high affinity and selectivity for the *Drosophila* nicotinic receptor: An anomaly for the nicotinoid cation-pi interaction model. *Biochemistry* 42: 7819–7827; © American Chemical Society.)

nithiazine in direct sunlight is complete within several minutes. The photodegradation products are inactive as insecticides. Replacement of the nitromethylene group with groups that absorb less, or do not absorb sunlight such as the nitroimine ($=N-NO_2$) in imidacloprid or cyanoimine ($=N-CN$) in acetamiprid, significantly improved photostability.

Metabolism

The metabolism of neonicotinoids in vertebrates, insects, and plants has many common features. It may result in cleavage and the separation of the heterocyclic and pharmacophore moieties, or modifications of a pharmacophore in an intact parent molecule. Oxidations, reductions, and elimination reactions are the major mechanisms that result mostly in a reduced or diminished insecticidal potency of the metabolites. Dehydration of the 4-hydroxyimidazolidinyl resulting in a formation of the imidazolinyl (olefin) or reduction of the *N*-nitroimine ($=N-NO_2$) to *N*-nitrosoimine ($=N-NO$) group (**Figure 5**) are examples of a limited number of metabolic conversions leading to increased insecticidal potency. *N*-desmethylation of *N*′-methyl in nitempyram pharmacophore and formation of a nonsubstituted imine represent detoxification reactions for insects but activation for mammals. Similarly, loss of an *N*-nitro-group from *N*-nitroimine in imidacloprid or *N*-cyano-group from *N*-cyanoimine in thiacloprid is a detoxification process in insects but an activation step in mammals. Oxidative metabolism catalyzed by cytochrome P450s can be prevented by using piperonyl butoxide-type synergists. Generally, excretion of the metabolites, some as conjugates, is fast with only low accumulation of the parent compound in organisms treated with neonicotinoids. Fast recovery from poisoning by neonicotinoids is in accordance with their high rates of absorption, distribution, and elimination.

The metabolism of imidacloprid (**Figure 5**) provides a typical example of the metabolic behavior of chloronicotinoids. In rats, imidacloprid is readily absorbed from the gastrointestinal tract and distributed into the organs within 1 h. Liver and kidney are the target organs. No residues of imidacloprid were

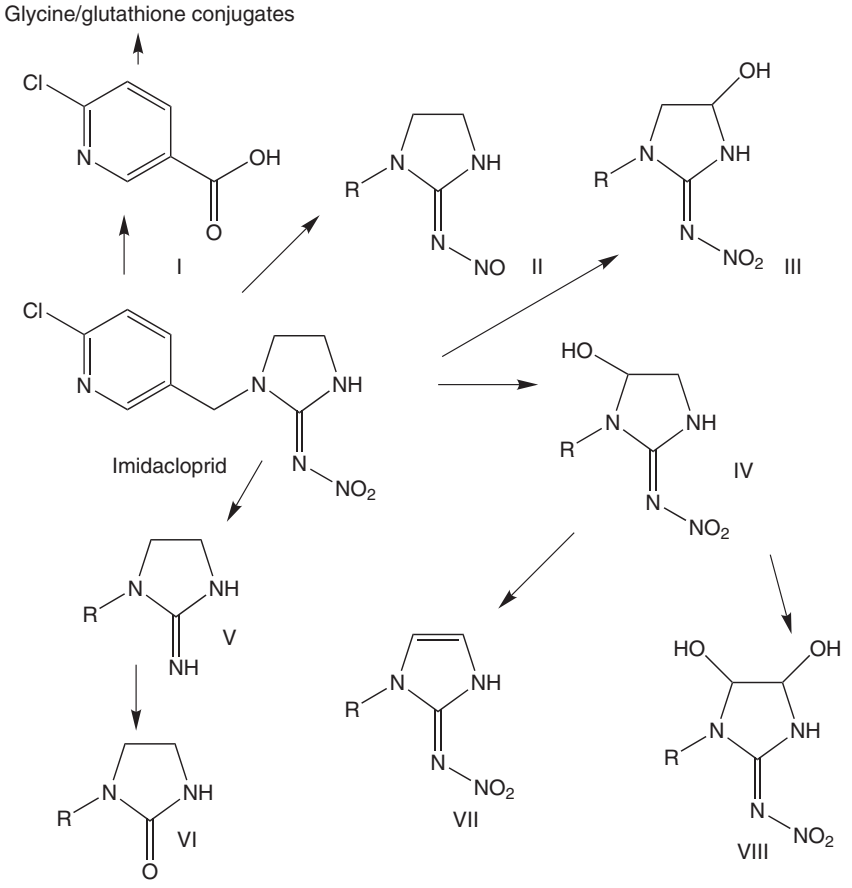


Figure 5 Imidacloprid metabolites in rats, insects and plants. I – 6-chloronicotinic acid (mammalian route of elimination); II – nitrosoimine; III – 4-hydroxy; IV – 5-hydroxy; V – guanidine; VI – urea; VII – olefin; VIII – 4,5-dihydroxy derivatives.

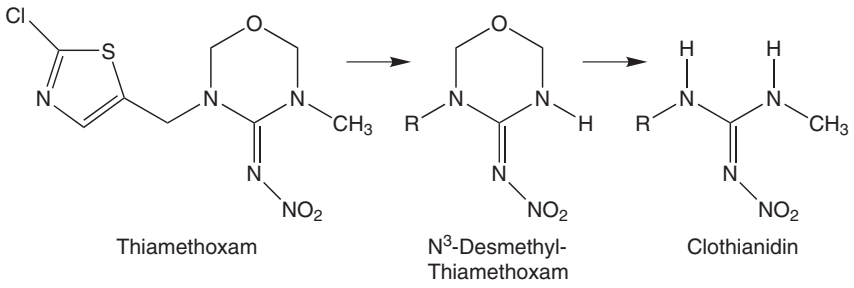


Figure 6 A proposed activation of thiamethoxam in insects and plants.

found in the central nervous system or in fat tissues or bones. Imidacloprid is metabolized in two distinct pathways in rats. In one pathway, it is oxidatively cleaved into 6-chloro-3-methylpyridine and 2-nitroiminoimidazolidine. The latter product is excreted directly in urine while 6-chloro-3-methylpyridine is eliminated as a glycine or glutathione conjugate. More than 90% of the administered compound is excreted within 24 h with the majority of the metabolites (~80%) in urine; the rest is eliminated in feces. In the second metabolic pathway, the imidazolidine ring of imidacloprid undergoes hydroxylation at the 4- and/or 5-position possibly followed by dehydration to imidazolinyl (olefin). The latter metabolite has higher affinity for the nicotinic acetylcholine receptors and is a more potent insecticide. Reductions of a nitroimino group to nitroso- and hydrazono-, or breaking the N–N bond and forming a guanidine-like derivative followed by a possible oxidation to urea-like derivative are the optional routes in insects, vertebrates, and plants.

The toxicokinetics of the thianicotinyl thiamethoxam is similar to that of imidacloprid. When applied orally to rats, goats, or chickens, thiamethoxam is rapidly and almost quantitatively absorbed. Its excretion, predominantly in urine, is fast. Accumulation in tissues is negligible. Thiamethoxam itself does not bind strongly to the neonicotinoid binding site of the nicotinic acetylcholine receptor but it is reported to be converted to clothianidin, a neonicotinoid with high affinity for the insect receptors, in insects and plants (**Figure 6**). It is possible that this activation proceeds via formation of an *N*-desmethyl thiamethoxam intermediate, another compound that acts at the neonicotinoid-binding site.

Uses

Nithiazine

The low photostability of nithiazine limits its commercial use to fly traps.

Imidacloprid and Other Neonicotinoids

Neonicotinoids are effective against homopterans, coleopterans, and lepidopterans. They act systemically because of their water solubility, being especially active against sucking insects. Their water solubility makes them useful for application in seed treatments. Low mammalian toxicity allows their use for flea control in dogs and cats. Their environmental stability at neutral or mild acidic media is valuable in soil applications, for example, against termites.

See also: Acetylcholine; Cholinesterase Inhibition; Nicotine.

Further Reading

Tomizawa M and Casida JE (2003) Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors. *Annual Review of Entomology* 48: 339–364.

Yamamoto I and Casida JE (eds.) (1999) *Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor.* New York: Springer.

Neoplasia *See* Carcinogenesis.

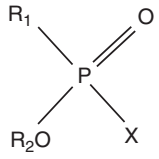
Nephrotoxicity *See* Kidney.

Nerve Agents

Harry Salem and Frederick R Sidell*

Published by Elsevier Inc.

- Preferred Name: Nerve agents. Also known as nerve gases
- Synonyms: Tabun (GA; CAS 77-81-6); Sarin (GB; CAS 107-44-8); Soman (GD; CAS 96-64-0); Cyclosarin (GF; CAS 329-99-7); G agents; Organophosphates (OP): VX (CAS 20820-80-8); V agents; Chemical warfare agents; Irreversible cholinesterase inhibitors; Anticholinesterase compounds
- Description: Nerve gases are clear liquids; therefore, the term 'gas' is a misnomer. The preferred term is 'nerve agents'. Because of the chiral phosphorus in their structure, the nerve agents contain various steroisomers. Soman, sarin, tabun, and VX contain equal amounts of (+) and (−) enantiomers. The G agents are volatile and thus present both vapor and liquid hazard. In decreasing order of volatility are sarin, soman, tabun, and VX. VX presents a negligible vapor hazard, but its volatility increases with increasing temperature. At temperatures above 40°C it also presents a vapor hazard
- Chemical Structures:



	X	R_1	R_2
GA (Tabun)	–CN	$-N(CH_3)_2$	$-C_2H_5$
GB (Sarin)	–F	$-CH_3$	$-CH(CH_3)_2$
GD (Soman)	–F	$-CH_3$	$-CH(CH_3)C(CH_3)_3$
GF (Cyclosarin)	–F	$-CH_3$	$-\text{cyclo-}C_6H_{11}$
VX	$-SCH_2CH_2N(CH(CH_3)_2)_2$	$-CH_3$	$-C_2H_5$

Uses

Nerve agents are used in chemical warfare.

Exposure Routes and Pathways

Casualties are caused primarily by inhalation; however, they can occur following percutaneous and ocular exposure, as well as by ingestion and injection.

Toxicokinetics

Nerve agents are absorbed both through the skin and via respiration. Because VX is an oily, nonvolatile liquid it is well absorbed through the skin (persistent nerve agent), although it can also be absorbed by inhalation. Thus, VX is more of a percutaneous threat than by inhalation, whereas the G agents (nonpersistent), which are also liquids, pose more of an inhalation hazard because of their vapor pressure. Sarin (GB) is the most volatile, but evaporates less readily than water, while cyclosarin (GF) is the least volatile of the G agents.

Nerve agents are hydrolyzed by the enzyme organophosphate (OP) hydrolase. The hydrolysis of GB, soman (GD), tabun (GA), and diisopropyl fluorophosphate occurs at approximately the same rate. The isomers of the asymmetric OPs may differ in overall toxicity, rate of aging, rate of cholinesterase inhibition, and rate of detoxification. The rates of detoxification differ for different animal species and routes of administration. The onset of effects from nerve agents depends on the route, duration, and amount of exposure. The effects can occur within seconds to several minutes after exposure. There is no latent period following inhalation exposure of high concentrations where loss of consciousness seizures has occurred within 1 min. At low concentrations; however, miosis, rhinorrhea, and other effects may not begin for several minutes. Maximal effects usually occur within minutes after contamination ceases.

Mechanism of Toxicity

The nerve agents inhibit the enzymes butrylcholinesterase in the plasma, acetylcholinesterase on the red blood cell, and acetylcholinesterase at cholinergic

*The views of the authors do not purport to reflect the position of the US Department of Defense. The use of trade names does not constitute official endorsement or approval of the use of such commercial products

receptor sites in tissues. These three enzymes are not identical. Even the two acetylcholinesterases have slightly different properties, although they have a high affinity for acetylcholine. The blood enzymes reflect tissue enzyme activity. Following acute nerve agent exposure, the red blood cell enzyme activity most closely reflects tissue enzyme activity. However, during recovery, the plasma enzyme activity more closely parallels tissue enzyme activity.

Following nerve agent exposure, inhibition of the tissue enzyme blocks its ability to hydrolyze the neurotransmitter acetylcholine at the cholinergic receptor sites. Thus, acetylcholine accumulates and continues to stimulate the affected organ. The clinical effects of nerve agent exposure are caused by excess acetylcholine.

The binding of nerve agent to the enzyme is considered irreversible unless removed by therapy. The accumulation of acetylcholine in the peripheral and central nervous systems (CNS) leads to depression of the respiratory center in the brain, followed by peripheral neuromuscular blockade causing respiratory depression and death.

The pharmacologic and toxicologic effects of the nerve agents are dependent on their stability, rates of absorption by the various routes of exposure, distribution, ability to cross the blood–brain barrier, rate of reaction and selectivity with the enzyme at specific foci, and behavior at the active site on the enzyme.

Red blood cell enzyme activity returns at the rate of red blood cell turnover, which is $\sim 1\%$ per day. Tissue and plasma activities return with synthesis of new enzymes. The rates of return of these enzymes are not identical. However, the nerve agent can be removed from the enzymes. This removal is called reactivation, which can be accomplished therapeutically by the use of oximes prior to aging. Aging is the biochemical process by which the agent–enzyme complex becomes refractory to oxime reactivation. The toxicity of nerve agents may include direct action on nicotine acetylcholine receptors (skeletal muscle and ganglia) as well as on muscarinic acetylcholine receptors and the central nervous system.

Recent investigations have focused on OP nerve agent poisoning secondary to acetylcholine effects. These include the effects of nerve agents on γ-aminobutyric acid neurons and cyclic nucleotides. In addition, changes in brain neurotransmitters, such as dopamine, serotonin, noradrenaline, as well as acetylcholine, following inhibition of brain cholinesterase activity, have been reported. These changes may be due in part to a compensatory mechanism in response to overstimulation of the cholinergic system or could result from direct action of nerve agent on the enzymes responsible for noncholinergic neurotransmission.

Human Toxicity

The human toxicity estimates for the nerve agents are summarized in **Table 1**.

Rhinorrhea may precede miosis as the first indication of exposure to even small amounts of nerve agent vapor. After exposure to high concentrations/doses by any route, rhinorrhea occurs as part of the generalized increase in secretions. Direct ocular contact to nerve agents may cause miosis, conjunctival injection, pain in or around the eyes, and dim or blurred vision.

Acute exposure of 3 mg min m^{-3} of GB vapor will produce miosis in most of the exposed population. Other routes of exposure may not cause any eye effects or cause a delayed onset of them, but will cause vomiting, sweating, and weakness.

The onset of miosis is within seconds to minutes following aerosol or vapor exposure but may not be maximal for up to 1 h, especially at low concentrations. The duration of miosis varies and is dependent on the extent of exposure. The ability of the pupil to dilate maximally in darkness may not return for up to 6 weeks. There is no correlation between miosis and blood cholinesterase levels.

Respiratory distress also occurs within seconds to minutes following vapor exposure. The symptoms include tightness of the chest, shortness of breath, and gasping and irregular breathing leading to apnea. Bronchoconstriction and bronchial secretions contribute to this. With larger concentrations, cyanosis and audible pulmonary changes occur, which can only be relieved by therapeutic intervention. Death due to nerve agent intoxication is

Table 1 Human toxicity estimates for nerve agents

Agent	*Inhalation LCt_{50} (mg min m^{-3})*	*Intravenous LD_{50} (mg kg^{-1})*	*Percutaneous LCt_{50} (mg min m^{-3})*	*Percutaneous LD_{50} (mg kg^{-1})*	*Oral LD_{50} (mg kg^{-1})*
GA	135	0.08	20 000	14	0.36–0.71
GB	70	0.014	12 000	24.3	0.07–0.29
GD	70			5	0.07–0.29
GF					0.14
VX	30	0.008		0.143	0.04–0.14

attributable to respiratory failure resulting from bronchoconstriction, bronchosecretion, paralysis of skeletal muscles, including those responsible for respiration, and failure of the central drive for respiration. Nerve agent intoxication causes skeletal muscles to fasciculate, twitch, and fatigue prior to paralysis.

The cardiovascular effects of nerve agent exposure are variable. Bradycardia may occur via vagal stimulation, but other factors such as fright, hypoxia, and adrenergic stimulation, secondary to ganglionic stimulation may produce tachycardia or hypertension. Following inhalation exposure to large amounts of nerve agent, the CNS effects will cause loss of consciousness, seizure activity, and apnea within 1 min.

Following skin contact with large amounts of liquid, the dermal effects may be delayed up to 30 min. Long-term exposure to an OP, diisopropyl phosphorofluoridate, used in the treatment of myasthenia gravis, caused side effects including nightmares, confusion, and hallucinations.

US Public Law 91-145 (50 USC 1521) and an International Treaty mandate that stored Chemical Warfare Agents (CWA) be destroyed by the US Department of Defense (DoD). Public Law 91-121 and 91-441 (50 USC 1512) mandate that the US Department of Health and Human Services (DHHS) review DoD plans for disposing of the stored munitions and make recommendations to protect public health. Thus, DHHS and Centers for Disease Control and Prevention (CDC) revised the airborne exposure criteria for GA, GB, and VX and proposed revisions for sulfur mustard. Worker population limits (WPLs), short-term exposure limits (STELs), general population limits (GPLs), and immediately dangerous to life or health (IDLH) values are used to protect workers and the general population during routine chemical demilitarization activities. The recommended airborne exposure limits for GA, GB, and VX (in $mg\,m^{-3}$) in demilitarization are as follows:

Agent	*GPL (24 h)*	*WPL (8 h)*	*STEL (15 min)*	*IDLH (30 min)*
GA	1×10^{-6}	3×10^{-5}	1×10^{-4}	0.1
GB	1×10^{-6}	3×10^{-5}	1×10^{-4}	0.1
VX	6×10^{-7}	1×10^{-6}	1×10^{-5}	0.003
HD (proposed)	0.000 02 (12 h)			0.7 (30 min)

Note: Five-minute ceiling level is 0.003.

Over the last two decades, two groups have addressed acute exposure guideline levels for hazardous substances. One group, sponsored by the American Industrial Hygiene Association, develops these advisory numbers for a 1 h exposure as emergency response planning guidelines (ERPG-1, ERPG-2, and ERPG-3). ERPG-1 is the level associated with slight irritation, ERPG-2 with developmental or subchronic toxicity, while ERPG-3 is associated with acute lethality. The other committee, sponsored by the US Environmental Protection Agency, developed 3 acute exposure guideline levels (AEGL) for 10 and 30 min, and for 1, 4, and 8 h. These are threshold exposure limits for the general public and are applicable to emergency exposure periods that would occur infrequently in a person's life. The AEGL-1 is the airborne concentration above which the general population, including sensitive individuals, could experience notable discomfort, irritation, or certain asymptomatic nonsensory effects. The effects are not disabling and are transient and reversible upon cessation of exposure. AEGL-2 is the airborne concentration at which the same population experiences irreversible or other serious, long-lasting adverse health effects or an impaired ability to escape. AEGL-3 is the airborne concentration predicted to cause life-threatening health effects or death. For GB and VX, these are as follows:

AEGL-1 (nondisabling)

	10 min	*30 min*	*1 h*	*4 h*	*8 h*
GB					
ppm	0.001 2	0.000 68	0.000 48	0.000 24	0.000 17
$mg\,m^{-3}$	0.006 9	0.004 0	0.002 8	0.001 4	0.001 0
VX					
ppm	0.000 052	0.000 030	0.000 016	0.000 009 1	0.000 006 5
$mg\,m^{-3}$	0.000 57	0.000 33	0.000 17	0.000 10	0.000 071

AEGL-2 (disabling)

	10 min	*30 min*	*1 h*	*4 h*	*8 h*
GB					
ppm	0.015	0.008 5	0.006 0	0.000 29	0.002 2
$mg\,m^{-3}$	0.087	0.050	0.035	0.017	0.013
VX					
ppm	0.000 65	0.000 38	0.000 27	0.000 14	0.000 095
$mg\,m^{-3}$	0.007 2	0.004 2	0.002 9	0.001 5	0.001 0

AEGL-3 (lethal)

	10 min	*30 min*	*1 h*	*4 h*	*8 h*
GB					
ppm	0.064	0.032	0.022	0.012	0.0087
$mg\,m^{-3}$	0.38	0.19	0.13	0.070	0.051
VX					
ppm	0.002 7	0.001 4	0.000 91	0.000 48	0.000 35
$mg\,m^{-3}$	0.029	0.015	0.010	0.005 2	0.003 8

The DoD in 1996 and 1997 originally set an army safety limit intended for monitoring potential public exposure to US chemical agents. This limit, termed GPL dosage was the smallest dose of an agent causing noticeable effects such as miosis. This was calculated by applying numerous safety factors to establish a level at which the general population could be exposed 24 h day^{-1} for a lifetime without experiencing any adverse health effects. DoD and the (US) Central Intelligence Agency selected the GPL as their threshold since it was considered a scientifically based standard. These GPLs were used for modeling nerve agents, and were revised in 2000 and 2001 as follows:

Agent	*Modeling year*	*Time basis (h)*	*Dosage (mg min m^{-3})*
GB	1996–97	72	0.013 0
GB	2000–01	24	0.043 2
GF	1996–97	72	0.013 0
GF	2000–01	24	0.014 4
HD	1996–97	72	0.432
HD	2000–01	24	0.288

Following the events of September 11, 2001, in the United States, concern with acts of terror have been heightened and resulted in President George W. Bush's declaration of war on terrorism. The President's Commission concluded that water supplies to US communities are potentially vulnerable to terrorist attack, especially if contaminants were inserted at critical points in the system. Contamination of water supplies goes back to antiquity and continues until today. In the United States and in areas around the world where US troops are deployed, concern for ensuing safety of water supplies can be traced back to at least the 1980s when Triservice Standards for some chemical warfare agents were established. These are presented in the following table:

Triservice Standards for military drinking water ($\mu g\,l^{-1}$)

Agent	*2 l day^{-1}*[a]	*5 l day^{-1}*	*15 l day^{-1}*
GA	175.0	70.0	22.5
GB	34.5	13.8	4.6
GD	15.0	6.0	2.0
VX	18.7	7.5	2.5
HD	350.0	140.0	47.0
BZ	17.5	7.0	2.2

[a]Civilian consuming 2 l of water per day.

These are guidelines established by the National Research Council and based on Triservice Standards developed by the US Army in collaboration with Lawrence Livermore Laboratory. These levels are those that should not cause acute adverse health effects or degrade military performance following ingestion for 7 days of 5 or 15 l of water per day. The 5 l day^{-1} consumption is considered the average for a soldier under normal working conditions, while under stress and exertion, consumption may rise to 15 l day^{-1}. The average daily drinking water for civilians is considered to be 2 l.

Animal Toxicity

Small doses of nerve agents can produce tolerance.

The cause of death is attributed to anoxia resulting from a combination of central respiratory paralysis, severe bronchoconstriction, and weakness or paralysis of the accessory muscles for respiration.

Signs of nerve agent toxicity vary in rapidity of onset and severity. These are dependent on the specific agent, route of exposure, and dose or concentration. At the higher doses or concentrations, convulsions, apnea, and neuropathies are indications of CNS toxicity. Following nerve agent exposure, animals exhibit hypothermia resulting from the cholinergic activation of the hypothalamic thermoregulatory center. In addition, plasma levels of pituitary, gonadal, thyroid, and adrenal hormones are increased during OP intoxication. The nerve agents are anticholinesterases and as such inhibit the cholinesterase enzymes in the tissues resulting in the accumulation of acetylcholine at its various sites of action in both the autonomic nervous system and the CNS. These include the endings of the parasympathetic nerves to the smooth muscles of the iris, ciliary body, bronchial tree, gastrointestinal tract, bladder, blood vessels, the secretory glands of the respiratory tract, the cardiac muscles, and the endings of sympathetic nerves to the sweat glands. Accumulation of acetylcholine at these sites results in characteristic muscarinic signs and symptoms, while the accumulation at the endings of the motor nerves to voluntary muscles and in the autonomic ganglia results in nicotinic signs and symptoms. The accumulation of acetylcholine in the brain and spinal cord results in the characteristic CNS signs and symptoms.

Nerve agents inhibit the activity of acetylcholinesterase by attaching to its active sites so that it cannot hydrolyze the neurotransmitter acetylcholine into choline, acetic acid, and regenerated enzyme. Thus, acetylcholine cannot attach to the enzyme, is not hydrolyzed, and continues to produce action potentials until the mechanism is fatigued. The biological effects of the nerve agents result from the excess of acetylcholine.

Animal toxicity values for nerve agents are listed as follows:

Agent	LCt_{50} (mg min m^{-3}) inhalation	LD_{50} (mg kg^{-1})			
		SC[a]	IV[b]	IP[c]	PC[d] (mg kg^{-1})
Rat					
GA	450	300	66	490	12.6
GB	220	103	39	218	2.5
GD	230	71	44.5	98	14.3
GF	180		5.3	400	1.8
VX	17	12	7.9		0.1
Rabbit					
GA	960	375	63		2.5
GB	120	30	15	275	4.4
GD	160	16	11		1.54
GF		63	15	550	0.3
VX	25	14	8.4	66	0.025
Dog					
GA	135		84		30
GB	60		19		10.8
GD		12	5		4.9
GF					
VX	15		63		0.054
Monkey					
GA	187		50		9.3
GB	74		22.3		
GD		13			
GF	130				
VX	50		8.4		0.065

[a]Subcutaneous.
[b]Intravenous.
[c]Intraperitoneal.
[d]Percutaneous (depilated).

Although there is a lack of information on the general toxicological effects of low-level, and sublethal repeated exposures, there are studies on the behavioral effects of such exposures to nerve agents in animals free of observed signs of intoxication. These were conducted in an effort to determine whether behavioral studies can provide markers of early neurotoxicity that are more sensitive than neurochemical and neuropathological changes.

Although blood cholinesterase activity correlations with nerve agent effects are equivocal, they are indicative of exposure but do not reflect changes in the CNS. In rodents repeated subcutaneous injections of GD over a 5 day period at doses less than one-half the LD_{50} caused a significant decline in cholinesterase activity in all regions of the brain examined. The regional sensitivities were in agreement with the studies employing acute high-level soman exposures. In all cases, the neostriatum was the area of the brain least sensitive to nerve agents. These results are consistent with those of GD, GB, and GA at doses of 30%, 40%, and up to 85% of the LD_{50}. No evidence of tolerance to the direct inhibitory effects of GD during 5 days of repeated injections was observed. However, a tolerance to GD-induced hypothermia was reported. GD at 35 mg kg^{-1} injected subcutaneously up to 36 days at regular intervals reduced body temperature after the third injection, and then a steady tolerance developed to the drop in body temperature even though brain cholinesterase levels were inhibited. Brain cholinesterase levels did not parallel the recovery of serum cholinesterase following cessation of GD injection. Red blood cell cholinesterase recovery more closely reflected brain cholinesterase recovery than did serum cholinesterase. Daily doses of 25–54 mg kg^{-1} of GD for 5 days with survival times of 7–35 days were consistent with previous studies in that the area of the brain most sensitive to nerve agents was the piriform cortex and the least sensitive areas were the hypothalamus and neostriatum. This was demonstrated in both neurochemical and neuropathological studies. GD-induced brain damage was similar in severity and locus whether administration was single or in repeated doses. However, the progression of brain degeneration following repeated dosing was more protracted. In rodents and nonhuman primates, the performance dose response was very steep, indicating that small changes in dose caused a large change in performance. Pretreatment plus use of antidote drugs was ineffective in preventing soman-induced performance decrements.

Clinical Management

Following exposure the victim should be removed from the area to avoid further contamination and decontaminated (water/hypochlorite) by adequately protected (protective clothing and gas mask) and trained attendants. Contaminated clothing should be removed carefully so as to avoid further contamination. Respiration should be maintained and drug and supportive therapy instituted. If exposure is anticipated, pretreatment with carbamates (pyridostigmine bromide) may protect the cholinesterase enzymes before GD and possibly GA exposures, but not for GB and VX exposures. The three types of therapeutic drugs to be administered following nerve agent exposure are (1) a cholinergic blocker, anticholinergic or cholinolytic drug such as atropine; (2) a reactivator drug to reactivate the inhibited enzyme, such as the oxime pralidoxime chloride; and (3) an anticonvulsant drug such as diazepam or benzodiazepine. Oxygen may be indicated in respiratory failure.

Miosis, pain, dim vision, and nausea may be relieved by topical atropine in the eye. Atropine, a cholinergic blocker or anticholinergic drug, is effective in blocking the effects of excess acetylcholine at peripheral muscarinic sites. The usual dose is 2 mg for minor exposures and 6 mg for severe exposures.

The dose should be repeated at 3–5 min until there is improvement. Pralidoxime chloride (protopam chloride) is an oxime used to break the agent–enzyme bond and restore the normal activity of the enzyme. This is most apparent in organs with nicotinic receptors. Abnormal activity decreases and normal strength returns to skeletal muscles, but no decrease in secretions is seen following the oxime treatment. The usual dose is 1000 mg (intravenous or intramuscular). The injection contains 600 mg, which is not a high enough dose for a severe exposure. In a severe exposure, three of these (1800 mg) would be given. This is not an item generally used by civilians. The administration of oxime may be repeated two or three times at hourly intervals either by intravenous or by intramuscular injections. Diazepam, an anticonvulsant drug, is used to decrease convulsive activity and reduce brain damage that may occur from prolonged seizure activity. It is suggested that all three of these drugs be administered at the onset of severe effects from nerve agent exposure, whether or not seizures occur. The usual dose of diazepam is 10 mg.

Pyridostigmine bromide is available as a pretreatment for GD and possible GA exposures, but not for GB and VX. It is available in 30 mg tablets, which should be administered every 8 h. When used prior to exposure, it should be followed by atropine and pralidoxime chloride after exposure. LD_{50} values in animals were increased several-fold and survival rates were also increased in experiments with GD and these therapies.

Supportive therapy may include ventilation via an endotracheal airway if possible and suctioning of excess secretions from the airways.

See also: G-Series Nerve Agents; Organophosphates; Sarin; Soman; Tabun; V-Series Nerve Agents: Other than VX; VX.

Relevant Websites

http://www.bt.cdc.gov – (US) Centers for Disease Control and Prevention, Agency for Toxic Substances and Disease Registry, Chemical Agents.

http://sis.nlm.nih.gov – (US) National Library of Medicine, Specialized Information Services, Chemical Warfare Agents.

Neurotoxicity

Peter S Spencer and Pamela J Lein

Introduction

Neurotoxicity refers to the direct or indirect effect of chemicals that disrupt the nervous system of humans or animals. Numerous chemicals can produce neurotoxic disease in humans, and many more are used as experimental tools to disturb or damage the nervous system of animals. Some act directly on neural cells, others interfere with metabolic processes on which the nervous system is especially dependent. Some only disrupt neural function; others induce maldevelopment or damage to the adult nervous system. Perturbations may appear and disappear rapidly, evolve slowly over days or weeks and regress over months or years, or have permanence if they are acquired during development. Neurotoxicity is usually self-limiting after exposure ceases and rarely progressive in the absence of continued exposure. Links between chemical exposure and long-latency neurodegenerative diseases are suspected but rarely proved.

Occurrence

Chemicals with the potential to disrupt the mammalian nervous system may occur naturally (neurotoxins) or arise by synthesis (neurotoxicants). While chemicals with neurotoxic potential are conveniently termed 'neurotoxins' or 'neurotoxicants', this is not an intrinsic property but rather the description of an effect that may occur if the tissue concentration exceeds a certain threshold. Biological chemicals with neurotoxic properties often have high target specificity and toxic potency, discrete biological actions, and are among the best understood mechanistically. Examples of chemicals with direct or indirect neurotoxic potential are found in bacteria, algae, fungi, plants, coelenterates, insects, arachnids, mollusks, amphibians, reptiles, fish, and certain mammals (**Table 1**).

Other less potent naturally occurring substances exhibit neurotoxic effects when encountered in large concentration for sufficient periods of time. Examples include metals (arsenic, lead, mercury) and certain compounds containing these elements (methylmercury) (**Table 2**). Some elements (manganese, selenium) and compounds (vitamin B_6) in this group, while neurotoxic in sustained heavy doses, are required in smaller amounts to support normal physiological function, including that of the nervous system.

Natural substances (thiaminase) that interfere with required substances (thiamine) are also associated with neurological disease in animals and humans.

Synthetic chemicals with neurotoxic potential (**Table 2**) are most commonly encountered in the form of prescription (ethambutol, isoniazid, vincristine) and over-the-counter pharmaceutical agents (bismuth preparations), domestic products used in antidandruff shampoos (pyridinethione), fragrance raw materials (2,6-dinitro-3-methoxy-4-*t*-butyltoluene), pyrolysis products in broiled, baked, or fried food (acrylamide), beverages (ethanol), workplace chemicals (*n*-hexane), pest-control agents (aldrin), environmental pollutants (mercury), and substances (methamphetamine) used to induce euphoria. Others are associated with special applications, such as chemical warfare in military and civilian settings (sarin).

Table 1 Naturally occurring substances with mammalian neurotoxic potential

Life form	*Substance with neurotoxic potential*
Bacterium	Diphtheria toxin
Alga	Anatoxin-a
Fungus	3-Nitropropionic acid
Plant	β-*N*-Oxalylamino-L-alanine
Coelenterate	Palytoxin
Insect	Apamin
Arachnid	Scorpion toxins
Mollusc	Conotoxins
Fish	Ciguatoxin
Amphibia	Batrachotoxin
Reptile	Dendrotoxin
Bird	Batrachotoxin
Mammal	Vitamin A

Direct-acting substances with neurotoxic potential are supplemented by other agents that initiate neurological change as a consequence of effects on another organ system on which the brain depends for normal function. Substances that target the liver, kidneys, or lungs fall into this category, as do agents that interfere with the continuous supply of oxygen (cyanide, azide) and glucose (6-chloro-6-deoxyglucose) required by the nervous system for normal function. Chronic liver failure and manganese intoxication are both associated with high signal abnormalities in the basal ganglia on T1-weighted

Table 2 Heavy metals and synthetic substances with neurotoxic potential

Substance	*Primary neurotoxic effects*
Acrylamide	Peripheral neuropathy (axonal degeneration); cerebellar ataxia
Arsenic	Acute encephalopathy (brain swelling and hemorrhage); peripheral neuropathy (axonal degeneration)
Barbiturates	Acute encephalopathy (sedation and coma), chronic encephalopathy, developmental neurotoxicity; facilitated GABA neurotransmission
Carbamate pesticides	Acute encephalopathy (cholinergic syndrome); neuromuscular transmission dysfunction; acetylcholinesterase inhibition
Carbon disulfide	Acute psychosis; chronic peripheral neuropathy (axonal degeneration); parkinsonism
Carbon monoxide	Encephalopathy/delayed parkinsonism; neuronal and tissue necrosis secondary to hypoxia
Carbon tetrachloride	Acute encephalopathy, visual dysfunction
Doxorubicin	Progressive ataxia (rodents); sensory neuronal degeneration
Ethanol	Fetal alcohol syndrome; acute encephalopathy (agitation, sedation, ataxia, coma); chronic encephalopathy (cognitive impairment, dementia); myopathy; peripheral neuropathy (vitamin B_1 deficiency?)
n-Hexane	Peripheral neuropathy (axonal degeneration)
Lead, inorganic	Peripheral neuropathy (axonal loss and demyelination); acute encephalopathy (seizures); cognitive dysfunction
Manganese, inorganic	Emotional disturbance, psychoses; parkinsonism/dystonia; neuronal degeneration in striatum and globus pallidus
Mercury, inorganic	Cerebellar syndrome (tremor, ataxia); psychobiological reaction (anxiety, personality changes, memory loss)
Methanol	Optic neuropathy (axonal degeneration, primary demyelination); extrapyramidal syndrome (necrosis of putamen); retinopathy (edema)
Methyl mercury	Developmental toxicity and teratogenesis; visual dysfunction (tunnel vision); cerebellar syndrome (ataxia); peripheral neuropathy; chronic encephalopathy (cognitive dysfunction)
Organophosphorus compounds (pesticides and warfare agents)	Cholinergic syndrome (certain compounds); peripheral neuropathy (certain compounds only); acetylcholinesterase inhibition
Phenytoin	Fetal phenytoin syndrome; cerebellar syndrome (ataxia, nystagmus); chronic encephalopathy (cognitive dysfunction); extrapyramidal syndrome (chorea, dyskinesia); peripheral neuropathy
Toluene	Acute encephalopathy (sedation, coma); chronic encephalopathy (cognitive dysfunction)
Tricyclic antidepressants	Seizure disorder (myoclonus); psychobiological reaction (serotonin syndrome, anticholinergic syndrome); tremor; extrapyramidal syndrome (dyskinesia)
Trimethyltin	Acute encephalopathy (neuronal degeneration of limbic system) – rodents; chronic encephalopathy (cognitive dysfunction, neuronal loss in hippocampus)

magnetic resonance images, suggesting that the metal accumulates because it cannot be cleared normally by the liver.

Neurotoxic Effects

The nervous system has a vast repertoire of functional reactions to chemical perturbation, and these responses give rise in the aggregate to a plethora of neurological and psychiatric phenomena, many of which recapitulate the clinical manifestations of disease of nontoxic origins. Large single doses of certain substances such as organic solvents (ethanol, toluene) induce functional changes in the organism that appear and disappear rapidly. Other agents, such as the anticholinesterase nerve agents, induce functional changes that reverse when the inhibited target protein is reactivated or replaced. Sometimes, as in the case of methanol, the latent period (hours) between exposure and effect is associated with the production and action of a toxic metabolite (formate). Single exposures to large amounts of other agents (arsenic, mercury, thallium) may be followed by a latent period of days or weeks before structural and functional changes become clinically evident. While certain substances (acrylamide) can induce neurological damage after single large exposures, smaller doses over a long period of time are also effective. The pattern of neurological deficit may be distinct in the two dosing scenarios. Neurotoxic disorders typically progress during the period of exposure and immediately following exposure, when the pathological events already in progress may take time to unfold before stabilization or recovery can begin. Prospects for functional recovery depend on the presence or absence of tissue damage, the extent of damage, and whether the central nervous system (CNS) is involved (poor prognosis).

An unanswered question is whether in some instances disease may progress or recur after chemical exposure has ceased. Certainly, catastrophic and fatal neurodegeneration may follow acute carbon monoxide poisoning, but this is an isolated example. Relapses occur in ciguatoxicity presumably because the offending agent is released from fat stores under certain physiological conditions. Progressive visual defects may occur from release of chloroquine stored in the choroid layer of the eye. There is also concern that certain substances (carbon disulfide) might predispose or accelerate the onset of age-related diseases of the nervous system, such as Parkinson's disease. Finally, research is underway to determine whether DNA-damaging agents (cycasin) may predispose neurons to tardive degeneration because of their low capacity for DNA repair.

Principles of Nervous System Vulnerability

There are many factors that determine the response of the nervous system to chemical exposure. Species, gender, genotype, age, and nutritional status are key determinants, as are chemical access, structure, and biotransformation. Metabolic activity of the brain may serve to activate a neurotoxic substance, as in the conversion of methylphenyltetrahydropyridine (MPTP) to the mitochondrial toxin *N*-methyl-4-phenylpyridinium ion (MPP+), which targets substantia nigral neurons and precipitates parkinsonism in humans and other mammals. Hepatic biotransformation is broadly concerned with the conversion of lipophilic chemicals to less toxic hydrophilic metabolites (phase 1) and their conjugation (phase 2) prior to excretion. Occasionally, phase 1 metabolism may increase the neurotoxic potential of an exogenous substance, as in the case of the stepwise conversion of *n*-hexane to 2,5-hexanedione, an agent that targets neuroproteins and causes nerve fiber degeneration in the CNS and peripheral nervous system (PNS). Coexposure to substances such as methyl ethyl ketone or toluene may impact phase 1 enzyme function and thereby markedly modify the quantitative neurotoxic response to *n*-hexane.

Most mammalian species reproduce the qualitative response of the human nervous system to chemical perturbation, but the sensitivity may differ markedly among species. For example, relative to humans, primates and cats, rodents are relatively refractory to organophosphate-induced axonal degeneration. Gender as a determinant of biotransformation may be less important in humans than in rats, where marked differences in the metabolism and consequent functional responses to individual chemicals such as parathion may be noted. Differences in genotype may determine the presence of neurotoxic responses, as in the rare mitochondrial polymorphism that results in hypersensitivity to aminoglycoside-induced deafness. Age in humans is generally associated with increased susceptibility to many chemical substances largely because of reductions in biotransformation, renal clearance, and biliary excretion, as well as factors such as reduced body weight, inanition, and polypharmacy that promotes drug and chemical interactions. In addition, neurons display age-related increases of DNA damage, regional nerve cell loss (e.g., substantia nigra), and axonal pathology (e.g., spinal roots). Nutritional status may predispose to human neurotoxicity as seen in minimally nourished Africans who develop spastic paraparesis from a combination of sulfur amino acid deficiency and exposure to cyanogenic substances, both of

which arise from dietary dependence on the root crop cassava (*Manihot esculenta*). Agents that interfere with vitamin production or utilization, including thiamine (pyrithiamine), riboflavin (quinacrine), niacin (3-acetylpyridine, 6-aminonicotinamide) or cyanocobalamin (nitrous oxide) produce various types of severe neurological deficit, as does excessive exposure to vitamin A. Other types of neurodegeneration are seen with substances that chelate physiologically important metals ions (pyridinethione, 8-hydroxyquinolines, ethambutol), such as zinc. Excessive dietary intake of sulfur and selenium produce neuronal lesions in ruminants and pigs, respectively.

The structure of chemicals and their differential access to the nervous system are of critical importance in determining the presence and nature of the neurotoxic response. While access to nervous tissue dictates the possibility of a direct neurotoxic effect, neurotoxicity ultimately depends on the ability of the substance to bind to neural tissue targets and interfere with functional or structural integrity. Structure–activity relationships are therefore of cardinal importance. For example, 1,2-diacetylbenzene but not 1,3-diacetylbenzene induces leg weakness because only the former binds to and crosslinks neuroproteins. Triethyltin targets the myelin sheath, trimethyltin damages neurons, but tributyltin lacks neurotoxic properties – another illustration of the critical importance of chemical structure in determining the presence and nature of the neurotoxic response.

The large majority of the nervous system is protected from direct exposure to chemicals in the bloodstream and the cerebrospinal fluid (CSF) by blood–brain/nerve/CSF barriers. These barriers separate the microenvironment of the brain parenchyma from changes in circulating ion and metabolite concentrations. Regulation of blood–brain/nerve interchange is a key function of capillaries coursing through nervous tissue. Structural specializations of capillary walls in the form of tight junctions between adjacent endothelial cells constitute a diffusion barrier. This allows the endothelium to regulate the selective transport and metabolism of substances from blood to brain and vice versa. While gases (oxygen, carbon monoxide, nitrous oxide) cross capillary walls with ease, many substances are excluded or their access to nervous tissue impeded by the presence of the capillary barrier. Lipophilicity and size are key elements in regulating the passage of macromolecules across the blood–brain barrier. Key nutrients and macromolecules required by the brain cross via facilitated diffusion or specific carriers. The epithelial junctions that constitute the blood-CSF barrier at the choroid plexus are somewhat more permeable and allow greater passage of drugs and toxicants into the interstitial fluid that bathes brain tissue. Many metals are required for normal CNS function and are thus transported across the blood–brain and blood-CSF barriers. However, excess metal may accumulate in their endothelial cells and give rise to toxic damage of the cellular barrier. Several metals are known to accumulate in both barriers, including substances with neurotoxic potential such as lead, mercury, arsenic, and manganese. Lead accumulates in the choroid plexus and alters key functions, including transthyretin, the binding protein for thyroid hormone. High concentrations of lead damage the blood–brain barrier, cause vascular leakage, and may result in brain swelling accompanied by herniation, ventricular compression, and petechial hemorrhages.

Some regions of the brain (e.g., hypothalamus) and PNS (spinal and autonomic ganglia) lack capillary barriers and are thus directly exposed to chemicals circulating in the bloodstream. Damage to the hypothalamus can have far reaching effects on somatic metabolism, reproductive function, and growth. The adult obesity of rats treated postnatally with monosodium glutamate exemplifies the effect and raises important questions for human health in regard to past exposure to glutamate-rich foods during postnatal development. In the PNS, the selective loss of sensory neurons in rats treated with doxorubicin arises because spinal ganglia lack a protective capillary barrier.

Certain other substances (tetanus toxin) reach nerve cells directly via distal axonal entry. Tetanus toxin is transported to the spinal anterior horn cell, subsequently translocates and binds to presynaptic inhibitory (glycinergic) nerve terminals impinging on the motor nerve cell, and thereby suppresses the inhibition of motor neuron activity leading to hyperexcitation. Violent and sustained muscle contraction (tetany) results in response to external stimulation. Another example of peripheral entry to the CNS is the transport and delivery of metals (manganese, aluminum) from the nose along olfactory neurons to the brain of laboratory animals.

The mammalian nervous system has functional design features that predispose it to chemical perturbation. Consequently, neurological dysfunction is among the most common of the toxic responses of humans to chemical substances. Some neurotoxic agents perturb energy generation by interfering with glycolysis (arsenic) while others (3-nitropropionic acid, cyanide) disrupt sites in the electron transport chain. Some agents (metronidazole, misonidazole) damage brain regions such as the brainstem nuclei that seem to have a high requirement for glucose.

Architectural design is another important determinant of vulnerability both at the cellular and organ level. Unlike most organs (liver, kidney, testes), the nervous system is regionally organized for functions such as memory, vision, audition, and olfaction. Whereas small lesions of the liver and kidney have little functional impact, a chemical that damages the hippocampus (domoic acid), visual cortex (methylmercury), or the peripheral vestibular and auditory system (streptomycin) may have devastating effects on the function of the organism. Moreover, whereas tissue repair follows damage to many organs, the CNS recovers from injury poorly. Some recovery may be afforded by reorganization of synaptic connections on surviving nerve cells, but regrowth and reconnection of damaged axons is impeded by postinjury proliferation of astrocytes.

Cellular architecture is yet another important factor in determining vulnerability to chemical attack. Unlike most types of mammalian cells, mature neurons and myelinating cells in the CNS (oligodendrocytes) and PNS (Schwann cells) possess elongated cellular processes that expose vast surface areas of membrane to chemicals present in extracellular fluid (**Figure 1**). Additionally, each of these cells has a segregated anabolic region (cell body) that is responsible for supplying the metabolic needs of proportionately huge volumes of cytoplasm (axons, dendrites, myelin). The unusual architecture of these cells demands the presence of a distribution system that efficiently transports materials from sites of production to sites of utilization. Within neurons, chemicals that disrupt axonal transport are known to induce axonal degeneration with consequent effects on sensory and motor function. The special vulnerability of the longest and largest-diameter axons leads clinically to sensory dysfunction and motor weakness in a stocking-and-glove distribution.

Neural cells are highly interconnected and dependent upon each other's presence and physiological activity for normal function. Thus, chemicals that disrupt Schwann cells (diphtheria toxin) or myelin (hexachlorophene) secondarily disturb nerve conduction along the axonal processes of neurons with which they are physically associated. Other agents interfere directly with electrical transmission (pyrethroids) or the orderly conveyance of signals via synapses (certain organophosphates) that connect nerve cells to each other. Similarly, agents (nitrofurantoin) that cause peripheral axons to degenerate thereby severe neuronal connections with muscle cells and cause muscle weakness.

Given the nervous system has built-in redundancy, the loss of neurons or axons must exceed a certain threshold for clinical effects to become apparent. That redundancy of nerve cells may be substantially reduced with the advance of age, such that regional loss of CNS striatal dopaminergic neurons is thought to be a factor in dictating susceptibility to agents (carbon disulfide) that can trigger parkinsonism. Similarly, age-related spinal nerve root demyelination and distal axonal degeneration of long and

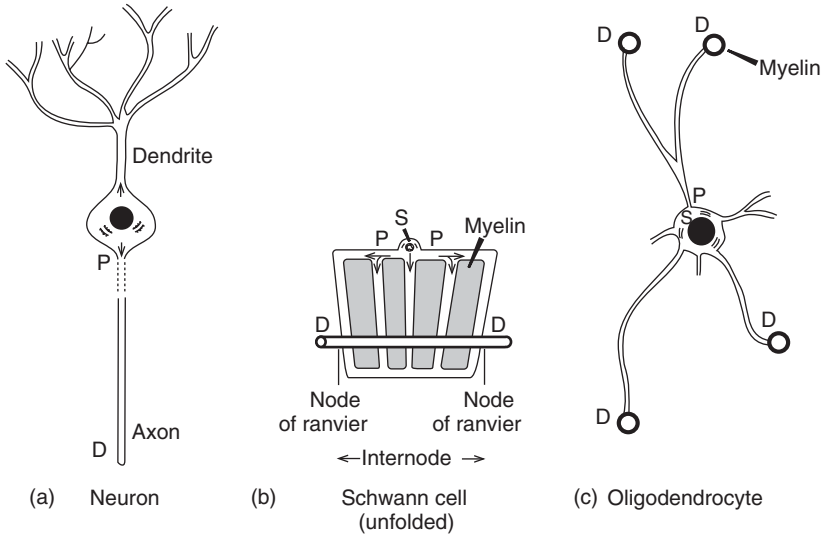


Figure 1 Diagram of (a) neurons, (b) Schwann cell with myelin sheath 'unrolled', and (c) oligodendrocyte, to illustrate that each cell has a restricted cell soma (S) and elongated processes that can be divided into proximal (P) and distal (D) portions. The elongated processes of these cells provide a huge area for chemical attack. (From Spencer PS and Schaumburg H (eds.) (2000) *Experimental and Clinical Neurotoxicology*, 2nd edn. New York: Oxford University Press; © Oxford University Press, Inc. Used by permission of Oxford University Press, Inc.)

large-diameter axons may predispose elderly individuals to substances that cause peripheral neuropathy (*vide infra*).

Vulnerability of Neurons and Their Processes

Nerve cells are vulnerable to chemical attack at many loci, including protein synthesis, mitochondrial and nucleic acid function. Somal DNA is predisposed to damage by reactive oxygen species because of the high oxygen consumption and metabolic activity of neurons. The cycad genotoxin methylazoxymethanol (MAM), an alkylating agent that promotes DNA adduct formation, interferes with neuronal development and has been implicated in a long-latency progressive neurodegenerative disease. Repair of DNA damage in neurons is poor relative to that in astrocytes, such that long-term neuronal effects might occur as a consequence of DNA damage. Mitochondrial DNA chain growth is blocked by certain anti-HIV drugs (2′,3′-dideoxyinosine, 2′,3′-dideoxycytidine) that cause painful axonal neuropathy. Interference with neuronal protein synthesis occurs with agents (ricin) that disrupt polypeptide elongation and trigger axonal degeneration. Chemicals that alter the mitochondrial electron transport chain (cyanide) have a propensity to induce basal ganglia damage. Substances (bromethelin, pentachlorophenol) that uncouple electron transport and oxidative phosphorylation elevate temperature and induce tremor and hyperexcitability. Several neuronal enzymes are key sites of chemical attack, including synaptic acetylcholinesterase (carbamates) and neuropathy target esterase (organophosphates), with resulting neuroexcitation and axonal degeneration, respectively. Lead interferes with oxidative phosphorylation by potently activating protein kinase C.

The excitable membrane of neurons is the target of a rich array of neurotoxins that interfere with ion channels required for the proper functioning of neurons, axons, muscle, and glial cells. These agents are often complex structures generated by invertebrate species and plants presumably for purposes of chemical defense. Sodium ion channels are common targets. Agents that bind to the outer surface of the Na^+ channel and prevent ion influx are found in dinoflagellates (saxitoxin); vertebrates, including fish, octopus and salamander (tetrodotoxin); and cone snails (geographutoxin). Lipid-soluble anesthetics (lidocaine, procaine) bind to a hydrophobic site in the channel and interfere with the gating mechanism. Activation of Na^+ channels by opening or impeding normal closure is seen with certain plant chemicals (grayanotoxin; pyrethrin), dinoflagellate chemicals (ciguatoxin) stored in fish, amphibian skin toxins (batrachotoxin), scorpion toxins, and synthetic pesticides (pyrethroids). Interference with Na^+ channel function is usually heralded by abnormal sensation (paresthesias) in the tongue, around the mouth, and in the extremities. While usually transient, ciguatoxin is a fat-soluble agent that may cause repeated neurotoxic events after single exposures presumably because of sequestration and periodic release within the affected subject. K^+-channel toxins with selective blocking actions have been identified in the venom of certain scorpions, bees, and snakes. Thallium and bromine ions are transported by K^+ and Cl^- channels, respectively, with significant neurological and psychiatric consequences for the affected subject. Calcium channel blockers are produced by certain plants, insects, spiders, snails, and snakes. Divalent lead ions interfere with intracellular processes regulated by Ca^{2+} ions and accumulate in the same intra-mitochondrial compartment as calcium.

Neurotransmitter Systems

Numerous substances target mechanisms involved in neurotransmitter (NT) synthesis, transport, synaptic release, re-uptake, the interaction between NT and postsynaptic receptor, or the removal of NT from the synaptic gap. Neurotoxicity occurs when the agent reduces or increases NT release, alters NT concentration or resident time, or acts as an agonist or antagonist at a postsynaptic receptor.

Acetylcholine synapses at neuromuscular junctions are targets for a number of biologic and synthetic substances (**Figure 2**). Neurotransmission is disrupted by agents that interfere with choline transport (hemicholinium, choline), with acetylcholine synthesis (triethylcholine), or synaptic vesicle uptake (vesamicol). Other agents (β-bungarotoxin, crotoxin) target mechanisms involved in presynaptic NT release. Botulism arises from the action of a zinc-endopeptidase (the light chain of the botulinum toxin dipeptide) in blocking synaptic transmission by cleaving synaptic-vesicle fusion proteins required for NT exocytosis. Botulinum-induced blockade of normal depolarization-induced NT release at the neuromuscular junction leads to flaccid muscle weakness. Conversely, α-latrotoxin in venom of the black widow spider causes massive NT release at the vertebrate neuromuscular junction resulting in a painful disorder featured by dysarthria, tremor, clonic muscle contraction, and paralysis. Numerous chemicals interfere with acetylcholinesterase, the enzyme that terminates NT action at the neuromuscular junction and other synapses within the CNS and PNS. Certain

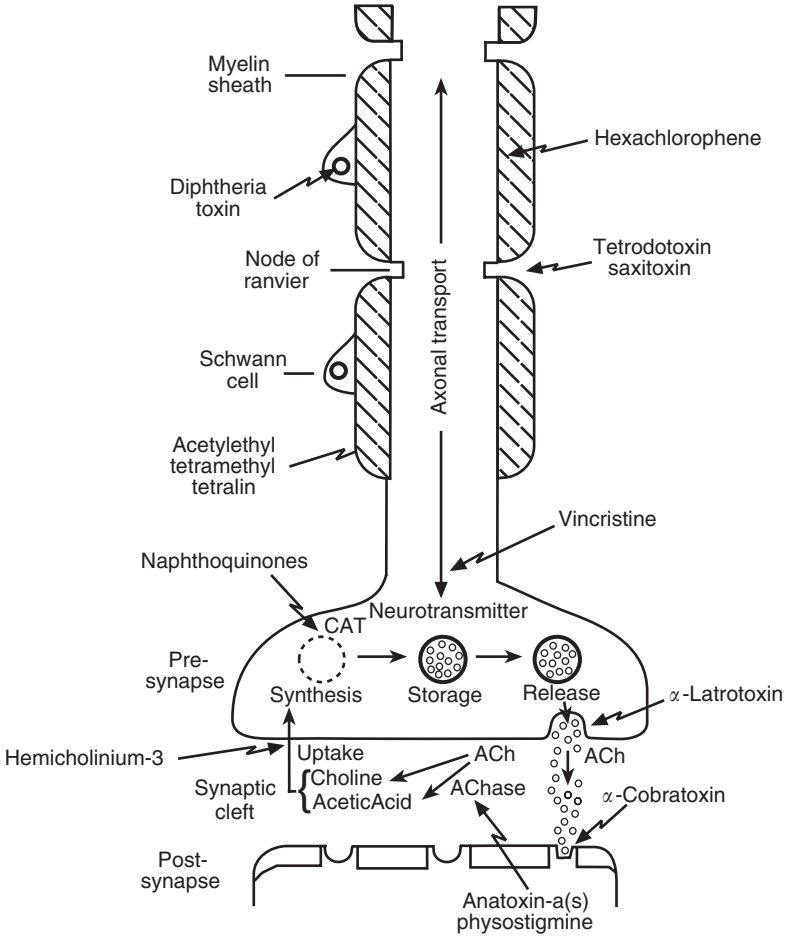


Figure 2 Targets of neurotoxic agents acting on PNS cholinergic nerve fibers (upper portion), terminals (lower midportion) and neuromuscular synapses (lowest portion). Ach, acetylcholine; AChase, acetylcholinesterase; CAT, choline acetyltransferase. (From Spencer PS and Schaumburg H (eds.) (2000) *Experimental and Clinical Neurotoxicology*, 2nd edn. New York: Oxford University Press; © Oxford University Press, Inc. Used by permission of Oxford University Press, Inc.)

anticholinesterases (edrophonium) bind directly to the active center of the enzyme and act rather briefly; others (physostigmine) carbamylate the enzyme and have long-lasting actions. The large class of organophosphates, which include high-potency agents used in chemical warfare and less hazardous materials employed as agricultural pesticides, interact with the anionic and/or esteratic sites in the active center of acetylcholinesterase to form complexes; the stability of the phosphorylated enzyme is further enhanced by the loss of one of the alkyl groups, a phenomenon known as (chemical) aging. Biological anticholinesterases include the product of a cyanobacterium (anatoxin-a(s)) and certain snake toxins (fasciculins).

Acetylcholine receptors provide another target for chemicals with neurotoxic potential; most of these act as antagonists. D-Tubocurarine is the classical nicotinic receptor antagonist, and curare-like substances are found in elapid and hydrophid snakes (α-neurotoxins) such as cobra (α-cobratoxin) and krait (α-bungarotoxin), mollusks (α-conotoxin), corals (lophotoxin), and certain plants such as delphinium (methyllcaconitine). Anatoxin-a is a potent agonist at neuromuscular, autonomic, and brain nicotinic receptors. Muscarinic receptor antagonists include atropine, scopolamine, and the synthetic warfare agent quinuclidinyl benzilate, which induces dryness of the mouth, blurred vision, confusion, delirium, and coma.

Glutamate receptors, which mediate most excitatory synaptic traffic in the CNS, are another important target of chemical substances linked to human disease. Fast synaptic transmission is mediated by DL-α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-type glutamate receptors, a target of the grass pea AMPA agonist *β-N*-oxalylamino-L-alanine that causes pyramidal tract degeneration and paraparesis (lathyrism) in heavy consumers. Seaweed (kainic acid) and dinoflagellate (domoic acid) toxins act as agonists at the kainate subclass of glutamate receptors, the latter causing significant CNS neuronal

excitotoxicity and CNS degeneration in humans. Glutamate receptors that respond to *N*-methyl-D-aspartate (NMDA) have numerous antagonists, including the anesthetic agent ketamine and phencyclidine (angel dust). Termination of excitatory neurotransmission by removal of synaptic glutamate is perturbed by dithiocarbamate pesticides. Ionotropic glutamate receptors also play an important role in mediating neuronal death secondary to energy deficits induced by hypoxia.

Gamma-aminobutyric acid (GABA), which is synthesized by glutamic acid decarboxlase (GAD), is the major inhibitory NT in the mammalian brain. GAD inhibition (2-amino-4-pentenoic acid) induces convulsions. Numerous compounds bind to and act as antagonists (picrotoxin) or agonists (muscimol) at $GABA_A$ receptors, which employ Cl^- channels to mediate fast inhibitory postsynaptic potentials. Enhanced GABAergic transmission occurs under the action of benzodiazepine and barbiturate drugs, while organochlorine insecticides (lindane, aldrin) exert convulsant effects mediated through picrotoxin-binding sites.

Glycine receptors, which mediate inhibitory neurotransmission in the brainstem and spinal cord, have a number of plant-derived antagonists (strychnine, hydrastine) that elicit hyperreflexia and tetanic muscle contraction among other signs. Tetanus toxin from *Clostridium tetanii* triggers generalized muscle rigidity by binding to glycinergic nerve terminals (and inhibiting glycine NT release) after crossing from anterior horn cells that retrogradely transport the agent from a peripheral wound site.

Several other NT systems and pathways are impacted by chemicals that exhibit neurotoxicity. Dopamine mediates communication in a number of important pathways, including the nigrostriatal tract (a key component of basal ganglia mechanisms for control of the quality of motor movement) that degenerates in Parkinson's disease and MPP+-induced toxicity. Extrapyramidal movement disorders occur as a side effect of a number of therapeutic drugs acting on dopaminergic pathways. Chemicals that perturb adrenergic function may interfere with NT synthesis (α-methyltyrosine), serve as false NTs (methyl-dopa), block vesicular uptake (reserpine), inhibit the cleavage enzyme catechol-*O*-methyltransferase (pyrogallol), or promote NT release (amphetamine). Substituted amphetamines (3,4-methylenedioxymethamphetamine) damage axons derived from the dorsal raphe serotonergic projection system of rodents and primates. Natural substances in plants interfere with substance P neurotransmission (capsaicin), cannabinoid receptors (cannabis), purinoceptors, and adenosine receptors (caffeine). Caffeine intoxication is characterized by anxiety, sleep disturbance, and mood changes; while caffeine withdrawal in sensitive subjects leads to vascular headache, drowsiness, and fatigue.

Axonal Transport

Disruption of the transport of materials along axons is another method by which chemicals perturb neural function and induce changes, including focal axonal pathology and axonal degeneration (axonopathy). Some agents interfere with the anterograde transport of materials from sites of synthesis (cell body) to sites of utilization (axon and terminal) (β,β′-iminodipropionitrile); some with retrograde transport (acrylamide), others with the return of materials from nerve terminals (zinc pyridinethione). Certain plant-derived chemicals (colchicine, vinca alkaloids) bind to tubulin, inhibit microtubule-based function, and thereby block bidirectional axonal transport. Retrograde transport may also serve to ferry foreign substances (ricin, tetanospasmin, certain metals) to targets in neuronal somata.

Neuroglia and Myelin

Neuroglia include (1) ependymal cells lining the ventricles of the brain and the central canal of the spinal cord, (2) cells of the PNS (Schwann cell) and CNS (oligodendrocyte) that wrap around axons to form compacted plasma membranes (myelin) that provide electrical insulation to speed nerve conduction, (3) cells (astrocytes) that interface between nerve cells and capillaries in the CNS, regulate interstitial water content, K^+ concentration, remove and metabolize certain NT molecules, and proliferate following injury.

Ependymal cells are susceptible to agents (amosconate) present in the CSF. Astrocyte foot processes investing cerebral capillaries undergo marked swelling in water intoxication, lead encephalopathy and in hypercapnia, and after the experimental administration of 6-aminonicotinamide, isoniazid, misonidazole, or ouabain. Astrocytes increase their glycogen content in a variety of insults (methionine sulfoxamine), form intranuclear inclusion bodies in lead intoxication, and greatly increase the relative size of the nuclear compartment in hepatic encephalopathy, which is thought to be triggered by hyperammonemia.

Myelinating cells are susceptible to agents that disrupt the synthesis of myelin components, the best example of which is diphtheria toxin, which has access to peripheral nerves where it inhibits Schwann cell protein synthesis and causes primary demyelination. Oligodendrocyte demyelination can be induced experimentally by diphtheria toxin and by other

protein synthesis inhibitors such as ethidium bromide and actinomycin D. The latter induces widespread status spongiosus of white matter, with edema fluid in the periaxonal space and between myelin membranes split open at the intraperiod line. Cuprizone (biscyclohexanone oxalylhydrazone) induces oligodendrocyte degeneration with intramyelinic edema. Several other agents (cycloleucine, hexachlorophene, triethyltin) trigger reversible changes in CNS and/or PNS myelin without apparent damage or loss of the myelin-forming cells.

System Vulnerability

Developing Nervous System

The intrinsic neural factors that determine or influence response to chemicals differ in development, at maturity, and in late life. The dominant host factor influencing response to exogenous chemicals is developmental state. As a result, neurotoxic effects observed following developmental exposure differ both quantitatively and qualitatively from those seen following adult exposure. Quantitatively, the developing nervous system is generally more sensitive to neurotoxic agents as exemplified by observations that NMDA causes a greater neurotoxic response in the immature rat brain than in the adult animal. Similarly, studies of both humans and rodents indicate that intrauterine exposure to lead or to polychlorinated biphenyls (PCBs) seem to be more damaging than exposure later in life. The nature of the neurotoxic effect may also differ as a function of developmental state. For example, infants of mothers treated with the anticonvulsant drug sodium valproate may display congenital malformations, including neural tube defects, whereas neurotoxicity in the adult is manifest as tremor, a confusional state and, in rare cases, parkinsonism.

The influence of developmental state on the response to neurotoxic agents largely reflects qualitative differences between the developing and adult nervous system. Neurodevelopment is a complex process that is precisely regulated in time and space, with the basic framework of the nervous system laid down in a step-by-step process in which each step is dependent upon the proper completion of the previous one. In both humans and rodents, normal neurodevelopment begins very early in the fetus and is not complete until puberty. Active organogenesis, which occurs during the period from implantation through mid-gestation, requires the concomitant and coordinated ontogeny of cell proliferation, migration, and differentiation. During late gestation and the early neonatal period, the processes of synaptogenesis, apoptosis, and myelination are predominant. These processes continue throughout later stages of neurodevelopment, and together with elimination of extraneous synapses via axon and dendrite retraction, function to refine patterns of neuronal connectivity. There are data to suggest that errors in timing, spatial resolution, or magnitude of any of these developmental events can have clinical consequences. For example, magnetic resonance imaging studies of schizophrenia indicate excessive pruning of axons and dendrites in the cortex during late adolescence, coincident with the onset of symptoms. Importantly, substances have been identified that interfere with each of these processes in animal models and, in some instances, humans, and the outcome of such interactions ranges from death to gross structural abnormalities to subtle defects in structure or function. For example, early gestational exposure to substances such as fumonisins can produce neural tube defects; exposure to ionizing radiation produces altered brain morphology and mental retardation; exposure to high concentrations of ethyl alcohol causes mental retardation, while moderate levels of alcohol exposure can delay motor development; intrauterine exposure to cocaine causes excessive outgrowth of dendrites, and exposure of infants and children to relatively low levels of lead is linked to reduced scores on tests of cognitive development and to increased aggressive tendencies.

The single most important determinant of the pattern of damage induced by neurotoxic agents is the timing of exposure relative to ongoing ontogenetic events. There are at least four scenarios to explain how timing of exposure influences neurotoxic outcome. First, because later stages of neurodevelopment depend on successful completion of early stages, even relatively minor disturbances early in neurodevelopment may cause significantly more damage than perturbations occurring at later stages. For example, inhibition of cellular proliferation by agents such as MAM, lead, mercury or ethanol, can subsequently impact migration, differentiation, and ultimately neuronal connectivity. Second, individual neurodevelopmental events may be differentially vulnerable to a specific substance. When proliferation is actively occurring within a given region of the brain, that region is vulnerable to anti-mitotic agents, such as MAM, but when cell proliferation ceases, the brain region is more resilient to MAM. Similarly, vitamin A and other retinoic acid derivatives can cause marked and irreversible abnormalities, including anencephaly and spina bifida, when exposure occurs during gestation days 5–10 in rats. In contrast, administration of retinoic acid on gestational day 12 fails to perturb brain development. Third,

since different brain regions develop on different time lines during prenatal and postnatal life, a chemical may produce impairment in different functional domains depending on the time of exposure. Thus, in a rat model of fetal alcohol syndrome changing the time at which neonatal rats are exposed to ethanol triggered neuronal cell loss via apoptosis from different brain regions, thereby giving rise to different profiles of functional deficits. Finally, the expression and/or function of many proteins targeted by neurotoxicants can vary with development. The α subunit of the glycine receptor exists in several isoforms that are transcriptionally regulated during development. The adult isoforms of the α subunit have a higher affinity for strychnine than the neonatal isoforms; thus, the developing nervous system is less vulnerable to strychnine intoxication than the adult. Another critical example includes NTs and enzymes that metabolize NTs, such as acetylcholinesterase. In the adult nervous system, these proteins function in neurotransmission; however, during development, NTs and acetylcholinesterase act as morphogenic factors that modulate patterns of neuronal connectivity. Therefore, substances that target NT systems, such as certain pesticides (*vide supra*), may have quite different effects on the developing fetus or child compared to the adult, and this has been demonstrated in animal models. As with the adult nervous system, the dose and duration of exposure also influence the response of the developing nervous system to exogenous chemicals. Physiological differences between developing and adult organisms underlie potentially significant differences in distribution, metabolism, and excretion of neurotoxic agents.

The potential for chemical exposure to the fetus begins before conception in that prior parental exposure to toxicants can have a major impact on the developing fetus. Parental exposures threaten fetal health by either altering maternal or paternal reproductive organs directly or via release of stored neurotoxic agents from maternal tissues during pregnancy. Yusho disease is a tragic example of preconception exposure influencing fetal neurodevelopment. Women in the Japanese town of Yusho who consumed cooking oil contaminated with PCBs, up to a year prior to conception, gave birth to infants exhibiting a constellation of symptoms including dysmorphism, skin lesions, hepatic dysfunction, and cognitive abnormalities. PCBs stored in maternal tissues were mobilized during pregnancy. Similarly, lead can be mobilized from storage depots in bone during pregnancy. Once in the maternal blood supply, chemicals may diffuse across the placenta and enter the fetal circulation. Some agents (organoarsenicals) accumulate in the placenta, which shields the offspring from exposure. However, the placenta does not block small molecular weight compounds (carbon monoxide), lipophilic compounds (PCBs, ethanol, methylmercury), or compounds using active transport mechanisms (lead). Once in the fetal circulation, these agents can readily enter the developing nervous system since the blood–brain barrier is not completely developed until after birth (6 months in humans). The lack of a fully formed blood–brain barrier explains why many systemically distributed compounds, such as lead salts, which generally do not elicit brain damage in adults, cause severe encephalopathy in newborn animals and humans. Prior to keratinization of the human fetal epidermis, beginning at 20 weeks of gestation, exogenous chemicals may also diffuse from the amniotic fluid into the developing fetus. After birth, exposure to potential toxicants may occur via breastfeeding and consumption of other contaminated foodstuffs, oral contact via hand-to-mouth activity, dermal contact, or inhalation. Compared with adults, children in all postnatal developmental stages have higher rates of respiration and energy consumption per kilogram of body weight, which increases their exposure rates. In addition, the skin is highly permeable during the newborn period and several epidemics of developmental neurotoxicity have been described involving percutaneous absorption of chemicals. These include hypothyroidism from iodine in povidone iodine (Betadine) scrub solutions and myelin disorders consequent to bathing infants in hexachlorophene. The expression of phase I and II metabolic enzymes is also developmentally regulated, resulting in altered abilities of developing organisms to detoxify and excrete chemicals relative to adults. This difference may confer increased resistance when a substance must be metabolized to an active metabolite. But, more frequently, the metabolic differences manifest as a decreased capacity of children to excrete toxins as compared to adults, and thus they are more vulnerable to neurotoxic agents. The lack of functional paroxonase, the enzyme that detoxifies many organophosphates, contributes to the increased vulnerability of the developing nervous system to the neurotoxic effects of these agents.

Very many chemicals are recognized teratogens in animals; a significantly smaller subset of these is known or suspected to be developmental neurotoxicants in humans. Some of the more significant of the latter group include ethanol, which causes a constellation of effects ranging from fetal alcohol syndrome to alcohol-related neurodevelopmental disorder; maternal smoking of tobacco (fetal tobacco syndrome); excess vitamins A and D; heavy metals, particularly

inorganic and organic mercury, lead and cadmium; anticonvulsants, such as phenytoin, valproate, phenobarbital, carbamazepine and primidone; drugs of abuse, including cocaine, cannabis and mescaline; persistent aromatic hydrocarbons, especially PCBs; and both organochlorine and organophosphate pesticides.

A significant challenge in the field of developmental neurotoxicity is to identify agents with developmental neurotoxic potential in humans. Detecting effects in the human population is difficult because they may be subtle (small shifts in IQ scores, slight changes in behavior) or because neurotoxicity does not become manifest until a significant period of time after the developmental exposure. Delayed neurotoxicity may arise via two different mechanisms. One of these involves the occurrence of a toxic insult early in neurodevelopment, but with manifestation of the pathological change much later in neurodevelopment when function of the affected cells is normally activated. An agent that causes this type of delayed neurotoxicity is the food additive, monosodium glutamate (MSG). MSG causes excitotoxicity via activation of glutamate receptors, and the developing brain is more sensitive than the adult brain to the toxic effects of glutamate agonists. Developmental exposure to MSG causes excessive apoptosis of neurons in the developing hypothalamus. However, the fetal loss of these hypothalamic neurons becomes evident (as hypogonadism and infertility) only in adolescence when the neuroendocrine function of these neurons is normally activated. A second mechanism of delayed neurotoxicity involves a developmental insult in which both anatomical and/or functional effects may be masked or attenuated initially because of compensatory mechanisms or plasticity. However, these developmental perturbations predispose the individual to neural deficits following subsequent insults such as chemical exposure, disease, or aging because of decreased reserve capacity. This phenomenon has been demonstrated in both animal models and humans following developmental exposures to methylmercury.

A current goal in developmental neurotoxicity is to develop screening methods to identify agents with the potential to cause developmental neurotoxic effects in humans. However, designing a screening method that is humane, scientifically valid and mechanistically driven represents a significant scientific and technical challenge in large part because there is a paucity of information regarding the molecular mechanism(s) by which agents perturb neurodevelopmental events. However, with the application of recent advances in the cell and molecular biology of normal neurodevelopment, these gaps in the database are being addressed. For example, the balance of activity between excitatory glutamate receptors and inhibitory GABA receptors modulates neuronal apoptosis in the developing brain. Developmental exposure of rats to ethanol significantly increases the percentage of apoptotic neurons via simultaneous inhibition of excitatory glutamate receptors and activation of inhibitory GABA receptors. Ethanol disrupts neuronal migration, axon outgrowth, and synaptogenesis in cultured neurons via interference with the L1 adhesion molecule. Significantly, prenatal exposure to ethanol mimics the brain defects observed in humans with congenital mutations in the L1 adhesion molecule. What is not yet clear is whether ethanol acts similarly in the developing human brain and under what conditions of dose and timing of exposure either of these mechanisms predominates. Also unanswered is whether these mechanisms are specific to ethanol or represent generalized mechanisms by which broad categories of agents cause developmental neurotoxicity.

Adult Nervous System

Chemicals generally perturb neurological function of the adult by interfering differentially with the structure and function of specific neural pathways, circuits, and systems. Vulnerable circuits within the brain include those that modulate and affect efferent output. Most commonly affected, however, are the peripheral neurons in pathways that relay information to and from the brain.

The special senses of vision, audition, balance, gestation, and olfaction depend on neural pathways that originate in peripheral receptors and terminate in the brainstem or cerebral cortex. Afferent pathways for taste, smell, hearing, and balance employ sensory neurons in ganglia that lack a blood-nerve barrier. However, chemicals that perturb the special senses seem most commonly to interfere with the structure or function of the peripheral sensory receptors. Olfaction and gustation are subserved by cilia-bearing sensory neurons that are continuously generated from stem cells, a process of cellular replacement that is disrupted by antiproliferative drugs such as vincristine and doxorubicin. For vision, the function of retinal cells is perturbed by a large number of substances, some of which produce reversible change (cardiac glycosides and trimethadone), while others (aminophenoxyalkanes) elicit morphological damage. For retinal ganglion cells, the nerve fibers that form the optic pathway are sites of vulnerability to toxic attack. Substances that impair energy metabolism (thallium, cyanide) tend to damage proximal regions of axons projecting into the optic nerve from the papillomacular bundle, while distal axonal

degeneration, with damage to the optic tracts, is seen with drugs such as clioquinol and ethambutol. Vestibular and auditory function may be affected concurrently by agents that target receptor cells in the inner ear of rodents (2-butenenitrile, 2-pentenenitrile) and humans (streptomycin). Other potentially ototoxic substances include cisplatin, furosemide and imipramine. Noise may exacerbate the neurotoxic effects of some ototoxic agents. Disturbance of human oculomotor function may take the form of nystagmus (carbon monoxide) or opsoclonus (chlordecone), two types of abnormal eye movement. Certain neurotoxic substances produce lesions of vestibular and cochlear nuclei in rodents (6-chloro-6-deoxyglucose) and primates (1-amino-6-chloropropane).

Sensorimotor function is altered by a number of chemicals that act at different sites in the neuraxis. Most affect the axons or somata of lower motor neurons in the anterior horn of the spinal cord or primary sensory neurons in dorsal root ganglia. Some substances target the nerve cell body of sensory neurons that detect touch and vibration (methylmercury), position sense (pyridoxine) or pain (capsaicin), or of neurons that regulate cardiac muscle (doxorubicin) or voluntary muscle (domoic acid, *β*-*N*-methylamino-L-alanine) function. Others target motor nerve terminals (botulinum toxin, α-latrotoxin) or the enzyme that targets acetylcholine (anticholinesterases), both of which produce acute alterations of neurotransmission associated with reduced or enhanced synaptic transmission. Temporary disruption of electrical impulse conduction is another neurotoxic effect. Agents (pyrethroids, ciguatoxin, tetrodotoxin) that perturb electrical activity in the excitable membrane (axolemmal) of the nerve cell produce rapidly reversible sensory abnormalities (paresthesias) in the distribution of trigeminal and elongate peripheral nerves. Those substances (hexachlorophene, ethidium bromide) that attack the myelin sheath or myelinating cell precipitate focal demyelination and remyelination, with consequent disruption and restoration of nerve conduction and associated sensory-motor phenomena over a period of several weeks. Focal demyelination and remyelination may also result from exposure to chemicals that block neurofilament transport and cause focal axonal swellings proximally (1,2-diacetylbenzene, 3,4-dimethyl-2,5-hexanedione) or distally (2,5-hexanedione, carbon disulfide, acrylamide). Chemicals that produce distal (acrylamide), but not proximal (*β*,*β*′-iminodiproprionitrile), giant axonal neurofilamentous swellings trigger retrograde distal axonal degeneration (distal axonopathy). Long and large-diameter myelinated axons in the PNS and CNS are vulnerable to distal axonopathy caused by a number of compounds (organophosphates, isoniazid), the resulting clinical picture being one of symmetrical sensory loss and muscle weakness in the distal extremities (stocking-and-glove polyneuropathy). Shorter and thinner nerve fibers, including postganglionic nerves of the autonomic nervous system, may become involved in distal axonopathies. This common type of neurodegeneration usually occurs after repeated exposure, evolves during and shortly after the period of intoxication, and then reverses as regenerating axons reestablish functional contact with denervated sensory terminals and muscle. Significant atrophy may result from prolonged skeletal muscle denervation, and recovery of sensory motor function may be slow, progressive and incomplete. When central motor pathways are heavily affected by distal axonopathy (leptophos, clioquinol), affected subjects may display permanent residual spasticity.

The motor pathway from brain to muscle is modulated by two other CNS structures that are vulnerable to substances with neurotoxic potential, namely the basal ganglia and cerebellum. Damage to the cerebellum may result in loss of coordination of limb and eye movement and in an ataxic gait (methylmercury, 3-acetylpyridine). The cerebellum and basal ganglia are both sensitive to hypoxia and related states induced by agents that impair energy metabolism (cyanide) and promote glutamate-mediated excitotoxicity. Other energy-disrupting agents elicit neuronal damage in the putamen (3-nitropropionic acid, methanol), pallidum (carbon monoxide), substantia nigra (MPTP), or other parts of the basal ganglia (manganese). These types of neurotoxicity may find clinical expression as parkinsonism, tremor, dystonia, and other extrapyramidal dysfunction. Movement disorders of various types may result from the side effects of several therapeutic agents (amphetamines, anticonvulsants, anticholinergics, neuroleptics, dopamine agonists, lithium, tricyclic antidepressants).

Autonomic regulation of the pupil, lacrimal and salivary glands, airway, heart, gut, bladder, genitalia, and blood vessels involves sympathetic, parasympathetic, and enteric neurons. Unlike their somatic counterparts in the spinal cord, efferent neurons of the autonomic nervous system are housed in peripheral ganglia with permeant blood vessels. Autonomic function is disrupted by agents that target synapses that utilize acetylcholine since this is the principal NT for all preganglionic autonomic fibers, all postganglionic parasympathetic fibers, and some postganglionic sympathetic fibers. Drugs that selectively block nicotinic receptors (curare) curtail

ganglionic output, while muscarinic agents (atropine) block transmission to effector cells. Anticholinesterase agents (fasciculins, organophosphates) stimulate sympathetic and parasympathetic activity, sometimes with the dramatic clinical consequences of a cholinergic crisis (nerve agents). Direct contact of anticholinesterase nerve agents (sarin, VX) with the eye, nasal passages, and bronchi leads to pupillary constriction (miosis), blurred vision, rhinorrhea, bronchoconstriction, and increased secretions. Systemic exposure results in increased salivation, bradycardia, enhanced lacrimation, urination, and defecation. Attendant muscle fasciculation, weakness, and seizures arise from peripheral somatic and CNS actions of anticholinesterase nerve agents.

The autonomic nervous system and endocrine function are regulated by the hypothalamus and associated limbic structures of the brain. Hypothalamic functions, including the regulation of temperature, heart rate, blood pressure, blood osmolarity, circadian control, and water and food intake, may be impacted by a range of chemicals. Parts of the hypothalamus lack a blood–brain barrier: infant mice treated with excitotoxic agents (glutamate, aspartate, cysteic acid) display extensive damage of the arcuate nucleus and develop a syndrome of obesity, skeletal stunting, reproductive failure, and gonadal hypoplasia. The hypothalamus receives major input from the hippocampus, which functions in the storage of declarative memory, uses cellular circuitry that involves glutamertergic synapses vulnerable to excitotoxins (domoic acid) and certain other agents (trimethyltin, trimethyl lead, soman), the latter possibly as a result of seizure-associated hypoxia.

See also: Carbamate Pesticides; Cholinesterase Inhibition; Metals; Nerve Agents; Organophosphates; Pesticides.

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New Drug Application *See* Investigative New Drug Application.

Niacin

Diana Ku

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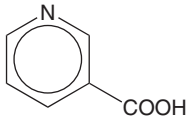
- Chemical Abstracts Service Registry Number: CAS 59-67-6
- Synonyms: Vitamin B_3, Nicotinic acid; Nicotinamide; Pellagra-preventative factor; 3-Carboxypyridine
- Chemical/Pharmaceutical/Other Class: Water-soluble vitamin
- Chemical Formula: $C_6H_5NO_2$
- Chemical Structure:



Uses

Niacin is a nutritional supplement used during periods of deficiency known as pellagra and for the treatment of dyslipidemia. Niacin needs may increase during chronic illness such as diabetes mellitus, malignancy, metabolic diseases, hyperthyroidism, infections, chronic fever, alcoholism, and during pregnancy and lactation.

Exposure Routes and Pathways

Routes of exposure are oral and intravenous. It can also be given intramuscularly or subcutaneously but intravenous administration is recommended when possible. Dietary sources of niacin are green vegetables, eggs, milk, and other dairy products, legumes, yeast, whole grains, lean meats, liver, and fish.

Toxicokinetics

Niacin is readily absorbed from the gastrointestinal tract. The peak serum concentration for an immediate release oral dosage form is usually seen within 45 min of niacin ingestion; 4–5 h for an extended release tablet. Niacin is hepatically metabolized and widely distributed into body tissues. Niacin is renally excreted. Excess amounts of niacin, beyond daily needs, are excreted largely unchanged in the urine. The plasma half-life is $\sim$45 min.

Mechanism of Toxicity

Niacin-induced vasodilation is believed to be mediated by prostaglandins. The mechanism of hepatotoxicity associated with niacin use is unknown.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute toxicity is not expected.

Human

Toxicity is unlikely even after acute ingestions of 50–100 times the recommended daily allowance. Side effects include nausea, vomiting, diarrhea, abdominal pain, headache, dizziness, and dryness of the skin. Niacin flush is a sensitivity reaction that causes warmth and finishing of the face and neck lasting $\sim$2 or 3 h. This event usually occurs with doses of $>$1 g. The symptoms are self-limiting and tend to occur less frequently with increased tolerance or premedication with aspirin or ibuprofen.

Chronic Toxicity (or Exposure)

Animal

It would be unlikely for animals to be given chronic niacin overdoses.

Human

Chronic megadoses of niacin may be associated with hyperglycemia, hyperuricemia, cardiac arrhythmias, hepatotoxicity, cystoid maculopathy, myopathy, peptic ulcers, and hyperkeratotic pigmented skin lesions. These problems may occur with doses exceeding 3 g day^{-1}.

In Vitro Toxicity Data

In one study of 87 infants born to women who were given therapeutic doses of niacin at any time during pregnancy, there were two infants born with congenital anomalies.

Clinical Management

Acute ingestions seldom require treatment. Reassurance that the niacin flush will gradually resolve over the next couple of hours should be given. In cases of chronic excessive use, the patient should be instructed to discontinue the supplement. Any toxic symptoms should be treated symptomatically.

See Also: Dietary Supplements.

Further Reading

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Nickel and Nickel Compounds

Shayne C Gad

This article is a revision of the previous print edition article by Betty J Locey and Arthur Furst, volume 2, pp. 415–416,

- Representative Chemicals: Metallic nickel (Ni); Nickel subsulfide (Ni_3S_2); Nickel sulfate ($NiSO_4$); Nickel carbonyl ($Ni(CO)_4$); Nickel oxide (NiO). Nickel can exist in a variety of other forms, but this text focuses on the primary environmentally relevant forms.
- Chemical Abstracts Service Registry Number: CAS 7440-02-0
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formula: $Ni^{+2,0}$

Uses

Nickel and nickel compounds are widely used in plated coatings, nickel–cadmium batteries, certain pigments, ceramic glazes, and as industrial and laboratory catalysts. Nickel subsulfide is used in refining certain ores and in smelting operations. Nickel is commonly used in alloys such as stainless steel, alloy steel, and nonferrous metal mixtures. Coins, costume jewelry, plumbing equipment, and electrodes are often made from nickel-containing alloys.

Background Information

Discovered in 1751, nickel is rare in earth's crust, but is believed to be more common in molten core.

Nickel is ubiquitous; it is found in air, water, soil, food, certain work environments, and in certain products. Low concentrations are found in soils and in plant and animal tissues. Nickel may be released into the ambient air with emissions from certain industrial processes and with smoke from the combustion of coal and petroleum products. Cigarette smoke may contain up to 3 μg of nickel per cigarette.

Exposure Routes and Pathways

Exposure to nickel and its compounds may occur through ingestion, inhalation, or dermal contact. The specific characteristics of the compound determined the likelihood of exposure through a particular route and the amount absorbed in to the body through that route.

Nickel is commonly ingested with food, and is found at low levels in drinking water. Elemental nickel, nickel oxide, and nickel subsulfide, may occur as a particulate or adsorbed onto other particles in ambient air. Nickel carbonyl is a highly reactive gas, with a half-life in air of ~100 s, and so exposure would occur only in the immediate vicinity of a release. Skin exposure may occur during contact with nickel-containing products (e.g., jewelry).

Toxicokinetics

The toxicokinetics of nickel compounds depends on their solubility in water and biological fluids. Nickel sulfate and nickel chloride are highly soluble in water, while nickel oxide is insoluble. Nickel subsulfide is poorly soluble, but is more soluble in biological fluids, presumably due to the effects of the proteins and other cellular components. Nickel and its inorganic compounds are not well absorbed through the skin or the gastrointestinal tract. Absorption is higher when soluble forms of nickel are administered in drinking water or to fasting subjects than when administered in food. The absorption of inhaled nickel particles depends, in part, on the size of the particles and the solubility of the compound, with soluble forms being rapidly absorbed and distributed, and insoluble forms being retained in the respiratory tract for much longer periods. Nickel carbonyl is rapidly absorbed from the lungs. Generally, absorption for nickel carbonyl > soluble compounds > insoluble compounds. Once absorbed, nickel is transported with the plasma, in a form bound to serum albumin, amino acids, polypeptides, and other small organic molecules. Nickel is found at elevated levels in the kidneys, liver, and brain. Nickel has been found in adipose tissue. Nickel may act at the point of contact (e.g., in the skin or in the lung) or systemically.

The half-life of nickel in nickel platers (exposed primarily to nickel sulfate) was found to be 20–34 h in plasma and 17–39 h in urine. In refinery workers (exposed to a mixture of soluble and insoluble forms of nickel), the half-life in the nasal mucosa was found to be several years.

The major route of excretion for nickel is in urine. Animal studies indicate that 60% of the nickel introduced into the body via injection is excreted in the urine and to a lesser extent through the bile into the feces. Some nickel is excreted in perspiration. Ingested nickel is primarily eliminated in the feces, with only ~10% excreted in the urine. Nickel crosses the placenta.

Mechanism of Toxicity

Skin sensitization is believed to occur as a result of nickel binding to proteins (particularly on the cell surface) and hapten formation. Essentially, the body perceives the nickel-protein complex as foreign and mounts an immune reaction to it. For example, sweat may react with the nickel in plated jewelry that comes in direct contact with skin; dissolved metal may penetrate and react with proteins in the skin and lead to immune sensitization. Nickel may substitute for certain other metals (especially zinc) in metal-dependent enzymes, leading to altered protein function. High nickel content in serum and tissue may interfere with both copper and zinc metabolism. It also readily crosses the cell membrane via calcium channels and competes with calcium for specific receptors.

Nickel carbonyl can cross-link amino acids to DNA and lead to formation of reactive oxygen species. Nickel carbonyl can also suppress natural killer cell activity and production of some interferons.

Responses in many of these assays were weak and occurred at toxic doses, and were affected by tissue culture conditions modifying uptake by the cell. The mechanism of nickel carcinogenesis is controversial, and is likely to vary with the form of nickel. The nickel ion (Ni^{2+}) alone does not form premutagenic DNA lesions, suggesting that nickel causes indirect DNA damage, perhaps due to oxidative stress or blocking DNA repair mechanisms.

Nickel is an essential trace nutrient in plants and certain animal species (e.g., rat and chick); however, it has not been shown to be essential in humans.

Acute and Short-Term Toxicity (or Exposure)

Human

The skin and respiratory tract are primary target organs. Nickel carbonyl is very reactive, and highly acutely toxic. Nickel carbonyl is very irritating to the respiratory tract, and exposure may lead to pulmonary edema, pneumonia, and death. Adverse reactions on exposure to other forms of nickel may occur at the site of contact (skin, respiratory tract, and gastrointestinal tract) or systemically (heart, blood, and kidneys). Ingestion of high doses of nickel and certain nickel compounds has been shown to cause stomach pain, increases in the number of red blood cells, and kidney damage.

Chronic Toxicity (or Exposure)

Animal

Different nickel compounds have been shown to have varying toxicity in animals. Both soluble and insoluble forms of nickel have been shown to damage the lung. Chronic inhalation studies (certain forms) have shown pulmonary inflammation, damage to certain regions of the respiratory tract mucosa and epithelium, and damage to the nasal olfactory epithelium. Nickel has been shown to be carcinogenic in animals via injection and implantation. Nickel subsulfide and nickel carbonyl have been shown to be carcinogenic via inhalation. Inhaled nickel oxide was carcinogenic in rats, but not in mice.

Nickel has been shown to adversely affect the blood (e.g., severe erythrocytosis) in experimental rats. Oral exposure to soluble nickel has been shown to cause increased prenatal or neonatal mortality.

Human

Nickel and nickel compounds are skin sensitizers, leading to irritation, eczema and allergic contact dermatitis. Oral exposure may elicit allergic dermatitis in sensitized individuals. Allergy-related asthma and skin reactions ('nickel itch' and contact dermatitis) have been associated with exposure. Skin sensitivity may even develop from contact with jewelry or coins made of nickel-containing alloys. Approximately 2.5–5% of the general population may be sensitized to nickel. A higher percentage of women than men is sensitized, probably because of direct contact with nickel-plated jewelry. Skin sensitization reactions can progress to erythema, some eruption, and in more extreme cases to pustules and ulcers. Severe skin reactions are most likely to occur in occupational settings where higher exposure is likely.

Exposure to certain nickel compounds is associated with development of cancer. Nickel particulate (e.g., elemental and subsulfide) has been associated with nasal and lung cancer after workplace exposures. Chromosomal aberrations have been noted in lymphocytes in occupationally exposed individuals. The American Conference of Governmental Industrial Hygienists (ACGIH) classifies inhalable nickel particulate – insoluble compounds as confirmed human carcinogens (A1). US Environmental Protection Agency (EPA) classifies nickel refinery dust and nickel subsulfide as known human carcinogens (A) and nickel carbonyl as a probable human carcinogen (B). The International Agency for Research on Cancer (IARC) classifies nickel and nickel compounds as having sufficient evidence of cancer in humans (group 1); however, IARC notes that the evaluation applies to the group in general and not necessarily to all compounds in the group.

In Vitro Toxicity Data

Nickel compounds are generally negative in bacterial gene mutation assays, but positive responses have often been found in *in vitro* mammalian cell assays.

Clinical Management

For inhalation exposure (typically to nickel carbonyl), the victim should be moved from the source of the exposure to fresh air. Contaminated clothing should be removed and contaminated skin washed. Blood, urine, and fecal nickel levels may be used as indicators of the level of recent exposure.

Chelating agents may be used to reduce the body burden after exposure. Diethyldithiocarbamate is the preferred chelating agent. D-Penicillamine and calcium ethylenediaminetetraacetate may also be effective in enhancing excretion of nickel.

Oral toxicity of elemental nickel is low. Treatment of illness caused by ingestion of nickel salts is usually limited to fluid replacement in cases of severe vomiting and diarrhea. Once sensitization has occurred, contact with nickel should be strictly avoided since reactions may occur after exposure to very low levels. This is particularly important in the workplace where high-level exposures are more likely to occur.

Environmental Fate

Nickel and its compounds are naturally present in the earth's crust, and releases to the atmosphere occur from natural discharges such as windblown dust and volcanic eruptions, as well as from anthropogenic activities. It is estimated that 8.5 million kilograms of nickel are emitted into the atmosphere from natural sources such as wind-blown dust, volcanoes, and vegetation each year. Five times that quantity is estimated to come from anthropogenic sources. Nickel releases are mainly in the form of aerosols that cover a broad spectrum of sizes. Particulates from power plants tend to be associated with smaller particles than those from smelters. Atmospheric aerosols are removed by gravitational settling and dry and wet deposition. Submicrometer particles may have atmospheric half-lives as long as 30 days. Monitoring data confirm that nickel can be transported far from its source. The form of nickel emitted to the atmosphere will vary according to the type of source. Species associated with combustion, incineration, and metals smelting and refining are often complex nickel oxides, nickel sulfate, metallic nickel, and in more specialized industries, nickel silicate, nickel subsulfide, and nickel chloride.

Nickel may be transported into streams and waterways from the natural weathering of soil as well as from anthropogenic discharges and runoff. This nickel can accumulate in sediment, with the adsorption of the metal to the soil depending on pH, redox potential, ionic strength of the water, concentration of complexing ions, and the metal concentration and type.

Ecotoxicology

The speciation and physicochemical state of nickel is important in considering its behavior in the environment and availability to biota. For example, the nickel incorporated in some mineral lattices may be inert and have no ecological significance. Most analytical methods for nickel do not distinguish the form of nickel; the total amount of nickel is reported, but the nature of the nickel compounds and whether they are adsorbed to other material is not known. This information, which is critical in determining nickel's lability and availability, is likely to be site specific.

In rainbow trout, 96 h LC_{50} values were from $\sim$8–36 mg Ni l^{-1} with highly soluble compounds (e.g., chloride, sulfate, and acetate salts). LC_{50} values in fathead minnows ranged from 3 to 90 mg l^{-1}. In studies with *Daphnia*, 48 h LC_{50} values ranged from 0.5 to 7 mg Ni l^{-1}.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration permissible exposure limit (PEL) time-weighted average (TWA) for nickel metal and other nickel compounds as nickel is 1 mg m^{-3}; the PEL TWA for nickel carbonyl is 0.007 mg m^{-3}. The ACGIH threshold limit values (TLVs) for nickel metal, insoluble compounds, soluble compounds, nickel carbonyl, and nickel subsulfide, all expressed as nickel, are 1.5, 0.2, 0.1, 0.12, and 0.1 mg m^{-3}, respectively. Except for nickel carbonyl, all of the TLVs are expressed as inhalable particulate. The US EPA reference dose for soluble nickel salts is 0.02 mg kg^{-1} day^{-1}, but this value is undergoing reevaluation, due to the availability of several relevant new studies.

See also: Hypersensitivity, Delayed Type; International Agency for Research on Cancer; Kidney; Metals; Respiratory Tract; Skin.

Further Reading

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National Toxicology Program (NTP) (1996c) Toxicology and carcinogenesis studies of nickel subsulfide (CAS NO. 12035-72-2) in F344/N rats and B6C3F1 mice (inhalation studies). *NTP TR 453. NIH Publication No. 96-3369*. US Department of Health and Human Services.

Relevant Websites

http://www.nickelinstitute.org – Nickel Institute.
http://www.epa.gov – US Environmental Protection Agency.

Nickel Chloride

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 7718-54-9 (Previously CAS 37211-05-5)
- Synonyms: Nickel dichloride; Nickelous chloride
- Chemical/Pharmaceutical/Other Class: Metal salt
- Chemical Formula: $NiCl_2$

Uses

Nickel chloride is used for nickel plating cast zinc, as an agent in electrolytic refining of nickel, as a chemical intermediate for nickel catalysts and complex nickel salts, as an absorber of ammonia gas in industrial gas masks, as a catalyst in diarylamine and silicon tetrachloride production, as an agent in electrodeless plating of nickel, as an agent in tin–nickel alloy plating, and as a fungicide for control of rust and rustlike disease. However, workers exposed to different forms of nickel have an elevated risk of lung cancer. Besides, Ni and its compounds have been reported to be potent carcinogens and toxic agents in humans and experimental animals. Therefore, Ni compounds are considered to be an industrial/occupational health hazard.

Exposure Routes and Pathways

Humans are frequently exposed to metals due to their ubiquity, wide use in industries, and persistence in the environment. Many nickel compounds are released into the atmosphere during mining, smelting, and refining operations. Although nickel is poorly absorbed from the gastrointestinal tract, exposure via food and drinking water provide most of the intake of nickel and nickel compounds. Humans and animals absorb ~1–10% of dietary nickel, and similar values were reported for drinking water exposure. Nickel metal is poorly absorbed dermally but some nickel compounds such as nickel chloride or nickel sulfate can penetrate occluded skin resulting in up to 77% absorption within 24 h. Nickel is excreted in the urine and feces, but because it is poorly absorbed, most ingested nickel is excreted in the feces. About 80–90% of nickel chloride is excreted and only a small amount is retained. The average daily intake of nickel in food is ~0.002 mg Ni kg^{-1} day^{-1} and the tolerable intake (TI) of nickel chloride is 0.0013 mg Ni kg^{-1} day^{-1}.

Toxicokinetics

Adverse effects can result from ingestion, skin contact, inhalation, or parenteral routes of exposure; nickel may be absorbed from the gastrointestinal and respiratory tracts as well as percutaneously; however, it is poorly absorbed orally. Nickel is bound to albumin and α_2-microglobulin in the circulation. Nickel chloride is a water soluble salt, and respiratory absorption with secondary gastrointestinal absorption of nickel (insoluble and soluble salts) is the major route of entry during occupational exposure. A significant quantity of inhaled material is swallowed following mucocillary clearance from the respiratory tract. Percutaneous absorption is negligible, but is important in the pathogenesis of contact hypersensitivity. Absorption is related to the solubility of the compound, and nickel given orally to rats as the chloride in the drinking water was eliminated mainly in the feces. Intubation of rats with nickel chloride led to 3–6% absorption of the labeled nickel, regardless of the administered dose. These studies suggest that

very little nickel in water or beverages is bioavailable, and that large doses are required to overcome the intestinal absorption-limiting mechanism.

Mechanism of Toxicity

As parent metal alters sodium balance and lipid metabolism; it induces metallothionein synthesis. Nickel chloride affects the T-cell system and suppresses the activity of natural killer cells. If given orally or by inhalation, nickel chloride has been reported to decrease iodine uptake by the thyroid gland.

Acute and Short-Term Toxicity (or Exposure)

Animal

The mouse oral LD_{50} for nickel chloride is $48\,mg\,kg^{-1}$ and the intraperitoneal LD_{50} is $11\,mg\,kg^{-1}$. Nickel chloride is a dermal sensitizer, and when injected interperitoneally into mice and rats caused a rapid decrease in body temperature. The alveolar macrophage was a cellular target for nickel toxicity following parenteral exposure to nickel chloride. Subcutaneous administration of nickel chloride to rats caused nickel uptake into and activation of alveolar macrophages, followed by reduced phagocytic capacity. Nickel chloride induces DNA damage in mouse leukocytes. The significant DNA damage observed after treatment with nickel chloride agrees with the results obtained for other metals like lead, chromium, and cadmium in mice and mercury in rats. The ability of nickel chloride to induce chromosome aberrations in male mice was tested by the micronucleus test and the dominant lethality test. Nickel chloride failed to produce micronuclei in polychromatic erythrocytes whereas cyclophosphamide, used as positive control, raised their incidence markedly. In contrast to the results obtained with cyclophosphamide, nickel chloride did not increase the rate of postimplantation death, but did decrease significantly the rate of pregnancy as well as the amount of preimplantation loss. Taking into account these results and the data in the literature, it was concluded that nickel probably has no clastogenic properties in mammals. Administration of nickel chloride to pregnant rats on days 7–11 of gestation resulted in significant embryotoxic effects such as increased resorption rate, decreased fetal weight, delays in skeletal ossification, and a high incidence of malformation.

Human

Inhalation of dust causes irritation of nose, throat, and eyes. Nickel poisoning has been reported in electroplaters who accidentally ingested water contaminated with nickel chloride, and there was rapid development of symptoms (e.g., nausea, vomiting, abdominal discomfort, diarrhea, giddiness, lassitude, headache, cough, dyspnea) that typically lasted a few hours, but persisted for 1–2 days in some cases.

Chronic Toxicity (or Exposure)

Animal

There is sufficient evidence in experimental animals for the carcinogenicity of metallic nickel, nickel monoxides, nickel hydroxides, and crystalline nickel sulfides. There is limited evidence in experimental animals for the carcinogenicity of nickel alloys, nickelocene, nickel carbonyl, nickel salts, nickel arsenides, nickel antimonide, nickel selenides, and nickel telluride. There is inadequate evidence in experimental animals for the carcinogenicity of nickel trioxide, amorphous nickel sulfide, and nickel titanate. In a two-stage carcinogenesis assay, orally administered nickel chloride enhanced the renal carcinogenicity of *N*-ethyl-*N*-hydroxyethyl nitrosamine in rats, but not the hepatocarcinogenicity of *N*-nitrosodiethylamine, the gastric carcinogenicity of *N*-methyl-*N*-nitro-*N*-nitrosoguanidine, the pancreatic carcinogenicity of *N*-nitrosobis(2-oxopropyl)amine, or the skin carcinogenicity of 7,12-dimethylbenzanthracene.

Human

Chronic exposure to aerosols of nickel chloride, emitted as mists from electroplating baths, may lead to chronic rhinitis, nasal sinusitis, anosmia, and perforation of the nasal septum. Asthma and chronic restrictive lung disease, nasal polyps and nasal septum perforation can also occur if nickel chloride is inhaled. Orally, nickel chloride can lead to cardiomyopathies. The International Agency for Research on Cancer deems nickel compounds to be carcinogenic to humans (group 1), that is, there is sufficient evidence in humans for the carcinogenicity of nickel sulfate, and of the combinations of nickel sulfides and oxides encountered in the nickel refining industry. There is inadequate evidence in humans for the carcinogenicity of metallic nickel and nickel alloys.

In Vitro Toxicity Data

Nickel chloride induced substantial induction of DNA repair synthesis in cultured Syrian hamster embryo and Chinese hamster ovary cells. Nickel chloride was not mutagenic in an *Escherichia coli* assay. The ability of nickel chloride to induce chromosomal

aberrations in Chinese hamster ovary and C3H1OT1/2-mouse cell lines was investigated, and nickel chloride induced chromosomal aberrations primarily in heterochromatin in both cell lines.

Clinical Management

Fluid replacement is indicated in the case of ingestion causing serious vomiting and diarrhea. For inhalation exposures, the patient should be moved to fresh air, and be provided with symptomatic and supportive treatment. Diethyldithiocarbamate is the chelating agent of choice. Disulfiram has been used to clear cases of nickel dermatitis.

Environmental Fate

Nickel chloride is water soluble and would be expected to release divalent nickel into the water.

Ecotoxicology

Numerous LC_{50} values are available for nickel chloride, for example, the LC_{50} for *Daphnia magna* (cladoceran) was $510\,\mu g\,l^{-1}$ for a 48 h test and $0.13\,mg\,l^{-1}$ for a 3 week test. Reproductive impairment of *Daphnia magna* was observed at 30–$95\,\mu g\,l^{-1}$ in a 64 h study.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value, 8 h time-weighted average is $0.1\,mg\,m^{-3}$ as inhalable fraction. The (US) Occupational Safety and Health Administration permissible exposure limit, 8 h TWA, is $1.0\,mg\,m^{-3}$. The (US) National Institute for Occupational Safety and Health (NIOSH) considers nickel metal and other compounds (as Ni) to be a potential occupational carcinogen. NIOSH usually recommends that occupational exposures to carcinogens be limited to the lowest feasible concentration.

Nickel chloride is listed as a US hazardous air pollutant, generally known or suspected to cause serious health problems. The Clean Air Act, as amended in 1990, directs the US Environmental Protection Agency (EPA) to set standards requiring major sources to sharply reduce routine emissions of toxic pollutants. EPA is required to establish and phase in specific performance based standards for all air emission sources that emit one or more of the listed pollutants. Nickel is also a toxic pollutant designated pursuant to Section 307(a) (1) of the Clean Water Act and is subject to effluent limitations.

See also: Metallothionein; Nickel and Nickel Compounds.

Further Reading

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Maibach HI and Menne T (1989) *Nickel and the Skin: Immunology and Toxicology*. Boca Raton, FL: CRC Press.

Relevant Website

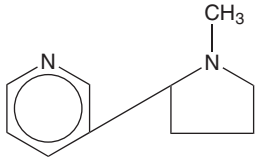
http://www.intox.org – (UK) National Poisons Information Service Centre of the United Kingdom. Nickel Chloride (an UKPID Monograph).

Nicotine

Brian Hughes

This article is a revision of the previous print edition article by Bonnie S Dean, volume 2, pp. 417–418, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 54-11-5
- Synonym: Methylpyridylpyrrolidine
- Chemical/Pharmaceutical/Other Class: Ganglionic cholinergic-receptor agonist
- Chemical Structure:



Uses

Nicotine is a highly toxic alkaloid found principally in tobacco products (cigarettes, 15–20 mg; cigars,

15–40 mg; snuff, 4.6–32 mg) and in smoking cessation products such as nicotine gum (2–4 mg per piece), lozenges (2–4 mg per piece), transdermal patches (7–52.5 mg per patch), nasal sprays (0.5 mg per spray), and inhalers (2–4 mg per use). It is also used as an insecticide.

Exposure Routes and Pathways

Exposure to nicotine occurs mainly through smoking tobacco products or inhalation of side-stream smoke. Dermal contact occurs most often through the use of therapeutic preparations (transdermal patch) designed for use in smoking cessation programs. Workers harvesting tobacco are also exposed dermally. Oral exposure occurs through the use of nicotine gum and accidental ingestion of tobacco products. Nicotine can also be absorbed through the nasal mucosa via the use of snuff.

Toxicokinetics

Absorption

Nicotine is a weak base with a pK_a value of 7.9. It is readily absorbed via the lung, oral mucosa, gastrointestinal (GI) tract, and skin. The dose of nicotine received via tobacco smoke varies a great deal and depends on the smoking behavior of the individual. Absorption of nicotine via deep lung inhalation of tobacco smoke is ~90% with a high amount of individual variability. Transdermal patches deliver 82% of the impregnated nicotine into systemic circulation. Absorption through the GI tract is limited in the stomach but extensive in the intestines due to a higher pH. However, extensive first pass metabolism by the liver limits the amount reaching the systemic circulation to 25–30% of the dose.

Distribution

Nicotine has an apparent volume of distribution in adults of ~1.7–3.0 l kg^{-1}. Plasma concentrations of nicotine appear to decline in a biphasic manner. Protein binding is ~4.9%. Under steady-state conditions, cotinine concentrations in the serum can be up to 10 times that of the parent compound. The half-life of nicotine in the initial phase is reportedly 2 or 3 min, and the half-life in the terminal phase is reportedly ~30–120 min. Nicotine is widely distributed in the body to the brain, lungs, adrenals, heart, GI tissue, spleen, thymus, kidney, skeletal muscle, saliva, and breast milk. Nicotine can also pass the placental barrier and enter the fetal tissue. Penetration through biological membranes occurs via passive diffusion rather than active transport.

Metabolism

Nicotine undergoes a large first-pass effect during which the liver metabolizes 80–90%. Small amounts are metabolized in the lungs and kidneys. The major metabolic pathway of nicotine is the C-oxidation to cotinine through a nicotine-Δ-1′-(5′)-iminium ion intermediate catalyzed by CYP2A6. Metabolism also occurs via *N*-oxidation, and glucuronidation of nicotine, cotinine, and *trans*-3-hydroxycotinine. Nicotine-1′-*N*-oxide is reduced to nicotine by bacterial flora in the large intestine via an *N*-oxide reductase system and subsequently undergoes enterohepatic circulation and repeat metabolism in the liver.

Excretion

Nicotine and its metabolites are quickly excreted in the urine. The half-life of nicotine in plasma is 1.6–2.8 h and that for cotinine is 8–29.3 h. Approximately 10–20% of the absorbed nicotine dose is excreted unchanged. Urinary excretion of nicotine is pH dependent, increasing in acid urine.

Mechanism of Toxicity

Nicotine exerts its effects by binding to nicotinic cholinergic receptors found in ganglia, the neuromuscular junction and the central nervous system. The prominent effects relate to an initial transient stimulation of the adrenal medulla, central nervous system (CNS), and cardiovascular system due to the release of catecholamines, gastrointestinal tract due to parasympathetic stimulation, salivary and bronchial glands, and the medullary vomiting center. Following these initial effects, nicotine causes blockade of the autonomic ganglia and the neuromuscular junction transmission, inhibition of catecholamine release from the adrenal medulla, and CNS depression.

Acute and Short-Term Toxicity (or Exposure)

Animal

Nicotine causes initial hyperexcitability, hyperpnea, salivation, vomiting, and diarrhea and then depression, incoordination, and paralysis in both small and large animals. The reported oral LD_{50} values for the male laboratory rat range from 50 to 188 mg kg^{-1}, and an LD_{50} of 288 mg kg^{-1} in female rats. The oral LD_{50} values for mice range from 3.34 to 24 mg kg^{-1}. Nicotine tends to be equally toxic through several exposure routes. The dermal LD_{50} for rabbits and mice are 50 and 140 mg kg^{-1}, respectively.

Human

Nicotine is a highly toxic alkaloid. It is water soluble, colorless, and bitter tasting. Few deaths have been reported from its use with the onset of symptoms much more rapid following the ingestion of liquid nicotine-containing products rather than with nicotine-containing organic material. Clinical manifestations include nausea, vomiting, abdominal pain, and increased salivation. Severe toxicity might result in headache, confusion, agitation, and restlessness, followed by lethargy, seizures, and coma. Severe toxicity with hypertension, tachycardia, and vasoconstriction has occurred from buccal absorption after biting a transdermal nicotine patch. The lethal oral dose of nicotine for adults has been established to be ~40–60 mg. Survival has been reported after ingestion of 1–4 g. Pediatric ingestion of 1–1.5 cigarettes resulted in episodes of weakness, limb jerking, and unresponsiveness. Children were asymptomatic if less than one cigarette was ingested.

Chronic Toxicity (or Exposure)

Animal

There are no apparent species differences in nicotine toxicity between animals and man. The toxic sequelae following chronic nicotine exposure to animals is similar to that in man including GI problems, cardiovascular effects, changes in neurochemistry, and reproductive effects such as decreased birth weight and length of gestation.

Human

Chronic exposure through tobacco use can produce nicotine dependence disorders. Tolerance may occur in some individuals. Effects that reinforce nicotine use include improved cognitive function and mood. Withdrawal symptoms following cessation of cigarette smoking may include anxiety, impaired concentration and memory, depression, hostility, sleep disturbances, and increased appetite. Correlation between smoking and alcoholism has been observed. Nicotine may also in part be responsible for cardiovascular diseases, lung cancer, and chronic pulmonary lung disease. Women who smoke during pregnancy also have greater risk of having children with low birth weight, and attention deficit disorders in latter in life.

In Vitro Toxicity Data

Nicotine was not found to be mutagenic in the Ames assay using several strains of *Salmonella typimurium* with or without S9 activation. Tests for aneuploidy/chromosome aberrations using *Neurospora crassa* were also negative. Nicotine did induce a slight increase in sister chromatid exchange rate in Chinese hamster ovary cells. Nicotine may be converted into other carcinogenic/mutagenic compounds such as nitrosonornicotine.

Clinical Management

Nicotine's rapid absorption, swift onset of symptoms, and quick metabolism and excretion necessitates the need for early supportive measures to be instituted in cases of severe acute intoxication. In mild toxicity, clinical effects may last 1–2 hours while in severe toxicity effects may persist for 8–24 h. Monitoring the patient's vital signs (pulse, blood pressure, respiration), and neurologic function, will enable one to determine if interventions are indicated. Such interventions may include continuous monitoring of the heart rate and rhythm with an electrocardiogram (if available) in the presence of an irregular and/or a rapid pulse, the administration of intravenous 0.9% NaCl, dopamine, or norepinephrine for hypotension and providing mechanically assisted respiration which may also include the use of supplemental oxygen (if accessible) for signs of respiratory depression. If seizure activity is noted, the use of diazepam or barbiturates should be considered as therapy for seizure control. Atropine may be used to control excess bronchial secretions, salivation, and diarrhea. For oral exposure to nicotine, GI decontamination procedures may be considered if performed soon after ingestion and only if the patient's level of consciousness allows this method to be used. If so, slurry of activated charcoal may be administered orally or via gastric lavage, since this will serve to decrease nicotine absorption in the intestinal tract. Emesis is usually spontaneous and the alkalinity of an antacid increases the absorption of nicotine. Therefore, neither ipecac for emesis nor antacid by mouth should be administered, as such treatment is contraindicated.

Environmental Fate and Toxicity

Environmental releases of nicotine occurred mainly from its use as an insecticide. At ambient temperatures, nicotine will exist as a vapor. The half-life in the atmosphere is ~4 h because it reacts with photochemically produced hydroxyl radicals. Nicotine's mobility in soil is somewhat dictated by the pH. Absorption on soil particles occurs in neutral and acidic soils when, as a weak base, nicotine is protonated. Nicotine has a high mobility in alkaline soils. Likewise, in water nicotine is not expected to bind to

sediment or suspended particles unless the pH is neutral or acidic. Information on the degradation of nicotine in soil is limited. The breakdown products include oxynicotine, 3-pyridylmethyl ketone, 2-3-dipyridyl, and *N*-methyl myosmine. The ability of nicotine to bioconcentrate in aquatic organisms is low (bioconcentration factor = 5).

Ecotoxicology

Nicotine was evaluated for acute aquatic toxicity in rainbow trout and dapnia. The mean 96 h LC_{50} for rainbow trout and the 48 h EC_{50} for dapnia are 4 and 0.24 mg l^{-1}, respectively. Nicotine sulfate was also evaluated in multiple aquatic species for lethality. The species and the corresponding toxicity are as follows: fathead minnow (96 h $LC_{50} = 19.7$ mg l^{-1}), rainbow trout (96 h $LC_{50} = 7.31$ mg l^{-1}), bluegill (96 h $LC_{50} = 4.31$ mg l^{-1}), goldfish (96 h $LC_{50} = 13.1$ mg l^{-1}), daphnia magna (48 h $EC_{50} = 3.25$ mg l^{-1}), midge (48 h $LC_{50} > 27$ mg l^{-1}), crayfish (96 h $LC_{50} > 38.2$ mg l^{-1}), and snail (96 h $LC_{50} > 38.2$ mg l^{-1}).

Other Hazards

There is a moderate explosion hazard when nicotine is exposed to heat or flame. Decomposition products include nitrogen oxides, carbon monoxide, and other highly toxic fumes. Nicotine is stable under normal conditions. Contact with oxidizing materials should be avoided. Heat or flames should be avoided.

Exposure Standards and Guidelines

The Occupational Safety and Health Association has set a permissible exposure limit of 0.5 mg m^{-3} (skin) as an 8 h time-weighted average. The American Conference of Governmental Industrial Hygienists guidelines are also set at this limit of 0.5 mg m^{-3}. The National Institute for Occupational Safety and Health recommended exposure limit is 0.5 mg m^{-3}. The level that is immediately dangerous to life or health is 5 mg m^{-3}.

Nicotine is a hazardous substance under the Comprehensive Environmental Response, Compensation, and Liability Act. Releases of 100 lb or more are reportable to the National Response Center. SARA Title III has established a threshold planning quantity requiring a facility that receives or produces 100 lb of nicotine to notify state emergency response and local emergency planning commissions. SARA Title III also requires state and local reporting of nicotine releases greater than or equal to 100 lb. The Federal Insecticide, Fungicide, and Rodenticide Act has established a tolerance of 2 ppm of nicotine on cucumber, lettuce, and tomatoes.

See also: Developmental Toxicology; Neurotoxicity; Tobacco; Tobacco Smoke.

Further Reading

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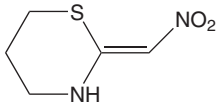
Nithiazine

Josef Seifert

- Chemical Abstracts Service Registry Number: CAS 58842-20-9
- Synonyms: (*E*,*Z*)-2-Nitromethylene-1,3-thiazinane (IUPAC); Tetrahydro-2-(nitromethylene)-2*H*-1,3-thiazine (CAS); 2*H*-1,3-Thiazine; Tetrahydro-2-(nitromethylene) (label)
- Chemical/Pharmaceutical/Other Class: Neonicotinoid insecticide
- Chemical Formula: $C_5H_8N_2O_2S$
- Chemical Structure:



Uses

Nithiazine is used as an active ingredient in fly strips (QuickStrike® Fly Abatement Strip). QuickStrike® strips are most effective against houseflies (*Musca*

domestica). The strips can be placed in or around the house, animal housing, recycling facilities, or other facilities where abundant houseflies are a nuisance.

Background Information

Nithiazine has historical importance as a compound on which neonicotinoids, a new class of insecticides, have been modeled. Its synthesis was accomplished by Shell (Modesto, CA) in the 1970s based on selection from a series of nitroalkyl heterocyclic compounds.

Exposure Routes and Pathways

Exposure due to inhalation is minimal because nithiazine has a low vapor pressure (4×10^{-7} mmHg). Accidental dermal contact and ingestion are two potential exposure routes.

Mechanism of Toxicity

Nithiazine is an agonist acting at the neural nicotinic acetylcholine receptor – Na^+/K^+ ionophore in mammals and insects. The nicotinic acetylcholine receptors regulate the flow of Na^+ and K^+ through the channels in the neural postsynaptic membranes. Opening and closing of the channels by acetylcholine maintains the dynamic ratio of the intracellular to extracellular concentrations of Na^+ and K^+ needed for the initiation of the signal in the postsynaptic neuron. The structural differences between insect and mammalian receptors determine the high selectivity of nithiazine toxicity to insects.

Acute and Short-Term Toxicity (or Exposure)

Animal

The data on nithiazine toxicity are scarce since nithiazine is being registered only for terrestrial nonfood use. High oral dosages ($>100\,mg\,kg^{-1}$ body weight) of nithiazine to male rats caused a decrease in body temperature, decreased locomotor activity (rearing), lower arousal, increased salivation and increased fecal boluses. These signs were more extensive in female rats. Additional functional observations in female rats were tremors, abnormal cage behavior, increased urination, ataxic gait, reduced reaction to visual stimuli and tail pinch, and reduced visual placing response. The results of gross necropsy and neurohistology were normal.

Human

Very little is known regarding acute toxicity of nithiazine in humans.

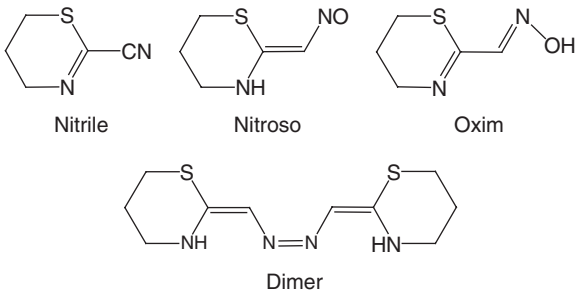


Figure 1 The major products of nithiazine photoreduction and dimerization.

Chronic Toxicity (or Exposure)

Little is known regarding chronic toxicity of nithiazine in humans or animals. It is reasonable to conclude, however, based on the data obtained in animal studies and *in vitro* tests, and on nithiazine affinity for insect nicotinic acetylcholine receptors, that nithiazine toxicity to humans and domestic animals or pets is low.

In Vitro Toxicity Data

Tests for genetic toxicity (Ames assay using *Salmonella typhimurium*, mouse bone marrow micronucleus assay, chromosome aberrations in human lymphocytes and/or Chinese hamster ovary cells, *in vitro* unscheduled DNA synthesis assay in primary rat hepatocytes) were all negative.

Environmental Fate

Nithiazine is extremely unstable in sunlight due to the nitromethylene chromophore. This group absorbs strongly in water at 365 nm with an extinction coefficient $\sim 40\,000\,M^{-1}\,cm^{-1}$. Direct irradiation results in a loss of the insecticidal activity and formation of a mixture of more than 40 degradation products. The major products of nithiazine photoreduction and dimerization from irradiation at 360 nm in water are nitrile, nitroso, and oxim derivatives, and dimers (**Figure 1**). Consequently, nithiazine half-time on foliage is only ~ 30 min. Low photostability is the primary reason that nithiazine is not used as an insecticide for field and other environmental applications.

See also: Acetylcholine; Neonicotinoids.

Further Reading

Kollmeyer WD, Flattum RF, Foster JP, *et al.* (1999) Discovery of the nitromethylene heterocycle insecticides. In: Yamamoto I and Casida JE (eds.) *Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor*, ch. 3, pp. 71–89. Berlin: Springer.

Nitric Oxide

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 10102-43-9
- Synonyms: Mononitrogen monoxide; Nitrogen monoxide
- Chemical/Pharmaceutical/Other Class: Gas
- Chemical Formula: NO
- Chemical Structure: N = O

Uses

Nitric oxide is used in the manufacturing of nitric acid. It is also used as a stabilizer for propylene and methyl ether, and to bleach rayon. Nitric oxide is a natural product of fuel combustion and a component of smog.

Exposure Routes and Pathways

Since it is a gas, inhalation is the most likely route of exposure. However, nitric oxide is also produced endogenously from arginine by humans.

Toxicokinetics

When exposed to air, nitric oxide may be converted to nitrogen dioxide or nitrogen tetraoxide, both of which are highly toxic. Conversion is slower at concentrations below 1 ppm. Other contaminants, such as ozone in the air, expedite the conversion process.

Mechanism of Toxicity

A cytotoxic free radical, nitric oxide, impairs mitochondrial ATP synthesis by inhibiting the citrate cycle and other cellular mechanisms of electron transport.

Acute and Short-Term Toxicity (or Exposure)

Animal

Inhalation can lead to the formation of methemoglobin and resultant toxic effects; for example, respiratory distress. The inhalation LC_{50} in rats is 1068 mg m^{-3}. In mice, $LC_{Lo} = 320$ ppm.

Human

Contact with skin and mucous membranes, such as those in the eyes and lungs, may be highly irritating. Inhalation leads to methemoglobin formation, which interferes with normal oxygen utilization. This can lead to fatigue, uneasiness, and respiratory distress.

Chronic Toxicity (or Exposure)

Animal

Nitric oxide is a mutagen in somatic cells. It has been shown to cause lung damage after long exposure periods.

Human

Effects similar to those seen on acute exposure are also seen chronically. In addition, if the compound is converted to nitrogen dioxide or nitrogen tetraoxide, toxicities associated with these compounds can also be observed.

Clinical Management

Oxygen therapy should be provided for cyanosis of dyspnea. Prednisone or predinsolone should be given at 5 mg, every 6 h, to reduce inflammation in the lungs.

Environmental Fate

Nitric oxide is converted spontaneously in the air to nitrogen dioxide; hence, some of the latter gas is present whenever nitric oxide is found in air (at concentrations below 50 ppm).

Exposure Standards and Guidelines

The Occupational Safety and Health Administration permissible exposure limit is 25 ppm.

See also: Photochemical Oxidants; Pollution, Air.

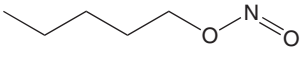
Further Reading

Bingham E, Cohrssen B, and Powell CH (eds.) (2001) *Patty's Toxicology*, vol. 3. New York: Wiley.
Wang T, El Kebir D, and Blaire G (2003) Inhaled nitric oxide in 2003: A review of its mechanisms of action. *Canadian Journal of Anaesthesia* 50(8): 839–846.
Weinberger B, *et al.* (2001) The toxicology of inhaled nitric oxide. *Toxicological Sciences* 59(1): 5–16.

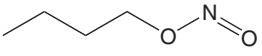
Nitrite Inhalants

Keiko Okamoto

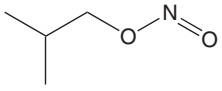
- Representative Chemicals: Amyl nitrite; Butyl nitrite; Isobutyl nitrite
- Chemical Abstracts Service Registry Numbers: Amyl nitrite (CAS 463-04-07); Butyl nitrite (CAS 544-16-1); Isobutyl nitrite (CAS 542-56-3)
- Synonyms:
  - Amyl nitrite: Isoamyl nitrite; *n*-Amyl nitrite; Nitramyl; Nitrous acid; Nitropentane; Nitrous acid; Pentyl alcohol nitrite; Pentyl ester; Pentyl nitrite
  - Butyl nitrite: 1-Butyl-nitrite; 2-Methylpropyl ester; Butyl ester; Isobutyl ester; Isobutyl nitrite; *n*-Butyl nitrite; *s*-Butyl nitrite; *t*-Butyl nitrite; Cyclohexyl nitrite; Aimies; Ames; Amys; Bang; Bolt; Bullet; Climax; Discoroma; Flash; Hardware; HiBall; Jack aroma; Jungle Juice; Lightning Bolt; Liquid Gold; Locker Room; Mama Poppers; Natural Brutes; Odor of Man; OZ; Poppers; Quick Silver; Ram; Rush; Satan's Secret; Snappers; Sweat; Thrust
- Chemical/Pharmaceutical/Other Class: Volatile organic nitrites; Aliphatic nitrites
- Chemical Formulas:
  - Amyl nitrite: $C_5H_{11}NO_2$
  - Butyl nitrite: $C_4H_9NO_2$
  - Isobutyl nitrite: $C_4H_9NO_2$
- Chemical Structures:



Amyl nitrite



Butyl nitrite



Isobutyl nitrite

Uses

Amyl nitrite has been used as a vasodilator drug, a diagnostic agent, and a cyanide treatment adjunct; it is an abused inhalant. Butyl nitrite is an abused inhalant. Isobutyl nitrite is an ingredient of various incenses or room odorizers, and it is also used as a jet propellant and in the preparation of fuels. It is an abused inhalant.

Exposure Routes and Pathways

Nitrites are usually inhaled but have been ingested, either accidentally or with suicidal intent.

Toxicokinetics

Effects following inhalation occur in 10 s, peak at 30–60 s, and last ~3–5 min. Nitrites are hydrolyzed to the nitrite ion and alcohol within seconds. Approximately 60% of the nitrite ion is biotransformed; ammonia is a metabolite. Almost 40% of the nitrite ion is excreted unchanged via the kidneys. Elimination follows first-order kinetics.

Mechanism of Toxicity

Nitrites produce relaxation of vascular smooth muscles, causing cardiovascular effects through coronary and peripheral vasodilation. They are oxidizing agents and, in excess, induce formation of methemoglobin, abnormal hemoglobin that is unable to bind and transport oxygen or carbon dioxide. Nitrites can also precipitate an intravascular hemolysis with formation of Heinz bodies. The resulting anemia can compound the hypoxic effects of methemoglobinemia.

Acute and Short-Term Toxicity (or Exposure)

Animal

Hearing loss, weight loss, lacrimation, changes in visual acuity, nausea, vomiting, decreased motor performance, tachypnea, seizures, immunosuppression, decrease in immune cells, methemoglobinemia, and alteration in hepatic angiogenic gene expression have been induced in animal studies.

Human

Users claim aphrodisiac effects from heightened stimulation, enhanced penile erection, and relaxation of the anal sphincter and rectum. Because of the rush of blood and oxygen to the brain, facial flushing and a temporary euphoria occur several seconds after inhalation. Perception of time is slowed.

Typical effects of inhalation abuse are dizziness, palpitations, blurred vision, headache, nausea, and stinging of the nasal passages, eyes, and throat. There is a fall in blood pressure with a reflexive increase in pulse rate. Pulmonary irritation, tachypnea, and shortness of breath are described. Cardiovascular

collapse, coma, anion gap acidosis, and seizures can occur in severely poisoned patients. Methemoglobinemia may occur, characterized by cyanosis and respiratory depression. Ingestion of nitrites seems to produce a more rapid and malignant methemoglobinemia than inhalation. Blood methemoglobin levels should be monitored in symptomatic patients. Methemoglobin levels of 20–30% produce mild symptoms, levels of 30–45% produce moderate effects, and levels of 50–70% are associated with severe toxicity. Levels $\geqslant$70% are often lethal if untreated. Plasma nitrite levels are not clinically useful.

Chronic Toxicity (or Exposure)

Animal

A significant evidence of increase in tumor growth and carcinogenic activity of isobutyl nitrite were observed in male and female rats in 2 year inhalation studies.

Human

Repetitive abuse can cause crusting skin lesions and telangiectasis (angioma or hyperemic spots). Tracheobronchial irritation with dyspnea and hemoptysis has been reported. Withdrawal from industrial exposure has resulted in respiratory failure, left ventricular hypertrophy, and myocardial infarctions. Damage to the lungs, liver, kidneys, bone marrow, and brain is possible. Nitrite inhalants are thought to be carcinogenic and immunosuppressive. Tolerance occurs.

In Vitro Toxicity Data

Isobutyl nitrite was found to be mutagenic in *in vitro* studies.

Clinical Management

Airway management, respiratory support, and high-flow oxygen are indicated for the cyanotic patient. The cardiovascular, neurological, and metabolic effects should respond to the usual therapeutic agents. Methemoglobinemia is treated with 1 or 2 mg kg^{-1} of methylene blue, given intravenously over a 5 min period and repeated if needed. Methylene blue is recommended for symptomatic patients and for patients with methemoglobin levels $\geqslant$30%. Patients with preexisting anemia or cardiovascular disease may need treatment even if their methemoglobin levels are as low as 15%. Exchange transfusion has been employed in severely symptomatic patients who were unresponsive to methylene blue treatment. Hyperbaric oxygen can be supportive while preparing for exchange transfusion. Gastric decontamination is indicated for oral exposure. It will be necessary to monitor complete blood count and arterial blood gases.

See also: Amyl Nitrite; Butyl Nitrite.

Further Reading

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Soderberg LS (1999) Increased tumor growth in mice exposed to inhaled isobutyl nitrite. *Toxicology Letters* 104(1–2): 35–41.

Tran DC, Yeh K, Brazeau DA, and Fung H (2003) Inhalant nitrite exposure alters mouse hepatic angiogenic gene expression. *Biochemical and Biophysical Research Communications* 310: 439–445.

Relevant Website

http://www.drugabuse.gov – US Department of Health and Human Services (2002) National Institute on Drug Abuse Research Monograph Series: Health Hazards of Nitrite Inhalants.

Nitrites

Betty J Locey

This article is a revision of the previous print edition article by Robin Guy, volume 2, pp. 420–421, © 1998, Elsevier Inc.

- Representative Chemical: Sodium nitrite
- Chemical Abstract Service Registry Numbers: CAS 14797-65-0 (Nitrite); CAS 7632-00-0 (Sodium nitrite)
- Chemical Formulas: NO_2^- (Nitrite); $NaNO_2$ (Sodium nitrite)

Uses

Nitrites have been used as vasodilators, as circulatory (blood pressure) depressants, as antidotes for cyanide and hydrogen sulfide poisoning (amyl and sodium nitrites), and to relieve smooth muscle

spasm. Topical silver nitrate is used in burn therapy. Sodium nitrite is commonly used to cure meats. It retards the onset of rancidity and the development of unpalatable odors and flavors during storage and delays the development of botulinal toxin. In addition, nitrites produce a desirable flavor and pink color in the cured meat. Sodium nitrite has also been used as an anticorrosive agent in cooling fluids. The greatest use of nitrates is as a fertilizer.

Exposure Routes and Pathways

Exposure may occur through oral, dermal, ocular, and inhalation routes. People may be exposed to nitrites through foods such as cured meats and selected vegetables (e.g., broccoli, spinach, cauliflower, collard greens, and root vegetables). Accidental poisonings have occurred when people have mistaken sodium nitrate for table salt.

People may also be exposed through contaminated drinking water. Individual wells drawing from shallow groundwater in areas where nitrogen-based fertilizers are used are at higher risk of contamination. Of particular concern are potential infant exposures both through water and through water used to prepare formula.

Nitrites and nitrates have a number of medicinal uses. Abuse of volatile nitrites (amyl, butyl, and isobutyl nitrites intended for medical use) as recreational drugs (e.g., psychedelics) has been reported. On the street, they may be called 'rush', 'poppers', and 'snappers'.

Toxicokinetics

Nitrites are absorbed orally, dermally, and through the lungs. Nitrites (NO_2^-) and nitrates (NO_3^-) are interconverted in the body. Ingested nitrates are rapidly absorbed through the upper intestine (proximal small bowel) and then rapidly distributed throughout the body. Nitrates in the blood then enter the lower, large intestine, where they are converted to reactive nitrites by bacteria in the gut (fecal organisms). Nitrites are then reabsorbed from the lower intestine back into the blood where they react with the ferrous ion (Fe^{2+}) in deoxyhemoglobin in red blood cells converting it to methemoglobin (containing ferric iron; Fe^{3+}). Some nitrate in the blood may be excreted in saliva through an active blood nitrate transport system and reintroduced into the beginning of the gastrointestinal tract. Nitrates are also metabolized in the liver. Nitrates and metabolites are excreted in the urine. The half-life of nitrate is generally less than 1 h; however, metabolites have half-lives from 1 to 8 h.

Transplacental passage of nitrite occurred in pregnant rats given doses of 2.5–50 mg kg^{-1} orally. In mice given 400, 800, or 1200 mg sodium nitrite orally in drinking water, 99.1–99.5% of the dose was eliminated. The remaining nitrite was transformed into nitrate and recovered from the liver and muscle.

Mechanism of Toxicity

Nitrites cause relaxation of smooth muscle and the conversion of hemoglobin to methemoglobin. Nitrites in the blood react with the ferrous ion (Fe^{2+}) in deoxyhemoglobin in red blood cells, converting it to methemoglobin containing ferric iron (Fe^{3+}). Methemoglobin with ferric iron (Fe^{3+}) cannot bind and transport oxygen to tissues and cells throughout the body. Oxygen is necessary for cells to generate energy and function. Systems in the body with the highest oxygen demand are most vulnerable. These include the central nervous system, particularly the areas that control breathing, and the heart.

In general, the effects associated with exposure to nitrite (NO^{2-}) and nitrite-containing compounds are the same whether ingested, inhaled, or produced *in vivo* (e.g., produced from nitrate). Ingested nitrate (NO^{3-}) is generally metabolized and excreted without producing adverse effects unless conditions are favorable for conversion to nitrite (e.g., higher pH, presence of certain intestinal flora).

Acute and Short-Term Toxicity (or Exposure)

Animal

Nitrite is irritating to eyes, skin, and respiratory tract. Exposure to sufficiently high concentrations can cause death. Many animal species lack the nitrate-reducing bacteria and therefore do not provide a good model for methemoglobin formation.

Significant levels of methemoglobinemia were produced when mice were exposed to butyl nitrites via inhalation. Pretreatment of the mice with methylene blue prevented the methemoglobin formation associated with the butyl nitrite exposure. A single intravenous dose of 30 mg kg^{-1} of sodium nitrite caused methemoglobinemia in dogs. The minimum lethal dose of sodium nitrite is estimated to be 150–170 mg kg^{-1} in cattle and ~70–75 mg kg^{-1} in pigs.

Human

Nitrite is a severe respiratory, eye, and skin irritant. Acute exposure to high levels of nitrites can be fatal. Exposure may cause visual field defects, hypotension, tachycardia, respiratory depression, and cyanosis due to formation of methemoglobinemia. Symptoms may

also include headache, confusion, dizziness, convulsions, unconsciousness, nausea, vomiting, and diarrhea.

Infants are much more sensitive to nitrite and nitrate toxicity. Infants have a higher stomach pH (generally greater than 4) than adults (generally a pH of ~ 2), generally have higher levels of nitrate reducing bacteria in their gut, have lower enzymatic capacity to reduce methemoglobin to hemoglobin, and the presence of hemoglobin F, which is more susceptible to oxidation by nitrites.

In addition, fetal hemoglobin (hemoglobin F) is oxidized by nitrite to methemoglobin at a rate twice as that of adult hemoglobin (hemoglobin A). Furthermore, enzymatic capacity of erythrocytes of newborn infants to reduce methemoglobin to hemoglobin appears less than that of adults. Methemoglobin anemia can cause the child's health to degrade in several days and can ultimately cause death. Symptoms include shortness of breath and a blue cast to the skin. In infants the condition has been referred to as the blue baby syndrome.

Chronic Toxicity (or Exposure)

Animal

In one study, rats received sodium nitrite at 100 mg kg^{-1} in drinking water daily during their entire life span over three generations and no evidence of chronic toxicity, carcinogenicity, or teratogenicity was observed.

Human

Long-term exposure to nitrites and nitrates at high enough levels may cause an increase in the formation of urine by the kidney (diuresis), increased starchy deposits, and bleeding of the spleen.

Nitrites are generally not classified as human carcinogens. Under certain conditions nitrites may combine with amines in the body to form nitrosamines. There are a number of different nitrosamines; many are regulated as human carcinogens. Certain chemicals, such as vitamin C (ascorbic acid), can limit the transformation of nitrites to nitrosamines. US Department of Agriculture (USDA) requires the addition of ascorbic acid or erythorbic acid to bacon cure to reduce the risk of nitrosamine formation.

In Vitro Toxicity Data

Nitrites have tested negative in DNA repair bacterial assays. Positive results have been reported in mammalian cytogenetics and sister chromatid exchange studies.

Clinical Management

If exposed via inhalation, the victim should be moved to fresh air and monitored for respiratory distress. Exposed eyes should be irrigated with copious amounts of tepid water for at least 15 min. Exposed areas of skin should be washed thoroughly with soap and tepid water. Medical attention should be sought.

Emesis and/or activated charcoal/cathartic may be indicated if the patient is conscious. For seizures, diazepam may be administered as an intravenous bolus. For hypotension, intravenous fluids may be indicated.

Methemoglobinemia may be noted and associated with a cyanosis. In general, plasma levels of nitrites and related compounds are not clinically useful. Methemoglobin concentration should be determined in all cyanotic patients or patients experiencing respiratory distress. Arterial blood gases should be monitored in symptomatic or cyanotic patients. Methemoglobinemia is often treated by intravenous administration of methylene blue (dose generally 1–2 mg kg^{-1}) over a 5–10 min period. Cyanosis should begin to improve within 15 min of treatment with methylene blue. A second dose of methylene blue may be indicated if improvement is not observed. Methylene blue is not indicated if the patient is G-6-PD deficient as hemolytic anemia can develop. If the condition is life-threatening, treatment may include transfusion and hyperbaric oxygen therapy.

Environmental Fate

Nitrite (NO_2^-) and nitrate (NO_3^-) are naturally occurring inorganic ions that can combine with various organic and inorganic species and compounds. Microbial action in soil or water decomposes wastes containing organic nitrogen first into ammonia, which is then oxidized to nitrite and nitrate (part of the 'nitrogen cycle'). Nitrite is easily oxidized to nitrate under environmental conditions and nitrates are more commonly found in surface water and groundwater. The levels of nitrate found in water are generally increased if impacted by organic wastes, fertilizers, and/or ammonia. In soil, nitrate-containing compounds are generally water soluble and readily leach from soil to groundwater.

Ecotoxicology

Nitrites are toxic to aquatic organisms. *Nitrosomonas* sp. bacteria oxidize ammonia to nitrite. Ammonia is produced by decomposing organic mater and excreted fish. Nitrites are generally less toxic to fish than ammonia; however, chronic exposure to low levels increase stress on the fish population leading to stress-related disease states such as fin rot and bacterial ulcers. At higher concentrations, nitrites can cause damage to fish skin and gills and increase

the likelihood of bacteria infections and the success of parasitic organisms. In addition, higher levels in the blood stream may lead to conversion of hemoglobin to methemoglobin, reducing the fish's ability to transport oxygen potentially leading to asphyxiation.

Other Hazards

Nitrites are generally incompatible with flammable materials, strong oxidizing agents, reducing agents, organics, and finely powdered metals. They tend to be hygroscopic (draw moisture from the atmosphere). Sodium nitrite is not combustible; however, it may enhance the combustion of other compounds. Reactions may lead to fire and/or explosions. Fires may produce irritating and toxic fumes.

Exposure Standards and Guidelines

The US federal primary drinking water standard, maximum contaminant level (MCL), and maximum contaminant level goal (MCLG) for nitrite (measured as nitrogen) are both set at $1\,mg\,l^{-1}$. The MCL was established to be protective of infants (below 6 months of age). The MCL and MCLG for nitrates are $10\,mg\,l^{-1}$.

The US Environmental Protection Agency Integrated Risk Information System provides a chronic oral reference dose (RfD) of 0.1 mg/kg-day (file last update September 1997). The RfD is based on a critical effect of methemoglobinemia in infants chronically exposed to nitrites in drinking water.

The US Food and Drug Administration (US FDA) is the federal agency responsible for monitoring proper use of nitrite by meat processors. USDA established guidelines to reduce/eliminate nitrosamine formation in nitrite and nitrate cured meats in 1973 (see *Federal Register*, Vol. 38, No. 221, Friday, November 16, 1973, p. 31 679). Levels of sodium nitrite that can be used in curing meat are defined in under the Meat Inspection Regulations (Title 9, Chapter 111, Subchapter A, Code of Federal Regulations, 1974). The final calculated concentration of sodium nitrite in cured meat products (processed with nitrites, nitrates, or combination) cannot exceed 200 ppm.

See also: Blood; Food Additives; Food and Drug Administration, US; Food Safety and Toxicology; Gastrointestinal System; Nitrite Inhalants; Nitrosamines; Respiratory Tract.

Further Reading

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Relevant Websites

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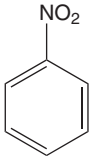
http://books.nap.edu – National Academy Press website.

http://www.epa.gov – USEPA. Integrated Risk Information System. Nitrite. CAS 14797-65-0. See also: USEPA. Office of Groundwater and Drinking Water. Consumer Factsheet on Nitrates/Nitrites.

Nitrobenzene

Robin C Guy

- Chemical Abstracts Service Registry Number: CAS 98-95-3
- Synonyms: Nitrobenzol; Essence of mirbane; Essence of myrbane: Mirbane oil
- Chemical/Pharmaceutical/Other Class: A chemical intermediate and solvent
- Chemical Formula: $C_6H_5NO_2$
- Chemical Structure:



Uses

Nitrobenzene is an industrial chemical. Most of the nitrobenzene produced in the United States is used to manufacture aniline. Nitrobenzene is also used to produce lubricating oils such as those used in motors and machinery. A small amount of nitrobenzene is used, sometimes as a solvent, in the manufacture of dyes, polishes, paints, drugs, pesticides, and synthetic rubber (Office of Pollution Prevention and Toxics, OPPT).

Exposure Routes and Pathways

Dermal contact, inhalation, and ingestion are possible exposure pathways.

Toxicokinetics

Nitrobenzene activation in rats to methemoglobin-forming metabolites appears to be mediated to a significant degree by intestinal microflora. In animal studies, the major part of nitrobenzene ($\sim$80% of the dose) is metabolized and eliminated within 3 days (WHO). The remainder is eliminated slowly. The slow compartment is likely due to erythrocyte recycling of nitrobenzene redox forms and glutathione conjugates. Covalent binding, presumably to sulfhydryl groups of hemoglobin, was demonstrated. In rodents and rabbits, *p*-nitrophenol and *p*-aminophenol are major urinary metabolites. In humans, part of the absorbed dose is excreted into the urine; 10–20% of the dose is excreted as *p*-nitrophenol (which thus may be used for biological monitoring). The half-life of elimination for *p*-nitrophenol is estimated to be ~ 5 h (initial phase) and > 20 h (late phase). The urinary metabolite *p*-aminophenol is significant only at higher doses.

Mechanism of Toxicity

Intestinal microflora present in animals may be responsible for reduction of nitrobenzene *in vivo* and subsequent methemoglobin formation.

Acute and Short-Term Toxicity (or Exposure)

Animal

In subchronic oral and dermal studies in mice and rats, central nervous system lesions in the cerebellum and brain stem were apparent and included petechial hemorrhages (WHO). These may be direct toxic effects or mediated by vascular effects of hypoxia or hepatic toxicity. Depending on the dose, these neurotoxic effects were grossly apparent as ataxia, head-tilt and arching, loss of righting reflex, tremors, coma, and convulsions. Other target organs included kidney (increased weight, glomerular and tubular epithelial swelling, and pigmentation of tubular epithelial cells), nasal epithelium (glandularization of the respiratory epithelium, pigment deposition in and degeneration of olfactory epithelium), thyroid (follicular cell hyperplasia), thymus (involution) and pancreas (mononuclear cell infiltration), while lung pathology (emphysema, atelectasis and bronchiolization of alveolar cell walls) was reported in rabbits.

Nitrobenzene causes toxicity in multiple organs by all routes of exposure. Methemoglobinemia results from oral, dermal, subcutaneous, and inhalation nitrobenzene exposure in mice and rats, with consequent hemolytic anemia, splenic congestion and liver, bone marrow and spleen hematopoiesis. In rodents, methemoglobinemia, hematological effects, impaired male fertility with significant testicular toxicity, and, in the inhalation studies, effects on the respiratory system were found at the lowest doses tested. Methemoglobinemia, bilateral epididymal hypospermia and bilateral testicular atrophy were observed at the lowest exposure level studied, 5 mg m^{-3} (1 ppm), in rats. The oral LD_{50} in rats was 640 mg kg^{-1}.

In mice, there was a dose-related increase in the incidence of bronchialization of alveolar walls and

alveolar/bronchial hyperplasia at the lowest dose tested of 26 mg m^{-3} (5 ppm).

Human

WHO reports that acute poisonings by nitrobenzene in consumer products have occurred frequently in the past. Significant human exposure is possible, due to the moderate vapor pressure of nitrobenzene and extensive skin absorption. Furthermore, the relatively pleasant almond smell of nitrobenzene may not discourage people from consuming contaminated food or water.

A small amount of nitrobenzene may cause mild irritation if it contacts the skin or eyes directly. Repeated exposures to a high concentration of nitrobenzene can result in methemoglobinemia, a condition in which the blood's ability to carry oxygen is reduced. During this condition, the skin may turn a bluish color and nausea, vomiting, and shortness of breath may occur. Effects such as headache, irritability, dizziness, weakness, and drowsiness may also occur. There is also some evidence that breathing high concentrations of nitrobenzene may damage the liver and spleen.

Nitrobenzene is toxic by all routes of exposure. Symptoms may be delayed for up to 1–4 h. Methemoglobinemia associated with headache, nausea, lethargy, depressed respiration, and cyanosis may occur. A bitter almond odor may be present, which suggests cyanide poisoning, but cyanide produces symptoms much more rapidly than nitrobenzene. Tachycardia, hypotension, respiratory depression/failure, and cardiac arrhythmias may be observed. Repeated exposure may be followed by liver impairment up to yellow atrophy, hemolytic icterus, and anemia of varying degrees, with the presence of Heinz bodies in the red blood cells. Pregnant women may be especially at risk due to transplacental passage. Individuals with glucose-6-phosphate dehydrogenase deficiency may also be special-risk groups (Environmental Protection Agency, EPA). Additionally, because alcohol ingestion or chronic alcoholism can lower the lethal toxic dose of nitrobenzene, individuals consuming alcoholic beverages may be at risk.

Chronic Toxicity (or Exposure)

Animal

Carcinogenic response was observed after exposure to nitrobenzene in rats and mice: mammary adenocarcinomas were observed in female B6C3F$_1$ mice, liver carcinomas in male Fischer-344 rats and thyroid follicular cell adenocarcinomas in male Fischer-344 rats. Benign tumors were observed in five organs.

Human

As repeat exposure to nitrobenzene in air over a lifetime causes cancer in animals, nitrobenzene may likewise cause cancer in humans (OPPT). The International Agency for Research on Cancer (IARC) has determined that nitrobenzene is possibly carcinogenic to humans.

In Vitro Toxicity Data

Nitrobenzene was nongenotoxic in *Salmonella typhimurium* and mammalian cells *in vitro* and in mammalian cells *in vivo*. Studies reported included DNA damage and repair assays, gene mutation assays, chromosomal effects assays, and cell transformation assays.

Clinical Management

Nitrobenzene is toxic by all routes including skin absorption. Systemic effects may be delayed a few hours. Poisoning closely resembles aniline. Initial care should include adequate gastrointestinal (gastric lavage as indicated and activated charcoal) and dermal decontamination. The patient should be given oxygen and monitored for cyanosis. Cardiac rhythm should be monitored in symptomatic patients.

Plasma nitrobenzene levels are not clinically useful. The metabolites in urine, *p*-nitro- and *p*-aminophenol, primarily in long-term exposure to nitrobenzene can be used as evidence of exposure. Methemoglobin levels should be determined in all cyanotic patients; cyanosis that does not respond to oxygen therapy may appear when the plasma methemoglobin level is 15%. Symptomatic methemoglobinemia should be treated with methylene blue. For seizures, diazepam should be administered via an intravenous bolus.

Exposed eyes should be irrigated with copious amounts of tepid water for at least 15 min and exposed areas should be washed extremely thoroughly with soap and water.

Ecotoxicology

Very low levels of nitrobenzene may be found in air. It may be present in water from industrial releases but in water, nitrobenzene is broken down by sunlight. Nitrobenzene is a liquid that does not bind well to soil; therefore, in the soil, it can move into the groundwater, be taken up by plants, evaporate to the air, and be broken down by bacteria. It does not appear to concentrate in fish or other aquatic animals. Most releases of nitrobenzene to the US environment are to underground injection sites. In 1992, only a small percent (6%) of environmental releases of

nitrobenzene was to air (OPPT). It can also evaporate slowly from water and soil exposed to air.

WHO summarized air quality data from various sources and reported that some measured levels in air in US cities in the early 1980s ranged between <0.05 and $2.1\,\mu g\,m^{-3}$ (<0.01 and 0.41 ppb). Data reported by the US EPA in 1985 indicated that less than 25% of air samples in the United States were positive, with a median concentration of $\sim 0.05\,\mu g\,m^{-3}$ (0.01 ppb); in urban areas, mean levels were generally less than $1\,\mu g\,m^{-3}$ (0.2 ppb), with slightly higher levels in industrial areas (mean $2.0\,\mu g\,m^{-3}$ (0.40 ppb)). Of 49 air samples measured in Japan in 1991, 42 had a detectable level, measured as 0.0022–$0.16\,\mu g\,m^{-3}$. Levels over urban areas and waste disposal sites were significantly lower (or undetectable) in winter than in summer.

WHO also summarized water quality data from various sources and reported that data on nitrobenzene levels in surface water appear to be more extensive than data on levels in air. While levels are variable depending on location and season, generally low levels (~ 0.1–$1\,\mu g\,l^{-1}$) have been measured. One of the highest levels reported was $67\,\mu g\,l^{-1}$, in the river Danube, Yugoslavia, in 1990. However, nitrobenzene was not detected in any surface water samples collected near a large number of hazardous waste sites in the United States (reported in 1988). Based on limited data, it appears that there may be greater potential for contamination of groundwater than of surface water; several sites measured in the United States in the late 1980s had levels of 210–250 and $1400\,\mu g\,l^{-1}$ (with much higher levels at a coal gasification site). Nitrobenzene has been reported in studies conducted in the 1970s and 1980s on drinking water in the United States and the United Kingdom, albeit in only a small proportion of samples, but was not detected in 30 Canadian samples (1982 report).

Exposure Standards and Guidelines

The estimated mean lethal adult dose is ~ 1–5 g. Children may be much more susceptible.

See also: Clean Air Act (CAA), US; Clean Water Act (CWA), US; Comprehensive Environmental Response, Compensation, and Liability Act, US; Consumer Product Safety Commission; National Institute for Occupational Safety and Health; Occupational Safety and Health Administration; Pollution Prevention Act, US; Toxic Substances Control Act, US.

Relevant Websites

http://www.who.int – World Health Organization (WHO), Environmental Health Criteria, No. 230: Nitrobenzene.
http://www.epa.gov – US Environmental Protection Agency. Chemical Fact Sheet on Nitrobenzene.
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Nitrobenzene.

Nitrocellulose

Dennis J Naas

- Chemical Abstracts Service Registry Number: CAS 9004-70-0
- Synonyms: Cellulose nitrate; Nitrocotton; Soluble gun cotton; Pyroxylin; Various trade names (C 2018, E 1440, H 1/2, BK2-W, BK2-Z, CA 80-15, Celex, Celloidion, Collodion Cotton, Collodion Wool, Xyloidin); Iodion cotton; Pyroxylin; Colloxylin; Paraladion
- Chemical/Pharmaceutical/Other Class: Nitric acid esters
- Chemical Formula: $C_6H_7N_3O_{11}$

Uses

Nitrocellulose is a product that has found many uses in everyday life. Its physical form can vary widely from white fibers to thin sheets to thick liquid. Nitrocellulose is used to make everything from smokeless gun powder to waterproof fuses in pyrotechnics, inks, adhesives, varnishes, resins, lacquer coatings, embedding sections in microscopy, photography, electrotechniques, galvanoplasty, and even certain plastics, such as what is used in ping-pong balls. It can be a white, yellow, or transparent plastic, which can be anywhere from brittle to flexible. It can have properties ranging from a strong, resistant plastic to an unstable class B (highly flammable, explosive when confined) explosive material, all determined by the nitrogen content. Other current uses include the making of membranes that are used to immobilize

DNA, RNA, or protein, which can then be probed with a labeled sequence or antibody (Western blot assays), microscopy embedding, electrotechniques, skin protectants, microfilters, and others. Nitrocellulose continues to be used in photography, the manufacture of lacquers, patent and natural leathers, artificial pearls, process engraving, and cements.

Background Information

In the 1830s and 1840s, European chemists discovered that cotton dipped in nitric acid produced an explosive material. This early form of nitrocellulose was too unstable to be used safely in explosives production. Scientists later converted nitrocellulose into a stable base for improved gunpowder known as smokeless powder. Further experiments revealed that a combination of nitrated natural fibers with ether and alcohol produced a nonexplosive solution that hardened into a film. This discovery led to a wide array of end uses for nitrocellulose including plastics, lacquers, and photographic film.

Exposure Routes and Pathways

Accidental oral exposure is most likely. Skin exposure and inhalation of airborne fibers are also possible in occupational settings but these are unlikely routes for the general public.

Mechanism of Toxicity

No information is available on specific effects of nitrocellulose exposure.

Acute and Short-Term Toxicity (or Exposure)

Human

Although the primary danger of nitrocellulose is physical harm from fire or explosion, there are a number of clinical case reports on ingestion of Collodion, which contains nitrocellulose along with ether (70%) and ethanol (24%). Symptoms are similar to ethanol intoxication (exhilaration, talkativeness, impaired motor coordination, slowed reaction time, ataxia, flushing, drowsiness, etc.) except that onset is more rapid and the stomach becomes promptly distended because of the volatility of Collodion. One or two ounces of Collodion may be fatal if swallowed.

Collodion is classified as moderately toxic. Probable oral lethal doses for humans are 0.5–5.0 g kg^{-1} or between 1 ounce and 1 pint (1 lb) for a 70 kg person.

Chronic Toxicity (or Exposure)

Human

Earlier proportional mortality studies of workers in a plastics-producing plant indicated excess mortality from certain digestive and genitourinary cancers. To more definitively examine mortality, a retrospective cohort study was conducted for 2490 male wage earners who worked at least 1 year during 1949–66. Possible associations warranting continued surveillance were found between rectal cancer and cellulose nitrate production.

Clinical Management

Recommended treatment for acute colloidon ingestions is similar to that recommended for ethanol or ether overdose, including gastric lavage.

Environmental Fate

Degradation of nitrocellulose involves a complex chemical dissociation into a wide variety of products. Extremely high concentrations of nitrate and nitrite (NO_3^-/NO_2^-) are present in leachate from nitrocellulose landfills. Low permeability of the sludge and especially soil/sludge mixture will attenuate effects over long period of time.

Ecotoxicology

No acutely toxic effects of nitrocellulose were observed among fish, invertebrate, or algal species except the green alga *Selenastrum capricornutum*. Sediments containing nitrocellulose indicated no adverse effects among chironomid populations exposed to 540 mg kg^{-1} in sediment over two generations. Four species of invertebrates and four species of fish were unaffected by nitrocellulose concentrations as high as 1000 mg l^{-1}. Four species of algae were exposed up to 1000 mg l^{-1}. Three were unaffected and *Selenastrum capricornutum* showed a 96 h EC_{50} of 731 mg l^{-1}.

Other Hazards

Nitrocellulose is very flammable and may explode or ignite without warning when dry.

Nitrocellulose is classified under Organization for Economic Cooperation and Development (OECD) standards as: R1 – Explosive when dry; R3 – Extreme risk of explosion by shock, friction, fire, or other sources of ignition; R11 – Highly flammable; S16 – Keep away from sources of ignition; S33

– Take precautionary measures against static discharges; S35 – This material and its container must be disposed of in a safe way; S37 – Wear suitable gloves; S39 – Wear eye/face protection.

NFPA (National Fire Protection Association) Hazard Classification: Health: 1. 1 = Materials that, on exposure, would cause irritation, but only minor residual injury, including those requiring the use of an approved air-purifying respirator. These materials are only slightly hazardous to health; only breathing protection is needed. Flammability: 4. 4 = This degree includes flammable gases, pyrophoric liquids, and Class IA flammable liquids. The preferred method of fire attack is to stop the flow of material or to protect exposures while allowing the fire to burn itself out. Reactivity: 0. 0 = This degree includes materials that are normally stable, even under fire exposure conditions, and which do not react with water. Normal firefighting procedures may be used.

Exposure Standards and Guidelines

According to the US Food and Drug Administration requirements, colloidon is an indirect food additive for use only as a component of adhesives that could be used in packaging.

See also: Phthalate Ester Plasticizers.

Further Reading

Gosselin RE, Hodge HC, Smith RP, and Gleason MN (1976) *Clinical Toxicology of Commercial Products*, 4th edn., p. II-167. Baltimore: Williams and Wilkins.

Marsh GM (1983) Mortality among workers from a plastics producing plants: A matched case–control study nested in a retrospective cohort study. *Journal of Occupational Medicine* 25(3): 219–230.

Tew RW and Jaffe LS (eds.) (1973) Mammalian toxicology and toxicity to aquatic organisms of nitrocellulose, a waterborne munitions waste pollutant: A review of literature concerning mammalian toxicology and toxicity to aquatic organisms of nitrocellulose. *US National Technical Information Service*, ad rep: 15 pp. 777807/9ga.

Relevant Website

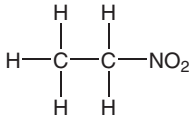
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Nitrocellulose.

Nitroethane

Christopher P Holstege

This article is a revision of the previous print edition article by Daniel Steinmetz, volume 2, pp. 423–424, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 79-24-3
- Chemical/Pharmaceutical/Other Class: Aliphatic nitro compounds
- Chemical Formula: $C_2H_5NO_2$
- Chemical Structure:



Uses

Nitroethane is used as a propellant; as a solvent for nitrocellulose; resins, waxes, and dyestuffs; and in chemical synthesis.

Exposure Routes and Pathways

Exposure to nitroethane may occur by inhalation, dermal contact, and ingestion.

Toxicokinetics

In studies involving animals, 50–70% of nitroethane vapors were absorbed through the upper respiratory tracts. Nitroethane is metabolized rapidly, with increased nitrite and nitrate levels detected in the blood. Animal studies suggest that absorbed nitroethane is eliminated within 48 h.

Mechanism of Toxicity

The mechanisms of toxicity for nitroethane are unknown. Methemoglobinemia associated with nitroethane is due to its metabolism to nitrite compounds.

Acute and Short-Term Toxicity (or Exposure)

Animal

Nitroethane produces similar effects in animals to those that appear in humans. Chronic nitroethane vapor administration has produced methemoglobinemia, pulmonary edema, narcosis, and liver and kidney damage in various animal models.

Human

Nitroethane is an irritant to the eyes and the respiratory tract. Vapors may exacerbate preexisting respiratory conditions, such as emphysema and asthma. Neither the odor nor irritation provide dependable warning properties. Overexposure to inhalation of the vapors may cause narcosis, headaches, and dizziness. The liquid is a mild irritant to the skin and can cause defatting and dermatitis. A number of cases of toxicity were reported in children who ingested nitroethane. These children developed prolonged methemoglobinemia following ingestions of small quantities of nitroethane-containing artificial nail removal products. Methylene blue therapy reduced the methemoglobin level in all of these children; however, methemoglobin levels increased again several hours later in some of the children and they required additional methylene blue.

Chronic Toxicity (or Exposure)

Animal

Chronic inhalation studies in rats and mice at doses of 0, 100, 350, and 1000 ppm for 6 h a day, 5 days a week demonstrated increased methemoglobin levels. At the highest dose tested, rats showed evidence of hepatic damage (vacuolization) and mice developed multinucleated spermatids.

In Vitro Toxicity Data

Assessment of mutagenicity using the Ames *Salmonella* and micronucleus assays has been negative or inconclusive.

Clinical Management

If dermal or eye contact with the liquid occurs, the affected areas should be flushed thoroughly with water for at least 15 min and the patient observed for resulting skin or eye irritation. In case of inhalation, the victim should be moved to fresh air and the patient should be monitored for respiratory irritation and pulmonary edema. If ingestion occurs, basic and advanced life-support measures should be utilized as necessary. Gastrointestinal decontamination procedures are unlikely to provide clinical benefit. The use of methylene blue should be considered in the treatment of nitroethane-induced methemoglobinemia. Repeat doses of methylene blue may be necessary for patients with profound methemoglobinemia.

Environmental Fate

Nitroethane has a wide variety of industrial uses. Because of the huge production and widespread use of this substance, releases into the environment can and have occurred. Release at ambient temperature and pressure will result in nitroethane existing solely as a vapor. Nitroethane is broken down in the atmosphere by hydroxyl radicals and has a half-life of 107 days.

See also: Dyes; Nitrocellulose.

Further Reading

Hornfeldt CS and Rabe WH III (1994) Nitroethane poisoning from an artificial fingernail remover. *Journal of Toxicology. Clinical Toxicology* 32: 321–324.

Osterhoudt KC, Wiley CC, Dudley R, Sheen S, and Henretig FM (1995) Rebound severe methemoglobinemia from ingestion of a nitroethane artificial-fingernail remover. *Journal of Pediatrics* 126: 819–821.

Nitrogen Mustard

Harry Salem and Frederick R Sidell*

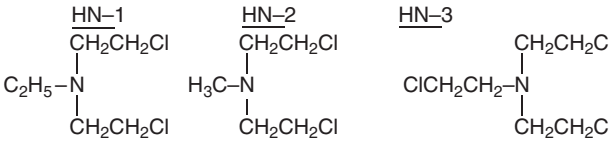
Published by Elsevier Inc.

- Chemical abstracts Service registry Numbers:
  CAS 538-07-8 (HN-1)
  CAS 51-75-2 (HN-2)
  CAS 555-77-1 (HN-3)

*The views of the authors do not purport to reflect the position of the US Department of Defense. The use of trade names does not constitute official endorsement or approval of the use of such commercial products.

- Synonyms:
  HN-1: Bis(2-chloroethyl)ethylamine; 2-Chloro-*N*-(2-chloroethyl)-*N*-ethylethanamine; 2,2[1]-Dichlorotriethylamine; Ethylbis (2-chloroethyl)amine; Ethyl-S
  HN-2: MBA; Mechlorethamine; Mustine; 2,2[1]-Dichloro-*N*-methyldiethylamine; Dichloren; Caryolysin; Chlormethine; Bis(2-chloroethyl)methylamine; Leukeran
  HN-3: Tris(2-chloroethyl)amine; 2-Chloro-*N*,*N*-bis(2-chloroethyl)ethanamine; Trichlorotriethylamine; Nitrogen mustard-3; Mechlorethamine; Mustargen

- Chemical/Pharmaceutical/Other Class: Vesicant; Alkylating agent
- Chemical Formulas: $C_6H_{13}CI_2N$ (HN-1); $C_5H_{11}CI_2N$ (HN-2); $C_6H_{12}CI_3N$ (HN-3)
- Chemical Structures:



Uses

Nitrogen mustards are among the blister agents/vesicants used in chemical warfare. HN-1 was originally designed to remove warts and later identified as a potential chemical warfare agent. HN-2 was designed as a military agent, but later used in cancer chemotherapy. HN-3 was developed as a military agent.

Exposure Routes and Pathways

Ocular, percutaneous, inhalation, ingestion, and injection are all possible routes of exposure. Effects may be local, systemic, or both. All of the nitrogen mustards are oily liquids that are colorless to pale yellow and evaporate slowly. They are more dangerous than sulfur mustard but, like sulfur mustard, they are derivatives of ammonia. The most toxic and most volatile of the three nitrogen mustards is HN-2, but HN-3 is used more because it is stable.

Toxicokinetics

Like sulfur mustard, the nitrogen mustards combine predominantly with the thiol group of molecules and are excreted as conjugated cysteinyl derivatives. Both nitrogen and sulfur mustards have structural similarities and have common chemical reactions. A key reaction is the intramolecular cyclization in a polar solvent such as water to form a cyclic onium cation and a free anion. Nitrogen mustards form the immonium cation, while the sulfur mustard forms the sulfonium cation. The cyclized form is responsible for the varied effects of mustards, which are similar. In nitrogen mustard the sulfur is replaced by nitrogen.

Animal Toxicity

The animal toxicity reported for HN-3 is described in **Table 1**. Evidence shows it causes leukemia and cancers of the lungs, liver, uterus, and large intestine in animals. Nitrogen mustards also produce developmental effects in animals.

Human Toxicity

Nitrogen mustards produce damage to the eyes, respiratory tract, skin, and suppress the immune system. Systemically they produce cytotoxicity, with the hematopoietic and lymphoid tissues being especially sensitive.

The estimated inhalation median lethal dosage (LCt_{50}) for HN-3 in humans is 1500 mg min m^{-3}. The estimated percutaneous vapor LCt_{50} is 10 000 mg min m^{-3}, and the estimated percutaneous liquid LD_{50} is 700 mg per 70 kg. The percutaneous median incapacitating dosage (ICt_{50}) in humans has been estimated at 2500 mg min m^{-3}; the dose to produce eye injury has been estimated at 200 mg min m^{-3}. The airborne exposure limit (AEL) for HN-1 is 0.003 mg m^{-3} as a time-weighted average, while none exist for HN-2 and HN-3.

Irritation of eyes following a single exposure to nitrogen mustards occurs at doses that do not affect the skin or respiratory tract and appears sooner than with sulfur mustard. Mild-to-moderate exposures cause slight smarting and lacrimation within 20 min,

Table 1 Animal toxicity values for nitrogen mustards

Species	*Toxicities*
Inhalation Time (min)	*LCt_{50} (mg min m^{-3})*
Mouse (10)	165 (vapor)
	345 (aerosol)
Rat (10–100)	670 (vapor)
Rat (0.25–2.0)	800 (aerosol)
Cat (10)	400–1000
Dog (10)	400–1000
Percutaneous	*LD_{50} (mg kg^{-1})*
Mouse	7.0
Rat	4.9
Rabbit	19.0
Dog	10.0
Intravenous	*LD_{50} (mg kg^{-1})*
Mouse	1
	2
Rat	0.7
Rabbit	2.5
Dog	1.0
Subcutaneous	*LD_{50} (mg kg^{-1})*
Mouse	2.0
Rat	2.0
Rabbit	2.0
Dog	
Intragastric	*LD_{50} (mg kg^{-1})*
Mouse	
Rat	2.5
Rabbit	
Dog	

becoming persistent ~2.5 h later, and reaching a maximum at 8–10 h. The effects include erythema and edema of the palpebral and bulbar conjunctiva with superficial, steamy haziness of the cornea, irritation, lacrimation, deep eye pain, miosis, and photophobia.

Following severe exposure these symptoms progress for 24 h or longer, and are followed by spotty hemorrhagic discoloration of the iris and roughened lusterless surface of the corneal epithelium, which demonstrate punctuate fluorescein staining. The corneal epithelium may exfoliate.

Clouding and edema of the cornea and necrosis may cause rupture of the globe.

There may be no skin lesions following mild vapor exposures. However, severe vapor or liquid exposure to nitrogen mustard will produce effects similar to those of sulfur mustard (but the onset is sooner than with sulfur mustard). These effects include erythema, irritation, and itching, with blisters developing in the erythematous areas.

Exposure of the respiratory tract to nitrogen mustard produces the same effects as sulfur mustard. These include the delay in onset, irritation of the nose and throat, hoarseness progressing to aphonia, and persistent cough, fever, dyspnea, and moist rales. After 24 h, chemical pneumonitis may appear.

Following oral administration or systemic absorption of nitrogen mustards, the intestinal tract may be damaged. In animals, severe diarrhea occurred, which may be hemorrhagic. The lesions were most marked in the small intestine and consisted of degenerative changes and necrosis in the mucosa. In humans, ingestion of 2–6 mg causes nausea and vomiting.

Following absorption of nitrogen mustard from intact skin or respiratory or gastrointestinal tract, the most specific effects are on the hematopoietic and lymphoid tissues. In bone marrow, the degenerative changes can be detected within 12 h and may progress to severe aplasia. The thymus, spleen, and lymph nodes involute rapidly with necrosis and phagocytosis of their lymphocytes. This is evident from the transient leukocytosis in the blood, which is followed by severe lymphopenia, granulocytopenia, and thrombocytopenia, for 5–10 days following exposure. The white blood cell count may fall to 500 cells mm^{-3} or lower. The various nitrogen mustards differ in their ability to produce these changes.

The chronic physiological effects may include, for severe exposure, scarring of the cornea, and the iris frequently becomes discolored and atrophied. Repeated skin burns may lead to hypersensitivity of the skin, which is an effect similar to that of sulfur mustard. That is, reexposure will cause erythema, with or without edema, and pronounced itching and burning occurring within 1 h. Lower concentrations will produce these effects in sensitized persons. Vesication heals more rapidly. Frequent manifestations of reexposure in sensitized individuals include a morbilliform rash and eczematoid dermatitis surrounding old lesions. The International Agency for Research on Cancer has classified nitrogen mustard as probably carcinogenic to humans (group 2A). Evidence shows it causes leukemia in humans.

Clinical Management

The victim must be removed from the source of contamination quickly by adequately protected attendants and then decontaminated using a solution of sodium hypochlorite, liquid household bleach, or fuller's earth. Oxygen and/or artificial respiration should be administered if dyspnea is present or breathing has stopped.

Erythema should be treated with calamine or other soothing lotions or creams to reduce burning and itching. Large blisters should be unroofed and covered with a sterile dry dressing if the patient is ambulatory or left uncovered if the patient is not ambulatory. Denuded areas should be irrigated with saline or soapy water and covered with a topical antibiotic (e.g., silver sulfadiazine or mefanide acetate). Multiple or large areas of vesication require hospitalization and whirlpool irrigation. Systemic analgesics are indicated especially prior to manipulation of the patient or irrigation of the burn areas. Systemic antipruritics (e.g., trimeprazine) may also be used.

Treatment of ocular injury includes thorough irrigation, application of homatropine (anticholinergic) ophthalmic ointment, and topical antibiotics several times daily. Vaseline or similar products should be applied regularly to the edges of the eyelids to prevent them from sticking together. Topical analgesics may be useful in severe blepharospasm for examination of the eye but should be used sparingly. Sunglasses may reduce discomfort from photophobia, and the victim must be reassured that complete healing and restoration of vision will result.

Steam inhalation and cough suppressants may relieve upper airway symptoms, sore throat, nonproductive cough, and hoarseness. Appropriate antibiotic therapy should only be instituted following confirmation of infection by positive sputum tests (Gram stain and culture). Intubation should be accomplished prior to the development of laryngeal spasm or edema so that adequate ventilation is established and suction of necrotic and inflammatory debris can be facilitated. Oxygen may be required as well. Early use of positive expiratory pressure (PEEP) or

continuous positive airway pressure (CPAP) may be useful. Bronchoscopy may be required if pseudomembrane has developed to permit suction of the necrotic debris by direct vision. Bronchodilators or steroids may also be used to relieve bronchospasm.

Death may occur between the fifth and tenth day postexposure because of pulmonary insufficiency complicated by a compromised immune response from mustard-induced bone marrow damage.

Atropine (0.4–0.6 mg intramuscular or intravenous) or other anticholinergic or antiemetic drugs may be used to control nausea and vomiting.

Sterilization of the gastrointestinal tract by nonabsorbable antibodies may reduce the possibility of infection from enteric organisms. Bone marrow transplants or blood transfusions may be indicated. The recent introduction of granulocyte colony stimulating factor may offer hope in the management of bone marrow depression.

A victim of nitrogen mustard exposure also requires the general supportive care given to a severely ill patient as well as the specific care given to a burn patient. This includes the liberal use of systemic analgesics and antipruritics and the maintenance of fluids and electrolyte balance. Parenteral food supplements and vitamins may also be beneficial.

See also: Bio Warfare and Terrorism: Toxins and Other Mid-Spectrum Agents; Blister Agents/Vesicants; Chemical Warfare Delivery Systems; Mustard Gas; Nerve Agents.

Relevant Websites

http://www.bt.cdc.gov – US Centers for Disease Control and Prevention, Agency for Toxic Substances and Disease Registry, Chemical Agents.

http://sis.nlm.nih.gov – US National Library of Medicine, Specialized Information Services, Chemical Warfare Agents.

Nitrogen Oxides

Lee R Shugart

- Chemical Abstracts Service Registry Number: CAS 10102-44-0
- Synonyms: Nitrogen dioxide (Nitrogen peroxide)
- Chemical/Pharmaceutical/Other Class: Inorganic, gas
- Chemical Formula: NO_2

Uses

Used in the nitration of organic compounds and explosives, in the manufacture of oxidized cellulose compounds, and as an oxidizing agent in rocket propulsion. It is an intermediate in the production of nitric and sulfuric acids.

Background Information

Nitrogen dioxide belongs to a family of highly reactive gases called nitrogen oxides (NO_x). These gases form when fuel is burned at high temperatures and come principally from motor vehicle exhaust and stationary sources such as electric utilities and industrial boilers. A suffocating, brownish gas, nitrogen dioxide is a strong oxidizing agent that reacts in the air to form corrosive nitric acid, as well as toxic organic nitrates. It also plays a major role in the atmospheric reactions that produce ground-level ozone (or smog).

Exposure Routes and Pathways

Inhalation, skin contact, eye contact, and/or ingestion are the main routes of exposure.

Mechanism of Toxicity

Nitrogen dioxide acts mainly as an irritant affecting the mucosa of the eyes, nose, throat, and respiratory tract.

Acute and Short-Term Toxicity (or Exposure)

Human

The acute toxicity of nitrogen dioxide by inhalation is high. Toxic effects may occur after exposure to concentrations of 10 ppm for 10 min and include coughing, chest pain, frothy sputum, and difficulty in breathing. Nitrogen dioxide at concentrations of 10–20 ppm is mildly irritating to eyes; higher concentrations are corrosive to the skin, eyes, and mucous membranes. Short-term exposure following ingestion includes nausea, vomiting, and stomach pain.

Chronic Toxicity (or Exposure)

Animal

Animal testing indicates that nitrogen dioxide does not have carcinogenic or reproductive effects and it does not produce heritable genetic damage.

Human

Chronic toxicities following exposure include shortness of breath and pulmonary edema, which may progress to respiratory infections, reduction in the blood's oxygen carrying capacity, lung disorders, eye damage, and digestive disorders.

In Vitro Toxicity Data

Nitrogen dioxide produces no genetic damage in bacterial and mammalian cells in cultures.

Clinical Management

If inhaled and breathing is difficult, the person should be moved to fresh air and administered oxygen. For skin contact, the exposed area should be washed with soap and water. For eye contact, the eyes should be flushed with water.

Environmental Fate

Highly reactive with air and decomposes in water.

Ecotoxicology

Nitrogen oxides significantly contribute to a number of environmental effects such as acid rain and eutrophication in coastal waters like the Chesapeake Bay as well as ozone formation, all of which can have adverse effects on both terrestrial and aquatic ecosystems. Fish toxicity: LC_{50} (hematological) for Red drum (*Sciaenops ocellatus*) is $3\,mg\,l^{-1}$ for 24 h. Invertebrate toxicity: LC_{50} (mortality) for Redtail prawn (*Penaecus penicillatus*) is $3.03\,mg\,l^{-1}$ for 144 h. Animal toxicity: LC_{50} (mortality) for rat by inhalation is 88 ppm for 4 h.

Exposure Standards and Guidelines

The US Environmental Protection Agency's health-based national air quality standard for nitrogen dioxide is 0.053 ppm (measured as an annual arithmetic mean concentration). National Institute for Occupational Safety and Health and Occupational Safety and Health Administration recommended exposure limit is 1 ppm ($1.8\,mg\,m^{-3}$).

See also: Chemicals of Environmental Concern; Nitrocellulose; Ozone.

Further Reading

Anyanwu E (1999) Complex interconvertibility of nitrogen oxides (nox): Impact on occupational and environmental health. *Reviews on Environmental Health* 14(3): 169–185.

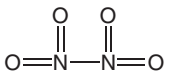
Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Nitrogen Oxides.

Nitrogen Tetraoxide

Samantha E Gad

- Chemical Abstracts Service Registry Number: CAS 10544-72-6
- Synonyms: Liquid nitrogen dioxide under pressure; Nitrogen peroxide; Dinitrogen tetroxide; Dinitrogen tetraoxide; Tetra oxide
- Chemical/Pharmaceutical/Other Class: Nitrogen oxides
- Chemical Formula: N_2O_4
- Chemical Structure:



Uses

Nitrogen tetraoxide is formed by pressurizing liquid nitrogen dioxide. It is a gas at normal temperature and pressure, and is used in the manufacture of explosives and rocket fuels. Nitrogen tetraoxide is used as a catalyst in oxidation reactions and in many other industrial applications. It is also a component of nitric and sulfuric acid.

Exposure Routes and Pathways

Principal routes of exposure are generally inhalation; for example, inhalation of industrial gases, fumes resulting from the welding process, vapors arising from the contact of nitric acid with organic materials, from the exhaust of metal cleaning processes, vapors associated with electroplating, engraving, and photogravure operations, dynamite blasting, diesel engine

exhaust, and polluted air resulting from internal combustion engine exhaust. Dermal exposure is rare.

Toxicokinetics

Toxic effects may result from inhalation exposures and from skin absorption.

Mechanisms of Toxicity

Nitrogen tetraoxide is absorbed through the respiratory system and reacts with blood, reducing fluid levels, inducing massive pulmonary edema, and a severe reduction in hemoglobin levels.

Acute and Short-Term Toxicity (or Exposure)

Animal

The inhalation LC_{50} is 315 ppm in rabbits. Nitrogen tetraoxide as a liquid can cause severe burns from even brief contact with the skin or eyes.

Human

The liquid is highly corrosive to the skin and may cause chemical burns. The vapor is extremely irritating to the eyes, and is capable of causing pain and severe conjunctivitis. A review by the American Conference of Governmental Industrial Hygienists (ACGIH) suggests that a 60 min exposure to 100 ppm leads to pulmonary edema and death; 50 ppm to pulmonary edema with possible subacute or chronic lesions in the lungs; and 25 ppm to respiratory irritation and chest pain. About 50 ppm is moderately irritating to the eyes and nose; 25 ppm is irritating to some people. The effects of exposure are insidious, leaving an exposed person asymptomatic for days, even at a fatal dosage. Only high concentrations show immediate toxic effects. The latent period may be from 5 to 72 h, and initial symptoms include coughing and nausea. Vapor can cause pain, severe conjunctivitis, and other effects to the eye. The liquid is highly corrosive to the skin. Nitrogen tetraoxide is a class A poison (US Code of Federal Regulations (CFR) 173, Section 173.326).

Chronic Toxicity (or Exposure)

Human

Long-term exposure of small levels can cause bronchitis, interstitial edema, epithelial proliferation, and possible emphysema and fibrosis. It is classified by the ACGIH as Category A4 (not classifiable as causing cancer in humans).

Clinical Management

Exposure to nitrogen tetraoxide in the missile industry can lead to symptoms identical to those from nitrogen dioxide. Medical assistance should be sought immediately after any inhalation exposure, however small. If eyes or skin are exposed these should be well rinsed with water.

Environmental Fate

Nitrogen tetroxide is released to the atmosphere where it can undergo reactions, including leading to air pollution.

Exposure Standards and Guidelines

The ACGIH threshold limit value, 8 h time-weighted average, is 3 ppm ($6\,mg\,m^{-3}$), and the short-term exposure limit (STEL) is 5 ppm ($10\,mg\,m^{-3}$). The US National Institute for Occupational Safety and Health (NIOSH) STEL, for a 15 min exposure, is 1 ppm ($1.8\,mg\,m^{-3}$), and the NIOSH Immediately Dangerous to Life or Health value is 20 ppm.

See also: Nitrogen Mustard; Nitrous Oxide.

Further Reading

Liekauf GD and Prows DR (2001) Inorganic compounds of carbon, nitrogen, and oxygen. In: Bingham E, Cohrssen B, and Powell CH (eds.) *Patty's Toxicology*, 5th edn., vol. 3, pp. 607–730. New York: Wiley.

Relevant Websites

http://www.intox.org – International Programme on Chemical Safety (IPCS). Nitrogen Oxides, 2nd edition (Environmental Health Criteria 188).

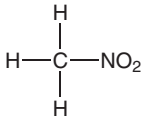
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Nitrogen Tetraoxide.

Nitromethane

Richard D Phillips

This article is a revision of the previous print edition article by Daniel Steinmetz, volume 2, pp. 427–428, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 75-52-5
- Synonym: Nitrocarbol
- Chemical Formula: CH_3NO_2
- Chemical Structure:



Uses

Nitromethane has been used as a chemical stabilizer for a variety of halogenated hydrocarbon solvents and aerosol propellants. In addition, it is a solvent, a chemical intermediate, a fuel for professional racing and hobby cars, an explosive when mixed with ammonium nitrate, and a rocket propellant.

Exposure Routes and Pathways

Nitromethane is a colorless, oily liquid with a moderately strong, somewhat disagreeable odor. Production of nitromethane and its use as a solvent, fuel additive stabilizer for halogenated alkanes and intermediates, may result in its release into the environment, principally the atmosphere. Human exposure to nitromethane may additionally occur via dermal contact and accidental ingestion.

Toxicokinetics

Toxicokinetic data on nitromethane are limited. Nitromethane may be metabolized to formaldehyde based on *in vitro* studies with liver microsomes, but only in trace amounts. In addition, nitromethane may form a cytochrome P450 NO complex. Nitromethane appeared to compete with carbon monoxide for a common binding site. However, nitromethane appears to undergo limited metabolic denitrification.

Mechanism of Toxicity

Nitromethane affects the central nervous system (CNS) via narcosis as a solvent. It is also a mild pulmonary irritant. In addition, nitromethane produces histidinaemia in rats by decreasing hepatic histidase activity, leading to increased tissue levels of histidine.

Acute and Short-Term Toxicity (or Exposure)

Animal

Dogs were more sensitive than other species following an oral dose with 125 mg kg^{-1} causing a toxic effect. Doses ranging from 250 to 1500 mg kg^{-1} caused death in all dogs tested. Pathologic lesions were seen in the liver and kidney.

Death occurred in one monkey exposed at 1000 ppm vapor for 48 h. A concentration of 5000 ppm for 3 h resulted in death to guinea pigs. Nitromethane concentrations of 500 ppm were tolerated for 140 h (6 h day^{-1} exposure) by guinea pigs, rabbits, and monkeys. The most common signs of toxicity were CNS depression and slight irritation in the respiratory tract. Histopathologic changes were mainly in the liver and kidneys with liver showing the most prominent injury.

The US National Toxicology Program (NTP) published the results from a 13 week subchronic inhalation study of nitromethane in both sexes of F344/N rats and B6C3F1 mice. Exposures were at 0, 94, 187, 373, 748, and 1500 ppm of nitromethane. At 1500 ppm, a significant decrease in body weight gain was observed for male rats. A similar trend (though not statistically significant) was observed in female mice exposed to 1500 ppm. Body weights were not depressed in the other rat groups or in the mice.

Neurological effects were observed in all male and female rats in the 1500 ppm groups and partially in the 748 ppm group. There were no exposure-related clinical signs of toxicity in mice. In addition, nitromethane caused exposure-related microcytic responsive anemia in male and female rats.

Exposure to nitromethane was also associated with minimal to mild hyperplasia of the bone marrow in both rats and mice.

In a 6 month inhalation study, New Zealand White rabbits and Sprague–Dawley rats were exposed by inhalation to 0, 98, or 745 ppm (0, 245, or 1860 mg m^{-3}) nitromethane for 7 h day^{-1}, 5 days per week for 6 months. Decreased body weight gain was observed in rats after 8 weeks of exposure to 745 ppm. The most notable response in rabbits was an effect on the thyroid: increased thyroid weight and decreased serum thyroxine levels. There were no exposure-related gross or microscopic lesions in either rats or rabbits exposed to 98 or 745 ppm.

Human

Signs and symptoms of toxicity include dermatitis due to solvent action of nitromethane. By analogy, with effects seen in laboratory animals, nitromethane may cause mild pulmonary irritation, weakness, and ataxia muscular incoordination at fairly high levels. More severe effects such as convulsions, liver and kidney injury are possible under conditions of severe overexposure. The American Conference of Governmental Industrial Hygienists (ACGIH) recommends a threshold limit value time-weighted average (TLV-TWA) of 20 ppm ($50\,mg\,m^{-3}$) for an occupational exposure to nitromethane to reduce the potential for adverse thyroid effects and pulmonary hemorrhage reported for inhalation exposed rabbits and rats.

Chronic Toxicity (or Exposure)

Animal

Nitromethane was administered $7\,h\,day^{-1}$, 5 days per week for 2 years via inhalation to male and female Long–Evans rats at 100 and 200 ppm. There were no pharmacologic effects for the exposure at either dose rate. The body weights of the female rats for both exposure groups were slightly less than the control. There was no effect on the hematology, no clinically significant effects of serum chemistry, no effects on organ weight, and no significant differences in the nonneoplastic or neoplastic pathology related to exposure.

The NTP-sponsored a 2 year chronic inhalation study on male and female rats (F344/N) and male and female mice (B6C3F1) with exposures at 6 h, 5 days per week for 103 weeks. The rats were exposed to 0, 94, 188, or 375 ppm; the mice were exposed to 0, 188, 375, or 750 ppm. Clinical findings consistent with mammary gland neoplasms were noted in female rats, but not in males, at some of the concentrations tested.

For mice, clinical findings at the highest doses included swelling around the eyes and exophthalmos in exposed males and females. This is consistent with harderian gland adenoma or carcinoma (combined) in exposed mice with increasing exposure. Female mice also had increased liver and lung tumors and nonneoplastic nasal damage. Males had lung and nonneoplastic nasal damage

The NTP study concluded that there was (1) clear evidence of carcinogenic activity from nitromethane in female F344/N rats, based on increased incidences of mammary gland fibroadenomas and carcinomas; (2) clear evidence of carcinogenic activity in male B6C3F1 mice, based on increased incidences of Harderian gland adenomas and carcinomas; (3) clear evidence of carcinogenic activity in female B6C3F1 mice, based on increased incidences of liver neoplasms and harderian gland adenomas and carcinomas; and (4) male F344/N rats showed no evidence of carcinogenic activity from nitromethane.

Human

There is no epidemiological evidence or case reports specific to exposure to nitromethane in the published scientific literature.

In Vitro Toxicity Data

Nitromethane has given consistently negative results in bacterial mutagenicity assays, and *in vitro* mammalian tests for sister chromatid exchanges and chromosomal aberrations. It was not mutagenic in *Drosophila*. It did not induce micronuclei *in vitro* in Syrian hamster embryo cells or *in vivo* in mice. However, nitromethane did show a positive response at high concentration in a cell transformation assay in Syrian hamster embryo cells. The results of short-term tests on nitromethane do not indicate that the compound has genotoxic activity.

Clinical Management

Appropriate procedures should be instituted for anyone overcome by nitromethane (e.g., removal from exposure to fresh air, washing skin areas, and irrigation of eyes with water). Treatment for pulmonary irritation or dermatitis may be needed if symptoms are present.

Exposure Standards and Guidelines

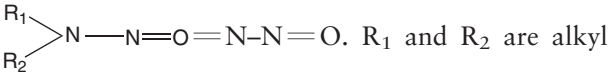
ACGIH TLV-TWA of 20 ppm ($50\,mg\,m^{-3}$).

Relevant Website

http://monographs.iarc.fr – International Agency for Research on Cancer (IARC, 2000) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, vol. 77 – Some Industrial Chemicals, pp. 487–502.

Nitrosamines

Heriberto Robles

- Preferred Name: *N*-Nitrosamines
- Chemical/Pharmaceutical/Other Class: Dialkylnitrosamines; Nitrosamines
- Chemical Structure: The name nitrosamines applies to a family of compounds that have an alkyl and an aryl group attached to the chemical group,  . R_1 and R_2 are alkyl and aryl groups

Uses

Nitrosamines have no known industrial use. However, they can be found in processed foods as unintentional by-products of food preparation and processing.

Background Information

Nitrosamines are formed by a reaction between nitrates or nitrites and certain amines. Nitrosamines and their precursors can be found in diverse consumer products such as processed meats, alcoholic beverages, cosmetics, and cigarette smoke. Nitrosamines are considered to be strong carcinogens that may produce cancer in diverse organs and tissues including lung, brain, liver, kidney, bladder, stomach, esophagus, and nasal sinus.

In 1957, a new malignant disease was reported in Norway's fur farms. Farmed mink fed a diet containing fish (herring) meal developed an unknown liver disease. Later, in the early 1970s, additional outbreaks of liver disease and cancer were reported in Norway's farm animals. Feeding experiments and extensive toxicological research using herring meal on cows and sheep resulted in liver damage and death of some animals. Upon examination, nitrite-treated herring meal was found to contain up to 100 ppm of dimethylnitrosamine. At the time of the incident, sodium nitrite was being used in Norway as a preservative for fish meal. It is now known that sodium nitrate reacted with amines normally present in fish to produce dimethylnitrosamine, a potent nitrosamine carcinogen.

Exposure Routes and Pathways

The most common route of exposure is by oral ingestion of nitrosamines in food. It has been estimated that the general population consumes approximately 0.1 μg of nitrosamines per day in their diet. Nitrosamines can be found in foods preserved with nitrates as well as in untreated foods such as mushrooms, alcoholic beverages, smoked fish, bacon, ham, and some cheeses. Nitrosamines have also been found in tobacco smoke and urban air. Nitrosamines can also be formed in the mouth or stomach if the food contains nitrosamine precursors. Under acidic pH in the mouth or stomach, nitrite or nitrates added to food or naturally occurring may combine with amines to form nitrosamines.

While the major route of exposure for the general population is through the consumption of nitrosamines in food, the total dose consumed by cigarette smokers is considerably larger. It has been estimated that cigarette smokers may inhale up to 17 μg of nitrosamines per day.

Toxicokinetics

The physical properties of nitrosamines vary widely depending on the nature of the substituent groups R_1 and R_2. Similarly, the nature of the substituent group has an effect on the toxicological properties of nitrosamines. For example, the LD_{50} of nitrosamine compounds is directly proportional to the carbon chain length of the substituent. Long-chain substituents have a higher LD_{50}. Also, the nature of the substituent group has an effect on the carcinogenicity properties of nitrosamines. Dimethyl and diethyl compounds cause predominantly liver tumors, while dibutyl compounds tend to cause bladder tumors.

Nitrosamines have a short half-life that has been measured in the order of minutes.

Mechanism of Toxicity

Nitrosamines are not carcinogenic at the point of application. They require bioactivation. One possible mechanism of biotransformation is by enzymatic transformation to a carbonium ion. Activation is known to proceed first by hydroxylation of an α-carbon. The resulting hydroxyalkyl moiety is eliminated as an aldehyde, and an unstable primary nitrosamine is formed. The unstable nitrosamine ultimately tautomerizes to a carbonium ion. The highly reactive carbonium ion readily alkylates with the nearby cellular macromolecules. Cancer and mutagenicity develop when reactive nitrosamine metabolites alkylate to genetic macromolecules.

Acute and Short-Term Toxicity (or Exposure)

Animal

Nitrosamines are strong hepatotoxic agents. Large, acute doses produce liver necrosis and hemorrhages in the liver and other tissues.

Chronic Toxicity (or Exposure)

Animal

Nitrosamines and *N*-nitroso compounds are strong carcinogens that produce cancer of the liver and kidneys. In experiments conducted to date, 75–80% of nitrosamines tested have been found to be carcinogenic to mammals. Dimethylnitrosamine, a member of the nitrosamine family, is highly carcinogenic to the liver and kidneys of almost all the mammalian species tested.

Rats exposed to tobacco-derived nitrosamines developed tumors at the nose, mouth, esophagus, lung, and pancreas. Tobacco-derived nitrosamines caused upper respiratory tract cancers in exposed hamsters.

It appears that all animals are susceptible to the carcinogenic action of nitrosamines. For example, dimethylnitrosamine given by gavage, in drinking water or in the feed, produced liver tumors in rats, mice, guinea pigs, hamsters, rabbits, dogs, and monkeys.

Human

Chronic, continuous exposure to low doses of nitrosamines in the diet is considered to be of toxicological importance to humans. There is a significant body of epidemiological data that links exposure to nitrosamines and human cancer.

Tobacco-derived nitrosamines are considered to be one of the major cancer-causing agents found in tobacco smoke and tobacco products. This is of importance as up to 90% of human lung cancers can be linked to cigarette smoking. The most potent carcinogen found in tobacco is the nicotine-derived nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone.

In Vitro Toxicity Data

In vitro studies have demonstrated the mutagenic activity of nitrosamines. For example, mutagenicity assays using dimethylnitrosamines have been positive for *Salmonella typhimurium*, *Escherichia coli*, and *Neurospora crassa*. Dimethylnitrosamine also produced mitotic recombination in *Salmonella cerevisiae*, recessive lethal mutations in *Drosophila melanogaster*, and chromosomal aberrations in mammalian cells.

Clinical Management

Nitrosamine exposure is not an acute hazard. Health hazards associated with nitrosamine exposure are limited to cancer, and liver and kidney damage associated with chronic exposure. No specific treatment exists for nitrosamine intoxication. Supportive and symptomatic treatment should be provided.

Since nitrosamines and their precursors are present in the food, exposure to nitrosamines cannot be avoided. However, recent studies have shown that ingestion of adequate quantities of vitamin E and selenium may reduce the risk of cancer. It is known that carcinogenic nitrosamines are formed from the reaction of some amines with nitrites and nitrates present in the diet. Vitamin E and selenium have been found to minimize or prevent the reaction of nitrites/nitrates with amines and hence prevent or reduce the formation of carcinogenic nitrosamines.

Vitamin C (ascorbic acid) is known to inhibit nitrosamine formation. For this reason, manufacturers of cured meat are now required to add vitamin C to their meat products.

Exposure Standards and Guidelines

The US Environmental Protection Agency (EPA) has included some nitrosamines under its B2 – probably human carcinogens – classification.

The US EPA has estimated oral cancer slope factors for some of the most common nitrosamines. Cancer slope factors published in the EPA's Integrated Risk Information System range from $4.9 \times 10^{-3}\,\mathrm{mg^{-1}\,kg^{-1}\,day^{-1}}$ for *N*-nitrosodiphenylamine (CAS 86-30-6) to $150\,\mathrm{mg^{-1}\,kg^{-1}\,day^{-1}}$ for *N*-nitrosodiethylamine (CAS 55-18-5). The same agency has established inhalation unit risk factors that range from $6.4 \times 10^{-4}\,\mu\mathrm{g^{-1}\,m^{-3}}$ for *N*-nitrosopyrrolidine (CAS 930-55-2) to $4.3 \times 10^{-2}\,\mu\mathrm{g^{-1}\,m^{-3}}$ for *N*-nitrosodiethylamine.

See also: Carcinogen–DNA Adduct Formation and DNA Repair; Carcinogenesis; Epidemiology; Nitrites; Tobacco Smoke.

Further Reading

Chow CK and Hong CB (2002) Dietary vitamin E and selenium and toxicity of nitrite and nitrate. *Toxicology* 180(2): 195–207.

Vermeer IT and Maanen JM (2001) Nitrate exposure and the endogenous formation of carcinogenic nitrosamines in humans. *Reviews on Environmental Health* 16(2): 105–116.

Weisburger JH and Chung FL (2002) Mechanisms of chronic disease causation by nutritional factors and tobacco products and their prevention by tea polyphenols. *Food and Chemical Toxicology* 40(8): 1145–1154.

Nitrous Oxide

Shayne C Gad

This article is a revision of the previous print edition article by Jayne E Ash and Shayne C Gad, volume 2, pp. 430–431, © 1998, Elsevier Inc.

- Chemical Service Registry Number: CAS 100-24-97-2
- Synonyms: Dinitrogen monoxide; Laughing gas; Hyponitrous acid anhydride; Factitious air; Nitrogen monoxide; Entonox; Nitronox
- Chemical/Pharmaceutical/Other Class: An oxide of nitrogen
- Chemical Formula: N_2O
- Chemical Structure: $-N=N^+=O$

Uses

Nitrous oxide is used therapeutically as an anesthetic or analgesic. It is also used in the formulation of rocket fuel and as a propellant for whipped cream. It occurs endogenously. Nitrous oxide is a common inhalant drug of abuse.

Exposure Routes and Pathways

Inhalation is the route of exposure.

Toxicokinetics

Nitrous oxide is rapidly absorbed from inspired air. Some patients lose consciousness when breathing 30% nitrous oxide in oxygen and most will become unconscious with 80%. It is almost entirely eliminated through the lungs, with small amounts through the skin and in urine.

Mechanism of Toxicity

High concentrations of nitrous oxide have a narcotic and/or asphixiant effect. By inactivating vitamin B_{12}, a critical cofactor in hematopoiesis and lipid membrane formation, nitrous oxide can cause anemia and neuropathy via selective inhibition of methionine synthase, a key enzyme in methionine and folate metabolism.

Acute and Short-Term Toxicity (or Exposure)

Animal

An asphixiant and narcotic at higher concentrations. The inhalation LC_{50} is $160\,m^{-3}$ in rats.

Human

Nitrous oxide causes drowsiness and headache. Anesthesia with nitrous oxide as the sole anesthetic in normal humans for periods of 2–4 h has induced tachypnea, tachycardia, increased systemic blood pressure, atrioventricular junctional rhythm, acute cardiovascular failure, mydriasis, diaphoresis, and occasional clonus and opisthotonus.

Chronic Toxicity (or Exposure)

Animal

Teratogenicity has been observed in studies of rats, rabbits, cat, and hamsters exposed to nitrous oxide. A carcinogen bioassay of nitrous oxide in mice exposed for $4\,h\,day^{-1}$, $5\,days\,week^{-1}$ for 78 weeks found no neoplastic or non-neoplastic lesions judged to be related to nitrous oxide.

Human

Occupational exposure has been associated with impairment of psychological functions, but these effects do not occur with trace concentrations. A recent review of the available data concluded that exposure to trace amounts of nitrous oxide is not associated with impaired fertility or an increased risk of developing cancer; however, recent studies seem to suggest a correlation between nitrous oxide anesthesia and hyperhomocysteinemia, an independent risk factor for coronary artery disease. Long-term exposure to high concentrations of nitrous oxide may cause megaloblastic bone-marrow depression and neurological symptoms. Bone-marrow depression was observed in humans exposed for 4 days to high concentrations of nitrous oxide in the treatment of tetanus. Nitrous oxide is a common inhalant drug of abuse, and severe myeloneuropathy has been observed as a complication. Nitrous oxide is listed as A4 (not classifiable as a human carcinogen) by the American Conference of Governmental Industrial Hygienists (ACGIH).

Clinical Management

Exposure should be terminated immediately. Oxygen therapy should be provided if respiratory difficulties are present.

Environmental Fate

Emission of nitrous oxide from medical use has been estimated to contribute less than 0.05% to total annual greenhouse gas emission.

Exposure Standards and Guidelines

The ACGIH threshold limit value, 8 h time-weighted average (TWA), is 50 ppm, and the US National Institute for Occupational Safety and Health recommended exposure level is 25 ppm as a TWA during the period of anesthetic administration.

See also: Nitric Oxide.

Further Reading

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Miller MA, Martinez V, McCarthy R, and Patel MM (2004) Nitrous oxide 'whippit' abuse presenting as clinical B12 deficiency and ataxia. *The American Journal of Emergency Medicine* 22: 124–126.

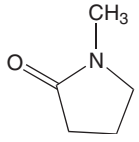
O'Sullivan I and Benger J (2003) Nitrous oxide in emergency medicine. *Emergency Medicine Journal* 20: 214–217.

Weimann J (2003) Toxicity of nitrous oxide. *Best Practice and Research. Clinical Anaesthesiology* 17: 47–61.

N-Methylpyrrolidone

Ralph J Parod

- Chemical Abstracts Service Registry Number: CAS 872-50-4
- Synonyms: *N*-Methyl-2pyrrolidone; 1-Methyl-2-pyrrolidone; NMP
- Chemical/Pharmaceutical/Other Class: Cyclic amide
- Chemical Structure:



Uses

N-Methylpyrrolidone (NMP) is used as an extraction solvent in the petrochemical industry, as a paint stripper in occupational (e.g., graffiti removal) and consumer (e.g., furniture) settings, as a solvent in the microelectronics industry, and as a chemical reaction medium. NMP enhances the penetration of topically applied pharmaceuticals and is used as a formulating agent in pigments, dyes, inks, and pesticides. It has been used increasingly as a replacement for chlorinated solvents that may pose a greater health risk.

Exposure Routes and Pathways

NMP is a liquid under normal environmental conditions. Due to its low vapor pressure, human exposures are primarily limited to dermal contact. Significant inhalation exposures to NMP are possible during applications that generate NMP aerosols (e.g., graffiti removal) or vapors (e.g., unventilated cleaning baths of heated NMP). Due to its complete miscibility in water, NMP vapor concentrations in the atmosphere are limited by the relative humidity, ranging from 0 ppm (100% relative humidity) to 315 ppm (0% relative humidity).

Toxicokinetics

NMP is freely soluble in both polar and nonpolar solvents and should readily cross biological membranes. This expectation is consistent with experimental studies indicating NMP is well absorbed following inhalation (40–60%), oral (about 100%), and dermal ($\leqslant$100% depending on conditions) exposures. Dermal absorption has been extensively studied as it typically poses the greatest potential for human exposure. Due to its irritant properties, neat NMP is unlikely to remain in voluntary contact with the skin for more than several hours. During this time, the flux of NMP through human skin is about $2\,\mathrm{mg\,cm^{-2}\,h^{-1}}$. The presence of cosolvents can

affect NMP fluxes. Water inhibits dermal absorption while other organic solvents (e.g., D-limonene) can increase it. For example, the dermal flux of 10% NMP in water is about $0.01\,mg\,cm^{-2}\,h^{-1}$. Prolonged NMP exposures can increase the permeability of the skin to NMP and other compounds; the dermal permeability to water increases sevenfold following a 4–6 h exposure to neat NMP.

Following absorption, NMP is uniformly distributed throughout all major organs in the rat with a volume of distribution (about $0.71\,kg^{-1}$) that approximates total body water. In both the rat and man, NMP is eliminated primarily by metabolism to other compounds via a saturable process; only about 2% of the absorbed NMP is excreted unchanged. The major metabolite is 5-hydroxy-NMP (50–70%) with lesser amounts of *N*-methylsuccinimde, 2-hydroxy-*N*-methylsuccinimide, and possibly other unidentified metabolites. The half-life of NMP in plasma is $\sim$4 h. Studies with radiolabeled NMP indicate the most of the radiolabel is excreted in the urine ($\leqslant$95%), with lesser amounts in the feces ($\leqslant$5%) and expired air ($\leqslant$2%). Ongoing studies are investigating the use of 5-hydroxy-NMP as a urinary biomarker for human exposures to NMP.

Mechanism of Toxicity

Developmental toxicity is the most sensitive endpoint associated with NMP exposures in experimental animals. While the mechanism responsible for this effect is unknown, available data suggest that NMP may be the proximate toxin.

Acute and Short-Term Toxicity (or Exposure)

Animal

NMP has a low acute toxicity. The oral LD_{50} values for NMP in multiple species range between 3500 and $7900\,mg\,kg^{-1}$; the dermal LD_{50} values in rats and rabbits range between 4000 and $10\,000\,mg\,kg^{-1}$. Respiratory tract irritation but no deaths were observed in rats exposed nose-only to a NMP vapor/aerosol mixture of $5100\,mg\,m^{-3}$ for 4 h. NMP is irritating to the skin and eyes. When instilled into the eye, NMP also causes corneal opacity, iritis, and conjunctivitis; however, these effects were reversible within 21 days. Sensitization studies have been negative. In 28 day feeding studies, body weight decrements, clinical chemistry changes, centrilobular liver hypertrophy, and testicular degeneration were observed in rats at $\geqslant 1230\,mg\,kg^{-1}$; in similarly exposed mice, toxicity was limited to epithelial swelling of the kidney distal tubules at $\geqslant 2130\,mg\,kg^{-1}$. The 28 day no-observed-adverse-effect levels (NOAELs) in rats and mice were about 450 and about $800\,mg\,kg^{-1}$, respectively. In 90 day feeding studies in rats, body weight decrements and changes in 3 of 36 neurobehavioral parameters occurred at $\geqslant 430\,mg\,kg^{-1}$ while increased liver weights (with centrilobular hypertrophy) and increased kidney weights (without associated histopathology) were observed at $\geqslant 1340\,mg\,kg^{-1}$. In 90 day feeding studies in mice, toxicity was limited to centrilobular liver hypertrophy at $\geqslant 620\,mg\,kg^{-1}$. The 90 day NOAELs in rats and mice were about 180 and about $280\,mg\,kg^{-1}$, respectively. NMP is not clastogenic *in vivo*.

Human

Few data on the acute toxicity of NMP in humans are available. Volunteers exposed to $\leqslant$12 ppm NMP for 8 h did not experience any eye or respiratory tract irritation, symptoms such as headache, dizziness, or nausea, or changes in pulmonary function measured by spirometry. At 12 ppm NMP, two of six subjects reported an acetone-like odor. NMP did not produce signs of sensitization in a repeated-insult patch test with NMP, although a minor and transient irritation was observed.

Chronic Toxicity (or Exposure)

Animal

Whole body exposure of rats to $\leqslant$100 ppm NMP vapor for $6\,h\,day^{-1}$, $5\,days\,week^{-1}$ for a lifetime resulted in only a slight decrement in male body weight at 100 ppm. In another study, rats receiving a lifetime dietary exposure to NMP exhibited decrements in body weight and an increase in the severity of chronic progressive nephropathy (males only) at the highest doses tested, $678\,mg\,kg^{-1}$ (males) and $939\,mg\,kg^{-1}$ (females); the lifetime NOAELs were $207\,mg\,kg^{-1}$ (males) and $283\,mg\,kg^{-1}$ (females). In both studies, sex organ histopathology was normal, and the incidence of cancer was not increased. Mice receiving a lifetime dietary exposure to NMP exhibited an increase in hepatocellular adenomas and carcinomas at the highest doses tested, $1089\,mg\,kg^{-1}$ (males) and $1399\,mg\,kg^{-1}$ (females); centrilobular liver hypertrophy was also noted in males at the high dose. Sex organ histopathology was normal. The lifetime NOAELs in mice were $173\,mg\,kg^{-1}$ (males) and $221\,mg\,kg^{-1}$ (females). The increased tumor incidence seen in mice may occur via a nongenotoxic mechanism given the negative results seen with NMP in both *in vitro* and *in vivo* genotoxicity tests. The human relevance of these positive results in mice is unknown.

In two multigeneration rat reproduction studies conducted under current guidelines, dietary exposures to NMP at the highest dose tested ($350\,mg\,kg^{-1}$) produced some signs of systemic toxicity in parental animals but did not affect reproductive performance or fertility; however, this dose resulted in decrements in pup survival and body weights. Sex organ histopathology and sperm parameters were normal. The NOAEL for reproduction was $>350\,mg\,kg^{-1}$; the NOAEL for developmental toxicity was $160\,mg\,kg^{-1}$. The normal sex organ histopathology noted in chronic and multigeneration reproduction studies combined with the normal reproductive performance in the latter studies suggest that NMP does not pose a significant reproductive hazard to humans.

Developmental studies have been performed via the dermal, oral, and inhalation routes of exposure in both rats and rabbits. Based on results of these studies, it appears NMP can sometimes cause developmental effects in the absence of maternal toxicity. Developmental toxicity is typically manifested by fetotoxic effects (e.g., decrements in fetal body weight), although malformations have been observed above fetotoxic doses. NMP also appears to be a more potent developmental toxin via the inhalation route (lowest-observed-adverse-effect level or LOAEL equivalent to about $120\,mg\,kg^{-1}\,day^{-1}$) than by either the oral (LOAEL about $250\,mg\,kg^{-1}\,day^{-1}$) or dermal (LOAEL about $750\,mg\,kg^{-1}\,day^{-1}$) routes; NOAELs associated with these exposure routes are about twofold lower than the LOAELs.

Human

No data on the chronic toxicity of NMP in humans are available.

In Vitro Toxicity Data

Data on the *in vitro* mutagenicity and clastogenicity of NMP are negative.

Clinical Management

Exposed skin and eyes should be irrigated immediately with water to minimize irritation and systemic absorption. Medical attention should be sought if symptoms or health concerns persist.

Environmental Fate

When released to the environment, NMP is expected to partition at equilibrium almost exclusively to water where it is readily biodegraded. Due to its low Henry's law constant ($1.6 \times 10^{-3}\,Pa\,m^3\,mol^{-1}$), significant volatilization of NMP from water is not expected. NMP that does reach the atmosphere will be removed by reaction with photochemically produced hydroxyl radicals (5.2 h half-life) and rain washout. NMP is not expected to adsorb significantly to soil and sediment matrices based on its calculated absorption coefficient (K_{oc}) of 9.6; the half-lives of NMP in various soil matrices are 4 days (clay), 8 days (loam), and 12 days (sand). Based on its low bioconcentration factor (0.16) and log octanol–water partition coefficient (-0.73), NMP should not pose a significant bioaccumulation hazard.

Ecotoxicology

The 96 h LC_{50} values for a variety of fish species range between 680 and $4000\,mg\,l^{-1}$. For a variety of aquatic invertebrates, the 48 h LC_{50} values are $>1000\,mg\,l^{-1}$; in algae the EC_{50} values are $>500\,mg\,l^{-1}$.

Exposure Standards and Guidelines

International occupational exposure limits (OELs) for NMP generally range between 5 and 50 ppm as an 8 h time-weighted average (TWA). The American Conference of Governmental Industrial Hygienists has not established an 8 h TWA OEL for NMP. Several countries have established a short-term excursion limit of 75 ppm. The California Office of Environmental Health Hazard Assessment has identified NMP as a reproductive toxin and established maximum allowable daily limits for exposure of $3200\,\mu g\,day^{-1}$ (via inhalation) and $17\,000\,\mu g\,day^{-1}$ (via dermal contact).

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N-Nitrosodimethylamine

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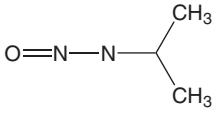
- Chemical Abstracts Service Registry Number: CAS 62-75-9
- Synonyms: Nitrosamine; Dimethylnitrosamine; *N*-Methyl-*N*-nitroso-ethanamine
- Chemical Formula: $C_2H_6N_2O$
- Chemical Structure:



Uses

Dimethylnitrosamine (DMN) is used commonly as an industrial solvent in the production of dimethylhydrazine; formerly used in the production of hypergolic rocket fuels, thiocarbonyl fluoride polymers, and as soluble cutting oil. It is presently used as an antioxidant, as an additive for lubricants, gasoline, and as a softner of copolymers. Nitrosamines have also been found in multiple cosmetic products, hand and body lotions, shampoos, and have been patented for use in pesticides and nematocides. Alarming levels have been found in soil, postulated to be from the use of triazine herbicide, which can react with ubiquitously used nitrogenous fertilizers. DMN is a research chemical, air and water pollutant, and tobacco smoke condensate. Presence of this compound has also been found in nonfat dry milk, gastric juices, rubber products, rubber manufacturing, metal industries, and certain chemical manufacturing.

Exposure Routes and Pathways

The common exposure routes are inhalation (contaminated air), skin contact, ingestion through food and water, and *in vivo* formation from amines and nitrates; it is classified as a potential human carcinogen. Maximal exposure occurs through food (e.g., nitrite cured meat, fish, or malt beverages), house hold goods (e.g., rubber), tobacco and tobacco smoke, cosmetics, drugs, pesticides, and indoor air (e.g., frying of nitrite cured meat with release of volatile nitrosamines).

No indication can be given about the rate in which a harmful concentration in the air is reached on evaporation of this substance at 20°C. DMN is used extensively in cancer research facilities. Human exposure occurs when unchanged DMN is excreted by the laboratory animals. DMN has been used as an industrial solvent and as a chemical intermediate in the production of 1,1-dimethylhydrazine. Other uses or proposed uses include: as a nematocide, as a lubricant additive, as an antioxidant, as a softener for copolymers, and in electrical condensers to increase the dielectric constant. Inhalation risks are very high in those settings.

Toxicokinetics

DMN is rapidly absorbed from all the routes of exposure. This chemical is dangerous because, in most instances, it is produced inside the body. *Biotransformation*: DMN (and many other related) is intrinsically not reactive, and requires metabolic activation by the P450 system for its biological effects. Upon metabolism, very reactive electrophilic carbonium ions (e.g., methyldiazonium) are formed, which in turn attack cellular macromolecules, e.g., protein, DNA, and RNA. *Distribution and elimination*: No clear-cut human data are available. An intravenous bolus dose of $1.35\,\mu mol\,kg^{-1}$ ($100\,\mu g\,kg^{-1}$) to Fisher 344 rats revealed a predominantly hepatic clearance of 2.5 min to 3 h. Orally administered DMN (2.02 or $4.05\,\mu mol\,kg^{-1}$) cleared between 5 and 120 min. Intravenously injected DMN concentrations declined in a biexponential manner with a terminal half-life of 10 min. The apparent total systemic blood clearance distribution was $39\,ml\,min^{-1}\,kg^{-1}$. The apparent steady-state volume of distribution was $297\,ml\,kg^{-1}$. The rate of metabolism of DMN after larger oral doses ($10\,mg\,kg^{-1}$) is $5\,mg\,kg^{-1}\,h^{-1}$ and follows zero-order kinetics. Terminal biological half-life is 11 min, and elimination predominantly by liver. The substance decomposes on heating producing nitrogen oxides. It reacts with strong oxidants and strong bases.

Mechanism of Toxicity

NDMA is a potent alkylating agent. It can cause DNA single-strand breaks, double-strand breaks, and fragmentation in the form of a ladder in cells and tissues. It is well recognized that metabolic activation of NDMA and other *N*-nitrosodialkylamines is required for the generation of electrophilic species that can elicit genotoxic or other damage in cells. This metabolic activation process is believed to be an important factor in determining the tissue and species specificities of some of these carcinogens. NDMA is activated to an alkylating electrophile by *N*-demethylation that is generally accepted as the rate-limiting step in the activation of DMN, which is catalyzed by hepatic cytochromes P450. Different cytochrome P450 species are involved in the metabolism of NDMA in microsomes. They show substrate

specificity and alkyl group selectivity in the metabolism of *N*-nitrosodialkylamines. Cytochrome P450IIE1 displays low K_m and high turnover numbers in catalyzing the demethylation and denitrosation of NDMA and other nitrosamines. It exists in untreated rats, rabbits, and other animals and is inducible by a variety of inducers such as acetone, ethanol, pyrazole, and isoniazid as well as by physiological conditions such as fasting and diabetes. Rat cytochrome P450j is involved in bioactivation of *N*,*N*-dimethylnitrosamine. The K_m value for NDMA demethylase determined in acetone induced rat liver microsomes was found to be 20–30 μM *N*-Nitrosodimethylamine1. Other P450 species contribute to the metabolism of NDMA when this substrate is present at high concentrations, especially cytochrome P450IIB1 suggesting high K_m values. Since animals and humans are rarely exposed to such high concentrations of NDMA, it is believed that P450IIE1 species are the predominant forms responsible for the metabolism of carcinogenic levels of these compounds. Microsomal activation of DMN is decreased by protein and protein-choline deficient diets and is increased by pretreatment with microsomal enzyme inducers. With human liver microsomes of differing cytochrome P450 contents, similar correlation is obtained. Oxidative demethylation of DMN by mouse liver microsomes and the activation of DMN to a mutagen follow similar kinetics. Micronutrients may have an effect on the levels of cytochrome P450 enzymes. Ascorbate deficiency can result in a depression of the levels of cytochrome P450 and cytochrome b5. But excessive intake of vitamin C does not significantly enrich microsomes in cytochrome P450 and b5 content. Different inducers are known to affect the metabolism of the two alkyl groups of an *N*-nitrosodialkylamine differently. Acetone/ethanol-inducible P450IIE1 is more efficient in catalyzing the α-oxidation of the methyl and ethyl groups of NDMA and other nitrosamines than other constitutive forms. The phenobarbital-induced P450(s), on the other hand, is less active in catalyzing the oxidation of these groups in NDMA and NEMA but more active in catalyzing the α-oxidation of the butyl group of NBMA. However, in comparing metabolic activities different results are obtained depending on the substrate concentration.

Acute and Short-Term Toxicity (or Exposure)

Animal

DMN is carcinogenic to all the 10 species tested in single bolus dose or multiple low doses. Primary target organs include liver, kidney, thymus, spleen, lung, skin, and trachea. Swiss mice fed a diet containing 0.005% DMN for 1 week developed tumors of the kidney and lung. Hamsters fed a diet containing 0.0025% for 11 weeks developed liver tumors. ICR mice injected 25–50 mg kg^{-1} (one dose) developed severe hepatotoxicity within 12 h. The LD_{50} in the rat for DMN is 26 mg kg^{-1} and the LC_{50} is 78 ppm over 4 h. A single dose of about 25 mg kg^{-1} DMN administered orally to the rat, or by intravenous, intraperitoneal, or subcutaneous injection produces serious destruction of liver tissue accompanied by hemorrhages into the liver and lungs. Often there occurs a serious accumulation of fluid in the abdominal area and blood in the lumen of the intestines. Death usually occurs in 2–4 days or the animal recovers completely. Rabbits, mice, guinea pigs, and dogs all develop similar liver damage.

Human

Usually does not involve irritation of skin or mucous membranes. Acute poisoning may invoke headaches, malaise, fever, or general weakness. Gastrointestinal effects include abdominal cramping, nausea, and vomiting, eventually leading to diarrhea. Hepatomegaly and jaundice may follow if the exposure is severe or prolonged.

Chronic Toxicity (or Exposure)

Human

Chronic exposure may cause liver disease with jaundice and swelling with a precipitous drop in the platelet count. It is a suspected human carcinogen. Nitrosamines can form in the gastric juice of the human stomach. This is commonly referred to as endogenous nitrosation. Bacteria in the mouth chemically reduce nitrate, which is prevalent in many vegetables, to nitrite, which in turn can form nitrosating agents. Many foods contain amines that can react with nitrosating agents in the acidic stomach to form nitrosamines. While it has been demonstrated that ascorbic acid can reduce nitrosation in the stomach, more research will be required for a fuller understanding of endogenous nitrosation and its ramifications for health and disease. It is carcinogenic to all the other species tested upon prolonged low level exposure. The lowest lethal oral dose in humans has been reported at 10 mg kg^{-1} per 80 week intermittent exposure.

Effects of Long-Term or Repeated Exposure

N-Nitrosodimethylamine (NDMA) produced liver tumors in rats when administered in drinking water

or in the diet. DMN is also known to produce many hemangiomatous tumors and some parenchymal cell tumors in the livers of rats after oral administration. NDMA acts as a transplacental carcinogen when administered to pregnant rats, mice, and Syrian golden hamsters by several routes. Increases in lung, liver, and kidney tumors were observed in both rats and mice exposed by inhalation. Mink are very sensitive to the tumerigenic effects of DMN.

Continuous low doses (adequate for animal survival) of DMN exposure for 7–8 months to the rat resulted in liver cancer. Higher concentrations for shorter periods, or as a single dose, resulted in kidney tumors. Continuous low-level exposures generally cause cancer (or tumor) of the liver and higher concentrations for short periods (or as a single dose) result in kidney tumors. Although, in the rat, cancers of the liver and the kidney are the most frequent outcomes, DMN can be immunotoxic (and immunocarcinogenic) and gastrointestinal-toxic as well. The fact that a single exposure to DMN can result in cancer has tremendous implications with respect to man. While there is no direct evidence that exposure to DMN leads to cancer in humans, indirect evidence, obtained from laboratory experiments that measured the relative metabolic rates of DMN by rat and human liver slices, indicate that man is probably about as sensitive to the carcinogenic action of DMN as is the rat.

NDMA is mutagenic for *Escherichia coli*, *Salmonella typhimurium*, and *Neurospora crassa*. It can produce mitotic recombination in *Sacharoyus cerevesiae* species, recessive lethal mutations in *Drosophilla melanogaster*, and chromosomal aberrations in mammalian cells. Mutagenic responses in bacterial cells are dependent upon the addition of a mammalian drug metabolism system (specific form of cytochrome P450).

Clinical Management

Quick removal of the affected workers from the site of exposure is required and respiration should be established. Absorption should be prevented by repeated flushing with water if exposure to mucous membranes is suspected. Parts of the body that were exposed should be decontaminated with soap and water. It is necessary to evaluate hepatic and renal function tests as thoroughly as possible, while paying special attention to liver size. Chest X-ray and cancer screening are necessary. Human exposure to nitrosamines results from contact with mixtures containing these compounds (e.g., cutting oils, tobacco products). Because of potential confounding by the other substances in these mixtures, data from human exposure is of limited use in the evaluation of carcinogenicity of individual nitrosamines.

Environmental Fate

Very little experimental data are available to predict the environmental fate of *N*-nitrosomethylethylamine in soil, atmosphere, or elsewhere in the environment. Nitrosamines photolyze rapidly in aqueous solution; therefore, photolysis is expected to occur on surfaces exposed to sunlight. Insufficient data are available to predict the relative importance of biodegradation or other transformation processes in soil. Based upon estimated K_{oc} values of 4–73 (2–3, SRC), *N*-nitrosomethylethylamine will be highly mobile in soil and can be expected to leach. Insufficient data are available to predict the relative importance of biodegradation in natural water. Volatilization from water is slow; the estimated volatilization half-life from a shallow, rapidly moving model river is 81 days (2, SRC). Since *N*-nitrosomethylethylamine is relatively soluble in water (30%), adsorption to sediment and bioconcentration in aquatic organisms are not expected to be important fate processes. Based upon an extrapolated vapor pressure of 1.1 mmHg at 20°C, *N*-nitrosomethylethylamine will exist primarily in the vapor phase in the ambient atmosphere (2, SRC). It will degrade rapidly in the vapor phase via direct photolysis in sunlight; the photolysis half-life at a solar zenith angle of 40°N is approximately 5.8 min (3, SRC). By comparison, reaction with photochemically produced hydroxyl radicals is a minor degradation process with an estimated half-life of 1.6 days (4, SRC). NDMA is released to the environment in mainstream and sidestream tobacco smoke. It may be formed in the nighttime atmosphere by reaction of atmospheric amines with nitrous acid. If released to the atmosphere, *N*-nitrosomethylethylamine will degrade rapidly through direct photolysis in sunlight (estimated half-life of 5.8 min at a solar zenith angle of 40°N). If released to soil or water, it will degrade rapidly at the water surface or on soil surfaces exposed to sunlight. Insufficient data are available to predict the relative importance of biodegradation within soil or water. NDMA is highly soluble in water and is therefore expected to leach in soil. The general population is exposed to *N*-nitrosomethylethylamine through inhalation of tobacco smoke and through consumption of various foods.

It is more difficult to determine point sources for DMN emissions than for other toxic agents because DMN is not extensively used by industry and most current occupational exposures will probably occur as a result of the chemical formation of the compound from its precursors rather than from the known utilization of the chemical. The chemical reaction, in the condensed phase, between nitrous acid

and dimethylamine (DMA) or trimethylamine (TMA) to form DMN is well known. Recently, it has been shown that DMA and TMA can react with oxides of nitrogen in the vapor phase to give DMN as a reaction product. This means that even though DMN is not used at a particular location, it may be formed from its precursors and therefore be found in the occupational environment. The fact that oxides of nitrogen can chemically react with amines to produce airborne nitrosamines is of special interest in light of the fact that there appears to be a statistical correlation between high concentrations of NO_2 and high incidence of cancer in some urban areas. NO_2, in itself, is probably not a carcinogen.

Exposure Standards and Guidelines

Current exposure level is $\sim 0.1\,\mu g\,day^{-1}$ due to successful efforts over the last two decades to reduce nitrosamine formation in foods and beverages. In contrast, the National Academy of Sciences report estimated an exposure of $17\,\mu g\,day^{-1}$ from cigarette smoking, although the use of filters has somewhat lowered smokers' exposure. Recent reports indicate that industrial exposure, such as found in a rubber or chemical manufacturing plant, can be relatively high. Nonoccupational exposures to DMN are predominantly via food products. Industrial effluents, automobile exhaust, and environmental auto reduction processes are additional natural burden.

The substance can be absorbed into the body by inhalation and by ingestion. The threshold limit value is A3, skin (American Conference of Governmental Industrial Hygienists, in 2000). MAK: Class 2 (in 2000).

Therapeutic Uses

There are no therapeutic uses. NDMA was a potent carcinogen to all the animal species tested and is a suspected human carcinogen. The lowest lethal oral dose in humans has been reported at $10\,mg\,kg^{-1}$ per 80 week intermittent exposure.

See also: Gasoline; Pesticides; Shampoo; Tobacco Smoke.

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Nonionizing Radiation *See* Radiation Toxicology, Ionizing and Nonionizing.

Non-Lethal Weapons, Chemical

Patricia M Nance

Non-lethal weapons (NLW) or less-than-lethal weapons have been defined by the US Department of Defense (DoD) to be "explicitly designed and primarily employed so as to incapacitate personnel or materiel, while minimizing fatalities, permanent injury to personnel, and undesired damage to property and the environment." NLWs are being developed and evaluated because the US military and other military and law enforcement organizations globally have increasingly been involved in operations that call for different types of weapons and tactics. For example, nontraditional military operations such as those conducted in the 1990s in Bangladesh, Haiti, Somalia, and Bosnia demanded greater flexibility because they commonly involve close and continual interaction between US forces and noncombatant civilians.

The DoD issued a formal Directive (3000.3) on NLWs in 1996. It states that NLWs should enhance the capability of US forces to accomplish objectives including "to discourage, delay, or prevent hostile

actions; to limit escalation; to take military action in situations where use of lethal force is not the preferred option; to better protect our forces; and to temporarily disable equipment, facilities, and personnel." The Directive also states that NLWs are not required to have a zero probability of producing fatalities or permanent injuries; rather they should significantly reduce fatalities and injuries when compared with use of lethal force. The concept of risk–benefit analysis is highlighted in the Directive, which calls for these weapons to "achieve an appropriate balance between the competing goals of having a low probability of causing death, permanent injury, and collateral materiel damage, and a high probability of having the desired anti-personnel or anti-materiel effects."

Riot control agents (RCAs), also known as 'crowd control agents', are a broad category of chemical NLWs that are in use by military and law enforcement agencies around the world. RCAs are intended to temporarily disable a targeted individual by way of irritating the skin and mucous membranes. These agents are generally regarded as safe, with low toxicity, when used as intended, but under increased exposure levels or prolonged durations of exposure they can have toxic effects. There is the potential of dermal, ocular, or pulmonary injury when exposed to high levels in enclosed areas. There is, however, a large margin between the dose intended to harass and the dose that would likely cause a serious adverse effect.

The chemicals in RCAs can be classified physiologically as lacrimators, vomiting agents, or respiratory irritants. The first group will cause eye irritation and lacrimation, the second group will also causing vomiting, and the final group produces uncontrollable sneezing, coughing, and sometimes vomiting. Typical characteristics of RCAs include rapid onset, short period of activity after exposure ceases, and relatively high margins of safety. The lacrimatory effects of many of the chemicals can range from mild to severe, including stinging of the eyes and tearing at low concentrations that can cause temporary disablement. At low levels these compounds will cause reversible effects with no serious injury. At higher levels or prolonged durations of exposure there is serious potential of injuries. At the ocular level such injuries as corneal edema, corneal ulceration and scarring, corneal opacification, and corneal vascularization may occur.

Oleoresin capsicum (OC), pelargonic acid vallinylamide (PAVA), and capsaicin are derived from the pepper plant. The ingredients in hot peppers that are responsible for 'the heat' are called capsaicinoids. Capsaicinoids are a family of chemicals and they come with various heat qualities. The mixture used contains the active ingredient capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) as well as other compounds. PAVA is a pepper derivative that is extremely hot. PAVA (capsaicin II) is the hottest of the capsaicin family. It generates immediate effects after exposure and usually begins to lessen with 15–30 min after removal from the exposure. Occasionally, ocular and mucous membrane effects can last up to 24 h. PAVA and OC are highly effective irritants, generally considered safe, although this is not necessarily accurate. Both sprays will quickly produce lacrimation and closure of the eyes as well as respiratory responses such as bronchoconstriction, severe coughing and sneezing, shortness of breath, and nasal irritation. Other potential effects are a burning sensation on the skin and loss of motor control. Pulmonary system effects of capsaicin and capsaicinoids are dominant, including bronchospasms, respiratory arrest, and pulmonary edema. OC can also cause hypertensive crises and hypothermia. At suprathreshold levels serious respiratory and cardiovascular effects as well as permanent damage to the sensory nervous system may occur. There have been a considerable number of deaths linked to OC although no actual causal relationship has been determined. Most of these deaths occurred within 1 h of exposure.

The compound *o*-chlorobenzylidene malononitrile (CS) is a potent, safe RCA. CS and other chemicals in this class of compounds can lead to toxic reactions in experimental animals and humans if there is a high air concentration. CS is highly irritating to the mucous membranes that cover or line the eyes, nose, throat, and stomach. It also causes intense eye irritation, excessive tearing, and the nose and mouth may feel a stinging or burning sensation as well as rhinorrhea. If the respiratory tract is irritated, as is frequent postexposure, the individual may also have excessive coughing and sneezing, increased tracheobronchial secretions, and tightness in the chest. CS can cause death by way of serious lung injury leading to respiratory and circulatory failure, when exposure lasts for long durations. Diarrhea and vomiting will occur if the gastrointestinal tract is irritated. Burning sensation on the skin followed by inflammation and erythema are results of skin exposure. If exposure occurs in hot, humid conditions the effects will be more severe. CS produces some or all of its effects within 30 s of exposure, some so severe that the individual will seek escape from exposure. CS is also less toxic than CR or CN (see below).

Dibenz[*b*,*f*]1:4-oxazepine (CR) is a potent sensory irritant with low toxicity. The effects of CR on the eyes and skin are more transitory than with other agents. CR is not associated with contact sensitization.

Experiments done on various species using various routes have shown CR to have a low acute toxicity, much less than CN or CS. Overdose in animals will cause rapid breathing, incoordination, spasms, and convulsions. The effects generally subside gradually over a period of 15–60 min at which point the animal will either appear normal or have respiratory distress leading to death.

Chloroacetophenone (CN) is a white crystalline solid with an apple-blossom odor. It is also known as tear gas or Mace®. CN acts directly on mucous membranes to produce intense ocular and respiratory irritation as well as burning and pain of the eye, nose, throat, and lungs. Effects can include blepharospasms (i.e., eye blinking), conjunctivitis, sneezing, coughing, secretions, nasal congestion, and a sense of suffocation. The onset of some symptoms is immediate and persists for up to 20 min after the individual leaves the contaminated atmosphere. The primary cause of death related to CN is a result of inhalation effects on the pulmonary system.

CS and CN are by far the most important irritants described above. CN was the primary pulmonary irritant after World War I until CS was developed in 1928. CS has replaced CN as the principal military and law enforcement RCA, while CN as Mace® is available over the counter for personal protection in some places. Capsaicin as pepper spray has somewhat replaced CN as a personal protective agent. Other chemicals in this class that are worthy of mention are chloropicrin (PS) and bromobenzenecyanide (CA). PS and CA were developed before World War I, but have largely have been replaced because they were too lethal for their intended effects but not lethal enough to compete with the more effective blistering and nerve agents. PS still is seen occasionally as a soil sterilant or grain disinfectant. The creation of CNB (CN, carbon tetrachloride, and benzene), chloroacetophenone in chloroform (CNC), and CNS (CN, chloroform, and PS) were attempts to make CN more effective. However, CS proved more effective and less toxic than any of the CN series and largely has replaced them. CR, as a more recent tear gas (first synthesized in 1962), is not yet used widely.

Diphenylaminochloroarsine (adamsite or DM) is one of several military vomiting agents used. This compound is more toxic than many other RCAs and is potentially dangerous.

See also: Arsenical Vomiting Agents; Riot Control Agents.

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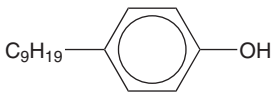
Nonsteroidal Antiinflammatory Agents *See* Acetaminophen; Acetylsalicylic Acid; Ibuprofen.

Nonylphenol

Alan L Blankenship and Katie Coady

- Chemical Abstracts Service Registry Numbers: CAS 25154-52-3 (mixed isomer); CAS 104-40-5 (4-nonylphenol); CAS 136-83-4 (2-nonylphenol)
- Synonyms: Nonylphenol; *p*-Nonylphenol-branched; 4-Nonylphenol; 2,6-Dimethyl-4-heptylphenol
- Chemical/Pharmaceutical/Other Class: Nonylphenol (NP) is a member of the alkylphenol class of chemicals. Alkylphenols are produced from cyclic intermediates during refining of petroleum and coal-tar crudes. Specifically, NP is manufactured by alkylating phenol with mixed isomeric nonenes in the presence of an acid catalyst. Thus, the product is a mixture of alkylphenols that vary in the length of carbon chain(s) and can vary in branching and substitution patterns. The product mixture contains predominantly *para*-substituted (4-nonylphenol) and occasionally *ortho*-substituted (2-nonylphenol), with various isomeric, branched-chain nonyl (nine carbon) groups. Theoretically, there can be 211 isomers present in a nonylphenol mixture due to different branching and substitution patterns

- Chemical Formula: $C_{15}H_{24}O$
- Chemical Structure:



Uses

Nonylphenol (NP) has not been widely used in commercial products, except in limited applications such as mixed with diisobutyl phthalate to color fuel oil for taxation purposes. However, it has been widely used as an intermediate in the production of nonionic surfactants of the nonylphenol ethoxylate (NPE) type. These nonionic surfactants are used as oil-soluble detergents and emulsifiers, lubricants, oil additives, and antioxidants for rubber manufacture. The occurrence of NP in the environment is mostly the result of metabolic degradation of NPEs and related alkylphenol ethoxylates (APEs). APEs are surfactants that are used in domestic and industrial detergents such as de-resinating agents, wetting agents, and degreasers. APEs are also components of biocides, plastics, and paints.

Background Information

NPs were first introduced in the United Kingdom in 1944 and they have subsequently been used in industry for over 50 years. Production of NP in the United States was ~147 million pounds in 1980 and grew to over 230 million pounds in 2000.

Exposure Routes and Pathways

Occupational exposure to NP may occur through inhalation and dermal contact with this compound at workplaces during its production and formulation into commercial products. Monitoring data indicate that the general population may be exposed to NP via dermal contact with products (e.g., nonionic surfactants) containing NP and ingestion of water containing NP. The primary route of environmental exposure to NP is through municipal wastewater treatment plants, as well as discharges of various industrial effluents from industries such as chemical plants, textile mills, and pulp and paper mills.

Toxicokinetics

By oral administration, nonylphenol is quickly absorbed in the gastrointestinal tract, distributed via the bloodstream, and finally excreted via urine and feces. In a human study in which one volunteer received 5 mg (66 $\mu g\,kg^{-1}$) ^{13}C-NP orally and a second volunteer received 1 mg (14 $\mu g\,kg^{-1}$) of ^{13}C-NP intravenously, NP was found to be ~20% bioavailable after oral application (relative to intravenous administration). The greatest concentration measured in blood after oral administration occurred after 60 min, followed by a distribution and elimination phase. By 10 h, NP could no longer be detected at a detection limit of 20 $pg\,g^{-1}$ in blood. From this very limited data, an elimination half-life of 163 min was calculated for humans.

Following dietary exposure to Sprague–Dawley rats, NP was rapidly absorbed and eliminated in the blood serum (absorption and elimination half-lives of 0.8 and 3.5 h, respectively). The predominant metabolite was a glucuronide, although NP aglycone was also observed. Other metabolic reactions of NP include sulfuration. In another investigation, ~70% of the orally administered NP dose in rats was recovered in feces and 20% in urine within 4 days.

Percutaneous penetration and absorption of ^{14}C-NP was found to be $<5\%$ and $<1\%$, respectively, in the skin of humans, pigs, and rats. NP was mainly present in the corneal layer of the skin.

Mechanism of Toxicity

There has been considerable interest in characterizing the biological effects of NP and related alkylphenols, ever since some were reported to exhibit estrogenic activity. Structure–activity studies with alkylphenols have shown clearly that the branching pattern, length of carbon chain, and substitution patterns on the phenolic ring have dramatic effects on the biological activity.

NP is able to interact and bind to the estrogen receptor, and is therefore capable of modulating estrogen receptor-mediated gene expression, such as that responsible for vitellogenin in fish. However, the estrogenic potency of NP is ~1000 times less than that of 17β-estradiol.

Acute and Short-Term Toxicity (or Exposure)

Animal

Published LD_{50} values for laboratory mammals include 1600 $mg\,kg^{-1}$ bw (rat, oral) and 2140 $mg\,kg^{-1}$ (rabbit, dermal), and 2031 $mg\,kg^{-1}$ (rabbit, oral). A number of acute aquatic toxicity tests have been conducted with 96 h LC_{50} values of 0.135 $mg\,l^{-1}$ (fathead minnow, flow through), 3.0 $mg\,l^{-1}$ (bay mussel, unspecified conditions), and 0.56–0.92 $mg\,l^{-1}$ (rainbow trout, unspecified conditions).

Some of the most sensitive biomarkers of effect to estrogenic chemicals are uterotrophic assays. For example, after oral and subcutaneous administration of 4-NP at 0, 25, 50, 100, and 200 mg kg^{-1} day^{-1} to female Long Evans rats (21 days old) for 3 days, an increase in uterine weight was observed at concentrations greater than or equal to 50 mg kg^{-1} day^{-1}. Some studies have also reported effects of NP in male rats. For example, male Sprague–Dawley rats (12 weeks old) exposed orally for 10 weeks experienced atrophy of seminiferous tubules ($\geqslant$100 mg kg^{-1} day^{-1}), decreased epididymis weight ($\geqslant$250 mg kg^{-1} day^{-1}), and decreased testis weight and decreased number of sperm ($\geqslant$400 mg kg^{-1} day^{-1}).

Human

Acute NP exposure, such as may occur in occupational settings, can produce severe irritation to the eye, skin, and respiratory system. Symptoms of such acute toxicity include a burning sensation, cough, labored breathing, sore throat, unconsciousness, abdominal pain, diarrhea, nausea, skin irritation, and burns. Other than these acute effects, there is no conclusive evidence that typical exposure to NP causes adverse health effects in humans.

Chronic Toxicity (or Exposure)

Animal

There are considerable data on the chronic toxicity of NP in laboratory animals. The focus of these investigations has typically been evaluation of the potential reproductive and developmental effects of NP, due to its ability to modulate estrogen receptor-mediated responses. Many endpoints are not consistently observed across studies. Some of this variability may be due to differences in the conditions, design, and other test-specific variables of the toxicity tests. For example, since phytoestrogens are abundant in most laboratory animal feeds (such as found in soy and alfalfa) and are known to modulate estrogen receptor-mediated responses, phytoestrogens may be confounding factors as a result of the feed selection.

In one study, Sprague–Dawley rats were exposed over three generations to 4-NP via diet at concentrations of 0, 200, 650, and 2000 ppm in order to assess potential reproductive effects. The F0 generation was exposed to 4-NP as adults and were bred once to produce the F1 generation, who were bred once to produce the F2 generation, who, in turn, were bred once to produce the F3 generation. Parameters evaluated over the course of the study included body weights, feed consumption, clinical observations, estrous cyclicity, reproductive performance, anogenital distance, pup survival, sexual development, sperm analysis, gross pathology, organ weights, and limited/selected histopathology. Feed consumption, clinical observations, and mortality were not adversely affected by NP administration. Nor were there any treatment-related changes observed in the litter data from all three mating trials. Effects that were observed included 7–12% reductions in terminal body weights, 14–18% increase in estrous cycle length, acceleration of vaginal opening by 1.5–7.3 days at 650 ppm and by 2.9–6.0 days at 2000 ppm in all three generations, a 8–13% decrease in epididymal sperm density in the F2 males at 650 ppm (sperm endpoints were unchanged in the F0 and F1 generations), increased relative kidney weight in adult males from the F0, F1, and F2 generations and in the F1 2000 ppm adult females, an increase in the incidence of renal tubular degeneration/dilatation in the males from all generations and in the 2000 ppm females from the F1, F2, and F3 generations, and in the 200 and 650 ppm females in the F3 generation, and a decrease in ovarian weights in the F2 generation and at 2000 ppm in the F1, F2, and F3 generations. The results of this study show that NP is a male and female reproductive toxicant at concentrations equal to or greater than 650 ppm based on decreased epididymal sperm density in males, as well as increased estrous cycle length and decreased ovarian weights observed in females.

Human

There are insufficient data to characterize chronic toxicity or exposure in humans.

In Vitro Toxicity Data

NP is an estrogenic compound that alters estrogen receptor-mediated gene expression, cell proliferation, and progesterone receptor responses in human estrogen sensitive MCF-7 breast tumor cells and other cellular models. For example, NP (10 μmol l^{-1}) induces mRNA expression of pS2 (a trefoil peptide expressed in breast cancer cells), MUC1 (a member of the mucin family), and estrogen receptor. Nonylphenols have been shown to be weakly estrogenic as indicated by elevated vitellogenin production in cultured rainbow trout hepatocytes ($ED_{50} = 16.15$ μmol l^{-1}).

Environmental Fate

NP partitions effectively into sediments following its release into aquatic environments ($K_{oc} = 31\,000$). In the mid-1990s, the average concentration of NP in US river sediments was determined to be $\sim$162 μg kg^{-1} (2960 μg kg^{-1}). In 1989, NP was detected in 17 of 30

US river samples at concentrations ranging from 0.11 to 0.64 $\mu g\,l^{-1}$. Concentrations of NP were greater (up to 600 $g\,l^{-1}$) in effluents from municipal wastewater treatment plants and industrial plants in the mid-1970s. While NP bioaccumulates in low-level aquatic organisms to a limited extent, NP is not expected to bioaccumulate appreciably in higher organisms ($\log K_{ow} = 4.48$). In freshwater fish for example, lipid-normalized bioconcentration factors ranged from 39 to 209 times the water concentration. Bioaccumulation was apparently greater in saltwater organisms where bioconcentration factors ranging from 78.7 to 2170 were measured.

Aerobic conditions favor the biotransformation and degradation of APE metabolites such as NP. Volatilization can be a major fate process for NP in moist soil due to a Henry's law constant of $1.1 \times 10^{-6}\,atm\,m^3\,mol^{-1}$. However, volatilization is likely not a major fate process from dry soil due to adsorption to soil particles ($K_{oc} = 31\,000$). NP is also susceptible to photochemical degradation (half-life 10–15 h in noon summer sun conditions).

Ecotoxicology

Due to concerns over potential exposure of aquatic organisms to NP, a number of acute and chronic toxicity tests have been conducted for both freshwater and saltwater species of invertebrates, fish, and aquatic plants. NP is considered an endocrine disruptor chemical and induces production of vitellogenin in male rainbow trout, a process that normally occurs only in female fish in response to estrogenic hormones during the reproductive cycle. NP also induces precocious development of ovaries and an intersex condition in some fish species.

Acute toxicity values (LC_{50}) for freshwater organisms ranged from 55.7 $\mu g\,l^{-1}$ for the amphipod (*Hyalella azteca*) to 774 $\mu g\,l^{-1}$ for the snail (*Physella virgata*). No relationships have been demonstrated between water quality characteristics (such as hardness and pH) and toxicity. The freshwater final acute value (FAV) for NP is 55.7 $\mu g\,l^{-1}$ which is equal to the LC_{50} for the most sensitive tested species, *H. azteca*. Acute toxicity values (LC_{50}) for saltwater organisms ranged from 17 $\mu g\,l^{-1}$ for the winter flounder (*Pleuronectes americanus*) to 209.8 $\mu g\,l^{-1}$ for the sheepshead minnow (*Cyprinodon variegatus*). The saltwater FAV for NP is 13.35 $\mu g\,l^{-1}$.

Chronic toxicity levels of NP for freshwater organisms ranged from 7.86 $\mu g\,l^{-1}$ for the rainbow trout (*Oncorhynchus mykiss*; based on growth), and 10.18 $\mu g\,l^{-1}$ for the fathead minnow (*Pimephales promelas*; based on survival) to 157.9 $\mu g\,l^{-1}$ for a freshwater cladoceran (based on reproduction). The chronic toxicity of NP for saltwater organisms was tested in only one species. A saltwater chronic value of 5.11 $\mu g\,l^{-1}$ was determined for the mysid (*Americamysis bahia*; based on reduced growth). Data were available to calculate a final acute–chronic ratio for NP of 9.41 based on a freshwater cladoceran, a saltwater mysid, and rainbow trout.

Two species of aquatic plants were exposed to NP and were found to be as sensitive as animals to NP exposure, showing effects that ranged from 27 to 410 $\mu g\,l^{-1}$.

Exposure Standards and Guidelines

Draft EPA water quality criteria for freshwater: 5.9 $\mu g\,l^{-1}$ (4 day average); 27.9 $\mu g\,l^{-1}$ (1 h average). Draft EPA water quality criteria for saltwater: 1.4 $\mu g\,l^{-1}$ (4 day average); 6.7 $\mu g\,l^{-1}$ (1 day average).

See also: Phenol.

Further Reading

Doerge DR, Twaddle NC, Churchwell MI, *et al.* (2002) Mass spectrometric determination of *p*-nonylphenol metabolism and disposition following oral administration to Sprague–Dawley rats. *Reproductive Toxicology* 16: 45–56.

US Environmental Protection Agency (USEPA), Office of Water. *Ambient Aquatic Life Water Quality Criteria for Nonylphenol – Draft*. EPA 822-R-03-029, December 2003.

No-Observed-Adverse-Effect Level *See* Levels of Effect in Toxicological Assessment.

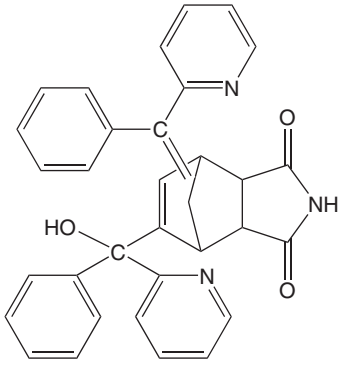
No-Observed-Effect Level *See* Levels of Effect in Toxicological Assessment.

Norbormide

Lynn Weber

This article is a revision of the previous print edition article by Tamal Kumar Chakraborti, volume 2, p. 438–439,

- Chemical Abstracts Service Registry Number: CAS 991-42-4
- Synonyms: 6-(α-Hydroxy-α-2-pyridylbenzyl)-7-(α-2-pyridylbenzylidene)-norbor-5-ene-2,3-dicarboximide; McN-1,025; S-6,999; Shoxin; Raticate; ENT 51,76
- Chemical/Pharmaceutical/Other Class: Heterocyclic dicarboximide
- Chemical Structure:



Uses

Norbormide was first introduced in the market in 1964 as a selective rodenticide. This compound is highly specific for rats. Norbormide is on the World Health Organization 'obsolete' pesticide list.

Exposure Routes and Pathways

Norbormide can enter the body through oral, dermal, and inhalation exposures.

Mechanism of Toxicity

Norbormide causes an extreme and irreversible vasoconstriction in small arteries in rats following both systemic and local administrations. However, large rat arteries (e.g., aorta), nonvascular rat smooth muscles (e.g., duodenum and trachea) and all smooth muscles from nonrat species are not constricted by norbormide even at high concentrations/doses. The peripheral vasoconstriction in small rat arteries subsequently reduces coronary blood flow rate, leading to cardiac arrhythmias, which can lead to death. The norbormide-induced vasoconstriction is mediated by activation of phospholipase C/protein kinase C and calcium influx via L-type voltage dependent calcium channels. In contrast, norbormide-resistant arteries and smooth muscles exhibit inhibition of L-type voltage dependent calcium channels and instead exhibit a mild relaxation response. Sex- and species-related differences in sensitivity to norbormide may be attributed partially to the differences in metabolism of this compound. A lethal dose of norbormide in rats ($1\,g\,kg^{-1}$) can also elevate the blood glucose level twofold with a decrease in both liver and muscle glycogens. Exposed animals became comatose within 30 min to 2 h after treatment. The hyperglycemic effect of this compound in rats is considered to be a secondary effect.

Acute and Short-Term Toxicity (or Exposure)

Animal

Laboratory rats showed difficulties in locomotion and ataxia but no hindlimb paralysis after treatment with norbormide. Death occurs within 15 min to 4 h after struggling, dysnpea, hypothermia, and convulsions. Oral LD_{50} values for Norway rats ranged from 5.3 to $15\,mg\,kg^{-1}$. Rodents other than rats have considerably higher oral LD_{50} values (e.g., hamster, $140\,mg\,kg^{-1}$; guinea pigs, $620\,mg\,kg^{-1}$; mice, $2250\,mg\,kg^{-1}$). However, no effect was detected with $1000\,mg\,kg^{-1}$ doses of norbormide in dogs, cats, monkey, sheep, pigs, or chickens. Interestingly, L-type voltage dependent calcium channels in guinea pig heart were relatively selectively inhibited by norbormide in the sino-atrial and atrio-ventricular nodes, suggesting a possible therapeutic benefit for norbormide in treating supraventricular arrhythmias in heart failure.

Human

Human toxicity due to norbormide exposure is highly unlikely because of its relatively selective toxicity in rats. Human volunteers given 20–300 mg showed only a minimal hypotensive effect, which returned to control levels within 2 h. A dose of 300 mg corresponds to 60 g of the 0.5% bait and 30 g of the 1% bait. Although a hypotensive effect of norbormide was cited as a potential toxic sign, the maximum reduction in body temperature was found to be 0.7°C following 20–80 mg of norbormide in human volunteers. The hyperglycemic effect could not be demonstrated in humans.

Chronic Toxicity (or Exposure)

Little information is available on chronic effects of norbormide in humans or animals. Due to the selective toxicity in rodents, in particular Norway rats, little persistent toxicity would be expected in other species.

Clinical Management

As mentioned earlier, human toxicity due to overdose of norbormide is not expected because of its selectivity for rats. The only sign identified was a mild reduction of systolic blood pressure for a short period of time; therefore, only symptomatic and supportive care has been recommended in cases of norbormide ingestion. However, emesis may be induced in case of recent substantial ingestion of norbormide. Exposed eyes should be washed with tepid water for 15 min. In case of dermal exposure, the contaminated area should be washed with a sufficient amount of soap and water.

See also: Pesticides.

Further Reading

Bova S, Cima L, Golovina V, Luciani S, and Cargnelli G (2001) Norbormide: A calcium entry blocker with selective vasoconstrictor activity in rat peripheral arteries. *Cardiovascular Drug Reviews* 19(3): 226–233.

Pelfrene AF (2001) Rodenticides. In Krieger R (ed.) *Handbook of Pesticide Toxicology*, 2nd edn., pp. 1793–1836. San Diego, CA: Academic Press.

Relevant Website

http://www.epa.gov – US Environmental Protection Agency. Chemical Profile on Norbormide.

Norepinephrine *See* Catecholamines.

Notorious Poisoners and Poisoning Cases

Joanna Willis, Thomas Holdsworth, and Katherine Cathrow

Introduction

In Greek mythology, Hercules was said to have dipped his arrows in snake venom to render them more deadly. This may explain the origins of the word 'toxic', thought to be derived from the ancient Greek word 'toxon', meaning arrow. Therefore, although most cases of poisoning seen today are either accidental or as an act of deliberate self-harm, the origins of toxicology bring to mind a more sinister role of poison, that is, its use in dispatching of enemies.

Poison has been called 'the coward's weapon' as it is administered by stealth without any chance of defense on the victim's part. Therefore, murderers who have made poison their weapon of choice are often seen as more sinister and cold-blooded than those who face their victims with a gun or a knife, and at the other end of the scale, chemical warfare is seen to betray the ancient principles of chivalry and bravery in battle.

What follows are a few famous cases through history where poison has been used intentionally to destroy life, from individual murders to chemical warfare. However, it must be pointed out that these cases only skim the surface of what is an extensive history of murder by poison.

Socrates (Greece, 420 BC)

Socrates was born around 470 BC in Athens and was taught his family's trade of sculpture, as well as receiving an education in geometry and astronomy. At first he was an enthusiastic student in the sciences. However, he soon came to regard his teachers as merely imparting received knowledge that they could not themselves prove, and he set out to seek true knowledge of 'causes' and 'good'.

Socrates became involved in Athenian life because of his ideas, and was friendly with many of those in power. As was required of all citizens, he also served as a soldier and we know that he was decorated for bravery. He came to be widely known and respected for his wisdom, although he famously said that his wisdom relied on the fact that he fully recognized his own ignorance.

Socrates made it his mission to seek out people whose reputation in society he felt was undeserved. He confronted these people and questioned them on their positions and views before leading them

through further questioning into inevitable contradictions. He called this questioning 'elenchus'. Despite the fact that Socrates was polite and considerate in these exchanges, they were often conducted in public places. The Athenian youth came to view these public humiliations as a form of entertainment, and others used the same method in a less polite and more personal manner.

In 399 BC Socrates was accused of impiety, neglect of the gods whom the city worships, practice of religious novelties, and corruption of the youth. It is probable that the charges resulted from resentment of influential figures towards Socrates methods of questioning, and the way in which younger members of society were using it to upset the establishment. Socrates had also openly ridiculed the method of election in parliament by lot. 'In no other craft', he claimed, "would the craftsmen be chosen in this way." In addition, references he made to his personal spirit or 'daimonion', raised public suspicion that he rejected the state religion.

Socrates was found guilty of these charges and sentenced to death. Though his friends were willing to organize his escape, Socrates accepted his fate despite feeling the sentence to be unjustified. The rule of law decreed death by drinking the state poison. The Greeks were fond of the poison Hemlock, and it was often used for suicidal purposes. The state poison was a species of Hemlock known as cicuta. However, the administered dose was often not fatal and had to be repeated. Plato famously describes how Socrates was put to death by drinking the state poison in prison in 402 BC.

The Borgia Family (Italy, Fifteenth and Sixteenth Century)

The Borgias, a noble Spanish family from Valencia, established themselves in Italy during the fifteenth and sixteenth centuries. Alfonso de Borgia pursued an ecclesiastical career and became Pope Calixtus III in 1455. Rodrigo Borgia followed in his footsteps and became a cardinal of the Roman Catholic Church and then Pope Alexander VI in 1492. Rodrigo and his mistress Vannozza Catanei had five children, two of whom, Cesare and Lucrezia, became notorious (along with their father) for their supposed use of a secret poison, known as 'La Cantarella', to dispatch of several of their rivals. Although the exact composition of 'La Cantarella' is not known, it is thought to have been a mixture of copper, arsenic, and phosphorus, prepared in the decaying carcass of a hog, itself poisoned with arsenic.

Cesare Borgia (1475–1507) rose to the level of cardinal as his father had done before him, but his skills lay in politics, and when his father became pope in 1492, Cesare became his personal advisor. However, when Cesare's younger brother Juan overtook Cesare in status by being made Duke of Gandía, Cesare reputedly became jealous, and when Juan was mysteriously murdered in 1497, many suspected Cesare of killing his brother. This was never proved and Cesare went on to marry and receive the title of Duke of Valentinois. As captain general of the papal army, and with his father's support, Cesare attempted to establish a secular kingdom in central Italy. He was determined to establish himself as an Italian Prince before his father's death deprived him of the papal support he relied on. Cesare was ruthlessly single-minded in this quest and he is reported to have assassinated numerous political figures who were standing in his way.

Cesare's sister Lucrezia Borgia (1480–1519) helped to raise the political profile of the Borgia family by marrying into prominent families. However, her first two marriages ended unhappily. Her marriage to Giovanni Sforza, Lord of Pesaro, Milan, was annulled by her father, the Pope, after Giovanni fled Rome in response to the Pope making an enemy of Milan. Lucrezia's second husband, Alfonso, Duke of Bisceglie (son of Alfonso II of Naples) was murdered by Lucrezia's brother Cesare. It is not known whether Cesare murdered Alfonso to sever Rome's ties with Naples or whether it was the result of a personal vendetta. In any case, it was seen as highly suspicious that Lucrezia allowed the murder to go ahead. Lucrezia's reputation was further soiled by the appearance of her son in 1501, rumored to be the child of either Alexander or Cesare Borgia. Despite this reputation, Alfonso d'Este, son of the Duke of Ferrara, married Lucrezia in 1501.

Lucrezia Borgia has long been accused of sharing in her father's and brother's crimes by poisoning rivals with 'La Cantarella' powder from a ring on her finger, and her reputation as a ruthless murderer has been immortalized in Victor Hugo's drama and Donizetti's opera. However, it would appear on hindsight that she was simply an instrument for the political ambitions of her family.

In 1503, Pope Alexander VI (father to Lucrezia and Cesare) died. Ironically, it is thought that his death was the result of poisoned wine, although it is not known whether he was accidentally served wine meant for one of his rivals, or whether one of his rivals had in fact intended to poison him. Following the Pope's death, an enemy of the Borgias, Giuliano della Rovere, was elected as Pope. Cesare Borgia was arrested and imprisoned under his rule and although he escaped in 1506, he died in battle in 1507. Lucrezia Borgia retired to the Ferrara courts after her

father's death and lived out her remaining days there as a patron of the arts. She died at the age of 39.

The Borgia family began to decline towards the end of the sixteenth century and by the mid-eighteenth century the name had disappeared completely.

The Murder of Sir Thomas Overbury (England, 1615)

Thomas Overbury was a poet and essayist who moved to London to seek his fortune. With his friend Robert Carr (page to Lord Dunbar) he managed to secure an appointment at the court of King James I, where they both received expeditious promotion. Carr acquired the title of Lord Rochester and Overbury was knighted.

Rochester soon became infatuated with the Countess of Essex, who divorced her husband for him. However, Overbury did not approve of the Countess and tried to oppose the match. Rochester married the Countess against Overbury's advice and he and his wife turned against Overbury.

Soon afterwards, Overbury, on the advice of Rochester, refused an invitation from the King to become Ambassador to Russia. The King was greatly offended by this refusal and sent Overbury to the Tower of London.

The Countess of Essex, still consumed by hate for Thomas Overbury, plotted to kill him. She procured the help of an apothecary named Franklin. Franklin supplied poisons, including rosalger (a compound of arsenic), sublimate of mercury and white arsenic, to Weston, the under-keeper of the Tower. Weston, under heavy bribery from the Countess, mixed small quantities of poison into Overbury's food over the course of 4 months. Overbury became very unwell and eventually died. At first his death was thought to be the result of syphilis, but after some time, suspicion was aroused and the case was brought to trial.

Franklin pleaded guilty and was hanged. Weston was also hanged although he maintained his plea of innocence throughout the trial. Rochester and the Countess were also found guilty but their death sentences were retracted and they were eventually pardoned by the King.

The Affair of the Poisons (France, 1679)

In seventeenth century France, it was fashionable among Parisians to seek advice and aphrodisiacs from fortune-tellers. These fortune-tellers, however, also sold poisons (often called 'inheritance powders' as they enabled those who made use of them to claim their inheritance ahead of time by dispatching of their parents or spouse).

An inquiry lead by Nicolas de La Reynie in 1679 exposed a number of these 'fortune-tellers' and as a result, a special tribunal, known as the Chambre Ardente ('burning court'), was set up for the trial of those accused of witchcraft and poisoning.

Perhaps the most famous case to be tried in the Chambre Ardente was that of the midwife and fortune-teller La Voisin (born Catherine Deshayes Monvoisin). She was exposed by La Reynie as a supplier of poisons, put to trial and sentenced to death by burning; a sentence carried out in 1680. La Voisin was supposed to have sold mixtures of arsenic, aconite, belladonna, and opium in many forms including cosmetics. Several members of French society were implicated following the exposure of La Voisin, including Madame de Montespan, the mistress of King Louis XIV. She was accused by La Voisin's daughter of seeking poison and black magic from La Voisin in her attempts to win the King's affections and then later in (failed) attempts to dispatch of her rivals in court and ultimately of the King himself. Although these charges against Madame de Montespan were never proved, a permanent stain was left on her name and she eventually left the King's court in 1691 to join a convent.

Other prominent French figures connected with La Voisin include Olympe Mancini (niece of Cardinal Mazarin and mother of Prince Eugene of Savoy), her sister Marie Anne Mancini, and Marshal Luxembourg (duke and peer of France and one of the military heroes of the time). The Duke of Buckingham was also rumored to have been one of La Voisin's clients.

Following the success of the Chambre Ardente in imprisoning and executing poisoners such as La Voisin, the poisoning epidemic that had taken hold of France for so long came to an end.

Toffana (Italy, 1690)

Perhaps the most notorious poisoner of the seventeenth century was an Italian woman named Madame Giulia Toffana. She invented an arsenical solution in 1690, called 'Aqua Toffana', which she sold in phials bearing the representation of a saint, usually Saint Nicholas of Bari (Bari was a town whose water was supposed to have had healing properties). The phials were sold to women under the pretence that Aqua Toffana was good for a woman's complexion (as arsenic is), but Toffana also sold her solution to women who wanted to rid themselves of their husbands. It was apparently colorless, tasteless, and miscible with wine, and therefore very easy to administer. Toffana is said to have been responsible for as many as 600 murders and for this she was executed in Naples in 1709. However, Toffana had

her followers and one of them, Hieronyma Spara, developed her own version of the poisonous solution, named Aquetta di Perugia, in Rome in 1695. She too was responsible for a number of deaths and, like Toffana, was executed for her crimes.

Thomas Wainewright (England, 1830)

At 30, Thomas Wainewright was a popular and successful gentleman in the literary and artistic circles of London society; he was a friend of William Blake and Charles Dickens, a published writer and an exhibited artist. At 40 he was working on a chain gang in a Tasmanian penal colony, shackled to thieves and murderers.

Born in 1794 and orphaned at a young age, Thomas Griffiths Wainewright was brought up by his grandfather, the editor of *The Monthly Review* (London's first literary magazine) into London's high society which was, at the time of the Romantic Revolution, a world of dandies and dilettantes, painters and poets. With an artistic temperament and considerable wit and charm, Wainewright seemed perfectly suited to the lifestyle of dinner parties and art galleries. He showed talent as a painter himself, exhibiting on several occasions at The Royal Academy, and wrote regularly for various magazines and journals. However, his glamorous lifestyle was expensive, and by his late 20 s, he found himself in financial difficulties.

In 1822, by which time he was married, Wainewright turned his artistic talents to forgery, counterfeiting signatures on documents to allow him immediate access to some of his inheritance, which was held in a trust fund. By 1824, he had got his hands on the full legacy of £5250. However, this money did not last long, and he was soon borrowing money from loan sharks and friends, and running up large debts.

In 1828 Wainewright, his wife Eliza, and their son moved into Linden House, the impressive country home in which he had grown up, and which was now owned by his uncle. Within a year, his uncle had died in mysterious circumstances, leaving his house and estate to Wainewright. The Wainewrights then invited Eliza's mother and two sisters to come and stay with them at Linden House. In the following months, Wainewright, Eliza, and Helen Abercrombie, the youngest sister, set up an elaborate insurance fraud. They insured Helen's life with five different companies and on false pretences, lying about Helen's age and the Wainewrights' financial situation.

Helen's mother seems, understandably, to have disapproved of this, but in 1830 she too died suddenly in mysterious circumstances. The fraud was completed in the same year, Helen's life being insured for a total of £16 000. It is hard to imagine why Helen would co-operate with a scheme that it seems could only have been successfully completed by her own death, but she was present at many of the negotiations and aware of at least some of the deception involved. It seems likely that she was being manipulated and deceived by Wainewright, and possibly by her sister, although the extent of Eliza's involvement is unclear. One actuary reported that, when asked why she wanted to insure her life, Helen said that "she had been told it was proper to do it." Almost as soon as the insurance policies were in place, Helen, up until then a healthy 21 year old, was taken ill. She died within a few days, in the grip of painful convulsions which one servant of the household reported were identical to those experienced by both Wainewright's uncle and Mrs Abercrombie. Helen's death certificate recorded the cause of death as "cerebral haemorrhage." However, the insurance companies refused to pay out on the basis of "misrepresentation", having identified the fraud. Wainewright promptly initiated legal action against them, but his reputation had taken a serious blow and there were widespread suspicions about the deaths. He decided it would be prudent to lie low, and moved to France, where he stayed for the next 5 years.

No charge of poisoning was ever brought against Wainewright, as there was insufficient evidence. It cannot be said with absolute certainty that he was responsible for his relatives' deaths, although the circumstantial evidence seems reasonably compelling. At this time forensic detection of poisons was difficult, and if Wainewright was indeed a poisoner, he was canny and skilful. The most widely accepted story of Helen's death is that she was poisoned first with antimony, causing her to suffer from symptoms such as nausea and vomiting. However, at the same time the Wainewrights served a relatively indigestible meal, providing an alternative explanation for her sickness. Then, after a couple of days and a visit from the doctor, she was fed jelly laced with strychnine. The bitter taste of the strychnine would have been masked by the sweet jelly, and she may have been told that the powder was an all-purpose remedy, such as a 'black draught' laxative, which was widely used at the time. In her weakened state, she would have died almost immediately. The convulsions described by the servant as being common to all three deaths are characteristic of poisoning with strychnine, which would have been easily available from an apothecary. There are also reports that Wainewright had several books on poisons in his library.

After five difficult and largely impoverished years in France, Wainewright returned to London, but was

promptly arrested for the forgery he had committed 10 years previously. He was held in Newgate Prison for some time, and then transported to Hobart Town in Tasmania, where he lived until his death in 1847. Initially he worked on a chain-gang building roads, but eventually he was allowed to work as a hospital orderly and even paint again. He painted portraits of many of the local dignitaries and their families, which are generally thought to be his most accomplished works as an artist.

William Palmer (England, 1855)

Lauded as the 'Prince of Poisoners' by the press of the time, William Palmer is believed to have murdered using strychnine. He was Britain's first recorded 'Serial Killer' and his effigy stood in the Chamber of Horrors at Madam Tussaud's Waxworks for 127 years.

Palmer was born in Staffordshire, England on August 6, 1824, the sixth of seven children. At the age of 10 he was send to Rugeley Free Grammar School, which he attended as a day scholar. Some accounts state that he was a boisterous child and a bully; others claim that he was the most well behaved of all the children. It would seem that his true personality fails to have been documented. When Palmer was 12, his father died. Without their father's strict governance, William and his siblings were now free to run wild.

At 17, Palmer left school and began an apprenticeship which his mother had arranged for him at a wholesale chemists in Liverpool. This job ended abruptly when his employers discovered that Palmer had been stealing money from them. He was in fact stealing money to buy gifts to impress his girlfriend. His mother intervened and paid back the money he had stolen to prevent his employers reporting him to the police. A second apprenticeship was arranged with a physician in a medical practice, but this too was doomed to fail as Palmer, still obsessed with the same girlfriend, stole money from his new employer. His mother's attempts to have William forgiven were futile and he was sent to Stafford Infirmary to serve an apprenticeship as a 'walking pupil'. His love of women and alcohol, however, continued to grow.

It was during his time at Staffordshire Infirmary that Palmer is thought to have become interested in poisons and he may have been behind a suspicious death of a patient whilst a student at the infirmary. It is claimed that Palmer laced brandy with poison and challenged his patient to a 'drinking contest' after which the patient died. Palmer continued his training at St Bartholomew's Hospital in London. However, things did not go well, and he persisted in drinking and womanizing. It was only after his mother intervened yet again and employed a private tutor that Palmer managed to settle, study and eventually qualify as a doctor in 1846.

Upon returning to Rugeley in 1847 Palmer set up a surgery from a rented house. Business went well and he was soon able to afford an assistant. However, he became heavily involved in horse racing and gambling, and at one point owned 15 racehorses. Eventually, he could no longer sustain his gambling addiction and fell into debt. His financial situation became so desperate that at one point he resorted to drugging a competing horse. This earned him the title of 'nobbler', and he was discredited by the racing authorities. Despite this, his gambling addiction grew and Palmer came to rely on moneylenders to finance his habit. In order to secure loans to buy more horses, he began to forge his mother's signature. By the autumn of 1855 he owed £15 000 and had outstanding bills for a further £11 500.

Palmer met his wife, Annie Thornton in 1845. She had completed her studies at finishing school, and Palmer was attracted to her beauty, charm, and wealth. They married in 1847, despite the audible displeasure of her mother, a rich widow. Palmer and his mother-in-law did not have a good relationship. She was a difficult woman by all accounts, drinking heavily and becoming violent. It is even supposed that she drove her own husband to suicide. Following a particularly heavy drinking binge, she became so ill that she needed to be nursed at her daughter and son-in-law's house. Within 2 weeks she was dead. At the time it was presumed that she had died from the effects of prolonged alcohol abuse, but later, as suspicions surrounding Palmer's activities grew, many began to suspect that Palmer was responsible for his mother-in-law's death.

Four of Annie and William Palmer's five children died within weeks of their birth. The death certificate in each case cited convulsions as the cause of death. However, the Palmers' housekeeper claimed that Palmer had murdered the children by dipping his finger in poison, then honey and then into their mouths. She claimed he had commented that he could not afford so many mouths to feed. Whether this was the case, or whether there was a medical explanation for the deaths of Palmer's children has never been established.

Palmer had a close friend, fellow gambler John Parsons Cook. Cook had inherited £12 000 and retired from his work as a solicitor, choosing to spend his time and money on horse racing. Having never enjoyed good health, his new-found wealth led him into a more 'riotous' lifestyle and he is reported to have caught syphilis, for which he was treated by Palmer.

In mid-November 1855, Palmer and Cook attended the Shrewsbury Races, where Cook won ~£3000. Palmer was not so lucky and he had begun to receive threatening letters from his money-lenders. Shortly after his friend's win, both were dining at a local inn, when Palmer was seen mixing up some kind of concoction in a room away from his friend. He returned to his friend and a tray of brandy was brought in. Upon drinking his brandy, Cook complained that it burnt his throat and thought it may be drugged. He retired to bed feeling unwell. Over the next few days, his condition deteriorated. Palmer attended to him, and even traveled to London with Cook's betting books to collect his winnings for him (money, Palmer later claimed, that was owed to him). By the 20th of November, Cook was very unwell, and in the early hours of the 21st, he suffered violent convulsions and subsequently died.

Palmer was arrested on suspicion of Cook's murder and taken to Stafford jail where he went on hunger strike before being threatened with force-feeding by the jail governor. On May 4, 1856, Palmer was transferred to London for his trial in Westminster.

Reports claiming that Palmer had bought strychnine around the time of Cook's death, and claims by maids in attendance of Cook that food sent by Palmer had made them sick resulted in Palmer being the first man in British history to be tried for murder by strychnine poisoning. However, strychnine was never found in the body of John Parsons Cook and although this was blamed on an inadequate postmortem, many, including Cook's own doctor who was present at the time of death, believed that Cook had died of tetanus.

During Palmer's trial, other suspicious deaths were investigated. Palmer had insured his wife's life for £13 000 in spring 1854 and by the autumn of the same year she was dead. Her death certificate stated that she died from English cholera. Her symptoms were recorded to include retching and vomiting, but no convulsions. Annie Palmer's body was exhumed for examination of her stomach contents. There were no traces of strychnine, but a small amount of antimony was found. Antimony can be used as a poison but at the time Annie Palmer died it was also often used to treat symptoms such as the ones she was suffering from.

Similarly, Palmer had insured his brother Walter's life. It is widely accepted that Palmer was defrauding the insurance companies as he actually employed someone to keep Walter sober while medical clearance was obtained for the insurance to be validated. Walter died soon after and the money went to Palmer. As with Annie, Walter's body was ordered to be exhumed when Palmer was awaiting trial for the murder of Cook. However, Walter's body was in such a state of decomposition that the coroner was unable to perform a satisfactory postmortem, and the case was dropped. Whether the death was due to alcohol or poisoning by Palmer is still unclear.

Other suspicious deaths were also investigated: an uncle of Palmer died shortly after a night of drinking brandy with him, and a friend of Palmer's died after being treated by him.

After a 12 day trial, the jury of 12 men took less than 2 h to reach a unanimous verdict of guilty. Palmer was sentenced to death for the murder of John Parsons Cook, and was publicly hanged in Stafford on of June 14, 1856, at the age of 31, still protesting his innocence.

It is supposed that Palmer poisoned at least 11 victims, and as many other suspicious deaths also carry his hallmarks. However, much debate still exists as to whether Palmer actually was a murderer, or whether he was simply labeled a killer by the misfortune of circumstantial evidence.

Madeleine Smith (Scotland, 1857)

In 1857, Madeleine Smith was tried for the murder, by arsenic poisoning, of her lover, Emile L'Angelier.

Madeleine was the 22-year-old daughter of a wealthy and well-respected family in Glasgow. Emile was a poor immigrant from Jersey, employed as a clerk at a Glasgow seed-merchant. They met in the spring of 1855 and became friends over the next few weeks. When Madeleine moved with her family to their summer house at Rowaleyn a few weeks later, Madeleine and Emile continued their evolving friendship through numerous letters. Madeleine's father soon found out about the friendship and, because of Emile's social standing, was not happy about it. However, Madeleine and Emile's relationship carried on in secrecy and they soon declared their love for each other. They had occasional clandestine meetings and Emile's letters reached Madeleine through her maid, Christina. During the summer of 1855 Emile and Madeleine became secretly engaged and planned their wedding for September of 1856.

Madeleine and Emile's relationship continued like this for over a year. However, in July of 1856 Madeleine was introduced to William Minnoch, a wealthy businessman Madeleine's father knew and intended his daughter to marry. Over the next few months Madeleine's letters to Emile began to lose their previous enthusiasm and Madeleine decided to postpone the wedding. She knew of her impending proposal from Minnoch and did not know what to do about it. Minnoch formally proposed to Madeleine in January of 1857 and Madeleine accepted.

Madeleine then made several attempts to end her relationship with Emile and asked him to return all of her letters so that no one would find out about the relationship and jeopardize her engagement. Emile refused to return Madeleine's letters and, instead, threatened to send them to her father. To prevent him from carrying out his threat and to attempt to pacify him, Madeleine agreed to continue seeing Emile.

On February 21 Madeleine went to a local apothecary and bought sixpenny worth of arsenic. She told the clerk that the poison was needed to kill rats and signed the Poison Book, as was required by law. It is not known whether Madeleine and Emile met that night, but the next morning Emile suffered from stomach cramps, nausea, and vomiting, which kept him at home for a week. Madeleine bought sixpenny worth of arsenic twice more in the next few weeks, again claiming that it was to kill rats. Madeleine and Emile met on the night of the 22nd of March; at half past two the next morning Emile arrived at his lodging house doubled-up in pain and he vomited many times over the next few hours. At 10 O'clock on the morning of March the 23rd, Emile died.

Over the course of the next week, Madeleine's letters to Emile were found and on postmortem examination, Emile's body was found to contain large amounts of arsenic. Madeleine was arrested for the murder of her lover. The trial began on June 30, 1857 and by this time there was considerable public interest in the case. Madeleine pleaded not guilty but was not allowed to speak for her defense in the trial, in accordance with the law. However, she recorded a statement before the trial began claiming that she had last seen Emile three weeks before his death and that the arsenic she had bought was for cosmetic purposes. In the course of the trial many people were questioned about Madeleine and Emile's relationship, about the events that occurred on the night before Emile's death, and about the fate of the arsenic Madeleine purchased. The prosecution concentrated on the fact that Madeleine's arsenic purchases coincided perfectly with Emile's periods of ill health. They suggested that Madeleine had become so afraid that Emile would jeopardize her engagement to William Minnoch that she poisoned cocoa with arsenic and gave it to Emile during one of their secret meetings. The defense concentrated on a number of people's accounts of Emile as an unstable man who was capable of suicide, and suggested that he was so angry about Madeleine's rejection that he tried to frame her for his murder. According to some of the defense witnesses, Emile had taken arsenic in small doses as a tonic.

On July 9, 1857 the jury returned a verdict of not proven on the charge of murder (a verdict unique to Scotland, which allows the defendant to go free but carries a stigma, as it not only states that the prosecution failed to prove its case, but also indicates that the defense failed to convince the jury of the defendant's innocence). Madeleine Smith walked free from the court and fled to Rowaleyn. Her engagement to William Minnoch was called off and the Smith family tried to forget about the unfortunate incident. However, public interest in the case refused to die down.

Not long after the trial, Madeleine moved to London and married a draftsman. They had two children but separated after 28 years of marriage. Madeleine emigrated to America a few years later and eventually married again and lived in New York. She died on April 12, 1928.

What actually happened to Emile L'Angelier in the early hours of March 23, 1857 will never be known, although speculation continues.

The Umbrella Assassination (London, 1977)

Georgi Markov was a Bulgarian writer who lived in his home country until 1969, when at the age of 40 he defected to the West. Living in London, he worked as a broadcast journalist for the BBC, Radio Free Europe, and the German Deutsche Welle.

He had a large audience in Bulgaria, and his outspoken views against the ruling communist party were seen as the inspiration for Bulgarian dissident movements. The leader of the Bulgarian communist party, Zhivkov Todor, decided in June 1977 that he wanted Markov silenced, and informed a politburo meeting of his wishes. The job of assassinating Markov was given to the interior minister Dimiter Stoyanov, who requested KGB assistance. The KGB chairman Yuri Andropov agreed provided there would be no trail left to the Soviet Union.

There were three attempts on Markovs' life. During a dinner party given by friends at Radio Free Europe, someone slipped a poison into his drink. However, this and another attempt on his life in Sardinia failed. The successful attempt took place in London on September 7, 1977.

Markov worked a double shift at the BBC, and after working the early morning shift, he went home to rest. On returning to work he parked his car South of Waterloo Bridge and made his way to the bus stop to catch the bus to the BBC headquarters. As he neared the people queuing for the bus, he felt a stabbing pain in his right thigh and turned to see a man facing away from him stoop and pick up an umbrella. The man apologized in a foreign accent and departed hurriedly in a taxi. Markov later described the man as thick set and ~40 years old. In pain, Markov

boarded the bus for work, where he told colleagues what had happened. He noticed a spot of blood on his jeans, and showed a friend a pimple-like red swelling on his thigh. When he returned home he became very ill, with a high fever.

The next day Markov was admitted to St James's hospital in Balham. Examination of his right thigh showed a central puncture wound of ~2 mm diameter, and a circular area of inflammation. A diagnosis of septicemia was made, due to the very high white cell count. Mr Markov died 3 days after he had been injured.

During the postmortem, a single metal sphere the size of a pinhead was excised from the wound. It was 1.52 mm in diameter and composed of 90% platinum and 10% iridium. It had two holes bored through it, with diameters of 0.35 mm, leaving 0.28 mm^3 available for toxin retention.

Dr. David Gall at the government chemical defence establishment Porton Down, hypothesized that ricin could be the only possible poison used, owing to the exceptionally small dose and the symptoms Markov had experienced.

After the fall of the Soviet Union it was revealed that ricin was used in an umbrella mechanism for injecting poison spheres into victims, a technique developed in the secret KGB laboratory 'the Chamber'. Two former KGB officers Oleg Kalugin and Oleg Gordievsky publicly admitted to Soviet involvement in Markov's death and it was reported that the Bulgarians had used a low-level Italian criminal to carry out the murder. The man was located in Denmark but questioning remained inconclusive; he then fled to Hungary and the Czech Republic and his current whereabouts are unknown.

Harold Shipman (England, 2000)

Harold Frederick Shipman was born to a working class family in Nottingham on the 14 of January 1946. Intelligent and successful at school, he endeavored to study medicine after the death of his mother from lung cancer when he was 17. In 1965, he realized this ambition and began a medical degree at Leeds University. Graduating in 1970, Shipman qualified as a General Practitioner (GP) in 1974, and went to work at the Abraham Ormerod Medical Practice in Todmorden, West Yorkshire.

It was during his time at this practice that colleagues discovered his addiction to the opioid pethidine. Shipman was disciplined by the General Medical Council (GMC), but was not struck off the medical register. It is now understood that Shipman murdered his first victim during his time there in 1975. As a result of his newly discovered history of opioid abuse, Shipman was dismissed from the Todmorden practice. However, he reappeared as a GP in Hyde, Greater Manchester in 1977, this time working at the Donneybrook House Practice.

Solid indications that he had been conducting murderous activities became apparent during his time at Donneybrook House, when the death rate amongst his patients was three times higher than that of his colleagues in the same practice. It is believed that Shipman murdered a further 71 patients whilst at this practice.

In 1993 Shipman set up his own practice on Market Street in Hyde, after falling out with the partners at Donneybrook House. He was to go on to murder another 143 patients whilst in this practice. It was not until 1998 that Shipman's crimes first came to light when the daughter of one of his victims grew suspicious following her mother's death.

Kathleen Grundy, an 81-year-old previous mayoress, was found dead in her home. Shipman had visited her house on the morning of her death. His visit had been prompted by a consultation the previous day, when he had requested her help in a survey of aging, as he proposed that she was extremely fit and well for her age. Mrs Grundy readily agreed, and Shipman arranged to take the required 'blood sample' for inclusion in the survey. Under the pretences of taking such a sample, he in fact injected her with a lethal dose of diamorphine, which led to swift respiratory depression and death. He was to state on her death certificate that she died simply from 'old age' – a strange conclusion to draw considering his conversation with her the previous day!

Mrs Grundy's last will and testament were scrutinized. A recently redrafted will replaced the original her family were familiar with, and to their surprise left all estate and monies to the sum of £386,000 to Dr Shipman, in recognition of his 'attentive care'. The new document was poorly drafted and the signature of Mrs Grundy did not correspond to her usual hand.

Mrs Grundy's daughter, Angela Woodruff, voiced her suspicions to the police that Dr Shipman may have forged her mother's will, and possibly undertaken more sinister actions. Owing to the severity of the allegations, the body of Kathleen Grundy was exhumed for postmortem 1 week after her burial in Hyde Chapel cemetery. Forensic teams took prints from the body looking for matches on the will to indicate her handling of the document: none were found. Tissue samples were taken from thigh muscle and liver for drug levels. Using mass spectrometry, it was ascertained that diamorphine was indeed present at highly toxic levels in the samples. The levels were consistent with those found in fatal overdose cases.

Rumours about Shipman spread, and on September 19, 1997, the *Manchester Evening News* published a story detailing these. In response, many concerned families came forward, and as a result, further bodies of elderly females who had lived alone, and died in 'suspicious circumstances' were also exhumed and tested for diamorphine. Similar results were found.

Shipman was arrested on suspicion of murder on September 7, 1998, just over a year since the investigation had begun. At no point did Shipman confess to having any knowledge regarding the murders. He claimed that many of these women had in fact been substance abusers. He cited mainly codeine as the probable drug of abuse and said that he could prove his claims by retracing medical records where he had made entries indicating his suspicions. Forensic document analysts and computer experts were later to show that Shipman had in fact tampered with records on the actual day of the deaths (some hours after he had administered lethal doses of opioid to them). The truth was uncovered when the in-built clock in his practice computer verified the exact time of new entries to medical records, regardless of their apparent chronological ordering to the onlooker. Shipman's 'perfect' plan was crushed. His fabricated suggestions that patients had suffered from life-threatening illnesses or drug abuse were thrown out in court on the basis of the computer evidence.

However, an important question remained: how did Shipman obtain so much diamorphine in order to murder so many? After his reprimand from the GMC in the 1970s following his pethidine addiction, he was banned from holding controlled drugs in his surgery. However, calling upon the help of local pharmacists, police discovered in controlled drug registers that Shipman had indeed prescribed morphine and diamorphine for many patients – both those with, and those without terminal illnesses.

When local registered nurses working in home care settings of the terminally ill were interviewed, it became apparent that Shipman had frequently failed to deliver the full prescription of controlled drugs to patient's homes when he had collected them from pharmacies on their behalf. Shipman was effectively stealing up to 60% of the diamorphine prescribed for his patients.

Shipman's claims that those patients who were found with opiates in their dead body tissues were habitual drug abusers still needed to be quashed. The prosecution brought in an expert forensic hair analyst. As hair grows at a rate of 1 cm per month, the expert could identify evidence of opioid use by quantifying levels in strands of hair. Using mass spectrometry, he discovered that the amount present in the victims' hair was consistent with opioid use on only one or two occasions. In a narcotic abuser, it is normal to find 2 ng of the substance per milligram of hair – this level was 200 times more than that found in the victims' hair samples.

Expert forensic psychiatrists believe that Shipman was indeed a psychopath, feeding a need to maintain a perfect public and personal perception of himself. His addiction to the power of a perfect murder grew in strength and momentum over time, and cost the lives of many of those who entrusted their care to him. John Pollard, a coroner who knew and worked with Shipman has theorized that "The only valid possible explanation for it is that he simply enjoyed viewing the process of dying and enjoyed the feeling of control over life and death."

On January 31, 2000, Dr Harold Shipman was given 15 life sentences and 4 years to serve in prison for 15 murders and the forging of a will. A public enquiry later concluded that Shipman had killed at least 215 of his patients over a period of 23 years. The youngest victim was a 41-year-old man, the oldest a 93-year-old woman.

Shipman never publicly accepted responsibility for the death of his victims and showed no remorse for his crimes. He was found hanging from bedsheets in his prison cell in the early hours of January 13, 2004, one day before his 58th birthday. He left behind his wife, Primrose, three sons and a legacy of misunderstanding, doubt and public loss of trust in the medical profession. The full extent of his crimes will never be known.

Chemical Warfare

Records of poisonous chemicals being used to maim and kill date back to ancient Greek times, when pitch and sulfur were combined to make 'Greek Fire', which was launched at enemies in battle. Poisons were also used in medieval battles although their use was seen to betray the tenets of chivalrous conduct. The First Hague Convention at the end of the nineteenth century resulted in prohibition of the use of chemical weapons in war. This seemed to have little effect however, as the first time chemical weapons were used on a large scale was in World War I.

From the beginning of the War in 1914, both sides made use of various tear gases, although the German chemist Fritz Haber was working on more effective chemical means of penetrating Allied defenses. In 1915, the German military's certainty of victory began to waver, and they began planning chlorine gas attacks. The first of these, and perhaps the most famous, took place in Ypres, Belgium on April 22, 1915, against French and Algerian troops. The Germans set up more than 5000 cylinders of chlorine and when the valves were opened, a dense green

cloud of chlorine gas at 1000 ppm settled over Allied lines, killing thousands of soldiers. Ironically, although a number of these attacks were carried out over the next year, German forces were unable to take advantage of the breach in Allied lines as they themselves had limited protective supplies.

By 1916, poison gas in the form of chlorine and phosgene (both respiratory agents) had become a standard weapon used by both German and Allied forces, and primitive gas masks were standard issue among troops. Research into agents for chemical warfare was heavily funded on both sides, and the British established a large facility at Porton Down on Salisbury Plain for this purpose. However, in practice, gas attacks rarely went according to plan and although the British used phosgene very successfully at the battle of the Somme in June 1916, many British soldiers were killed by their own gas.

The race was on to develop the most effective poisons and the best methods of delivery as well as the most impenetrable gas masks. By 1918, gas mask technology had rendered chlorine and phosgene ineffective at normal concentrations, but the Germans had started using 'mustard gas', a liquid blistering agent (vesicant) that was rarely lethal but could incapacitate men who came in contact with it. Again, within months, mustard gas was being heavily used by both sides. Ill-prepared forces, such as the Russian troops on the Eastern Front, always suffered the most.

Chemical warfare continued until the armistice was declared in November 1918. By this time gas was estimated to have killed more than 100 000 men and injured more than a million (a small proportion, nonetheless, of the total number of casualties and deaths in the First World War).

By the end of World War I, a number of other agents had been developed, including the arsenical Lewisite (another blistering agent developed by the Americans), and agents that blocked the absorption of oxygen in the blood, such as hydrogen cyanide. Despite the Treaty of Versailles in 1919 and the Geneva Protocol in 1925, both of which forbade the use of chemical agents in warfare, research and development continued in secret. Fritz Haber continued his research into poisonous gases under the guise of 'pest control'. He developed an insecticide called 'Zyklon B' in the form of a crystalline material that released hydrogen cyanide fumes. Hydrogen cyanide had never achieved widespread use in World War I as it dissipated too easily in the open air. However, in enclosed spaces, it was very effective (see discussion on Holocaust gas chambers, below). Because of his Jewish background, Haber resigned when the Nazis came to power in Germany in 1933. However, he was soon replaced by Gerhard Schrader, who had accidentally come across a highly lethal organophosphate compound in December 1936 while carrying out genuine research into insecticides. Named 'tabun', it became the first in a long line of nerve agents. Tabun was invisible, odorless, and could kill in very small quantities by being absorbed through the skin (thereby rendering gas masks ineffective). With Nazi funding, Schrader soon developed an even more lethal nerve agent, which he named 'Sarin'.

Chemical warfare was beginning to resurface all over the world in the few years before World War II. The Italians dropped mustard gas from planes during their campaign in Abyssinia (now Ethiopia) in 1937 and the Japanese reportedly used mustard gas against the Chinese. The fear of chemical warfare in the imminent war resulted in exhaustive civil defense programs in Britain with the distribution of 30 million gas masks (useless of course in the face of a nerve agent attack).

When World War II broke out in 1939, stockpiling of poison gases recommenced. However, contrary to the predictions made before the war, chemical weapons were hardly used. Battles moved more rapidly than they had done in World War I, rendering many agents useless, and new explosives were capable of far more destruction than poison gases. The Germans produced nerve agents and, by 1944 they possessed enough tabun to kill vast numbers of people. However, they never used it, possibly because they believed (incorrectly) that the Allies knew about nerve agents and had their own supplies.

After World War II, large quantities of chemical weapons that had been stockpiled but never used, were very publicly disposed of. However, the existence of nerve gas was no longer a secret as Russian and British intelligence had uncovered its production in Germany at the end of the war. The Cold War saw massive development of nerve agents all over the world and while the British renounced the use of offensive chemical weapons in 1965, the Americans continued to develop new agents. By 1967 vast quantities of the nerve agent 'VX' had been produced. VX was less volatile and more toxic than previous nerve agents.

The Americans, who had not ratified the original Geneva Protocol in 1925, eventually ratified it in 1975. They then began an international campaign to limit the use of chemical weapons.

By 1980, although chemical weapons were still the subject of much debate and suspicion (including accusations of the use of 'Yellow Rain' in South East Asia and the use of 'knock down agents' by USSR forces in Afghanistan) they had not been used on a large scale since World War I. However, all that was to change during the Iran–Iraq war in the 1980s when the Iraqis resorted to chemical warfare to

compensate for their small numbers. Mustard gas, Lewisite and nerve agents (including VX) were all produced and mustard gas and sarin were used in attacks on Iranian forces. This was the first time in history that the use of chemical weapons had actually contributed to the defeat of a side (the Iranians). Then, following the success of these attacks, Sadam Hussein used mustard gas and the nerve agents sarin, tabun, and VX against Iraqi Kurds as part of a campaign aimed at depopulating rural Kurdistan. The most deadly of these attacks (of which there were more than 100) took place in Halabja in March 1988 and took the lives of thousands of civilians.

During the Gulf War in 1991, there were widespread fears that Saddam Hussein would use chemical and biological weapons on Coalition forces. However, these fears were not realized and no weapons of mass destruction were deployed. UN Inspection teams proceeded to destroy many of Iraq's stockpiles following Iraq's defeat, although whether stores remain is still a matter of considerable debate today.

The collapse of the USSR in the late 1980s and early 1990s was a step forward in controlling chemical weapons. However, by this time, destruction of chemical weapons was becoming increasingly difficult due to environmental issues. Incineration is now the only acceptable method of disposal and as yet only a limited number of suitable incinerators exist as the process is dangerous and expensive.

Although there have been no large-scale attacks using chemical weapons since the 1980s, a number of smaller attacks have occurred. Perhaps the most famous of these recent attacks took place on March 20, 1995, when containers of a liquefied form of sarin were placed on five different subway cars on three different lines in the Tokyo subway system by members of a Japanese religious sect named the 'Aum Shinrikyo' (Supreme Truth). Five thousand people were injured and 12 died. The leader of the sect, Shoko Asahara (born Chizuo Matsumoto) was arrested along with other members. Shoko Asahara confessed to the subway attack (as well as a number of other terrorist attacks, many of which had been thought to be accidental) and has since been sentenced to death.

In the aftermath of the September 11, 2001 attacks in the United States, many of the captured Islamic terrorists revealed how they had been trained in methods of dispersing hydrogen cyanide into the ventilation systems of buildings.

Clearly the threat of chemical warfare is still very real, and research continues into defensive technology such as nerve gas vaccines and chemical sensors. The Chemical Weapons Convention came into effect in 1997 and it has taken the Geneva Protocol one step further, banning the manufacture and storage of lethal and nonlethal chemical weapons as well as their use.

Holocaust Gas Chambers, World War II

Perhaps the most atrocious killings of the two World Wars took place not on a battlefield, but in the Nazi concentration camps. By 1941 prison camps were filling up with POWs and Jews but the Nazis had no efficient method of disposing them. Then, in September 1941, as an experiment, they tried using Zyklon B in Block 11 at Auschwitz I. Six hundred Soviet POWs and 250 Poles were killed and the Nazis had found an efficient and very effective method of destroying their prisoners.

Zyklon B was the trade name of a pesticide developed in the 1920s by the German (and ironically, Jewish) chemist Fritz Haber. It was originally used in concentration camps to delouse prisoners and control typhus. It consisted of small pellets or discs of wood pulp or diatomaceous earth impregnated with hydrocyanic acid (HCN). When the pellets were removed from their airtight containers they evolved hydrogen cyanide as a gas. The pellets normally contained an irritant warning chemical, but when the Nazis began to experiment with it in gas chambers they ordered Zyklon B to be produced without the warning chemical. After the War two directors of the company that supplied this modified Zyklon B were sentenced to death by a British Military court.

Zyklon B achieved its most widespread use in the gas chambers at Auschwitz II, Birkenau. There were four chambers at Birkenau, each capable of killing up to 2000 people at once. The system worked very efficiently; a death train would arrive at the camp in the morning and by the afternoon, the prisoners would have been poisoned and then either buried in mass graves or burned.

Other camps, such as Treblinka and Chelmno used carbon monoxide in their gas chambers.

The use of the word 'zyklon' (German for cyclone) continues to prompt angry reactions from Jewish groups. In 2002, both Bosch Siemens and Umbro were forced to withdraw from attempts to use or trademark the term for their products.

Conclusions

Murder by poison has changed a great deal over the centuries. In ancient times, poisons were often thought of as spiritual or witchcraft, and were used with little knowledge of how they worked; there was no such thing as toxicology, let alone forensic toxicology. For this reason, detection of these murders was

often very difficult. However, poisoning is no longer a mystery to us. Toxicology has given us insight into the mechanisms by which poisons act, allowing us to detect their use and prevent and treat their effects.

However, with knowledge comes the power to misuse it. This is demonstrated by the (thankfully rare) cases of medical professionals, such as Harold Shipman, who abuse their position of trust and murder patients, and it is also seen on a far larger scale when countries at war resort to the use of chemical weapons. Most of the chemical warfare agents used recently, such as sarin, have been developed with a full understanding of the potentially devastating effects they have on the human body.

It is clear that murder by poison, from individual cases to chemical warfare, will continue in the future, and advancements in toxicology will inevitably aid as well as hinder its use.

In fact, dioxin was the poisoning agent in a high profile political incident in 2004. It was ultimately identified as the cause of the disfiguring acne-like skin condition suffered by Ukrainian opposition leader Viktor Yushchenko a few months before the first presidential election. The suspicion is that the dioxin was placed into soup ingested by Mr. Yuschenko. Despite his condition, Mr. Yuschenko continued to campaign and was the ultimate victor when a second election was held after the first outcome was invalidated. The acne-like skin condition is the most recognizable hallmark of dioxin poisoning in humans. It is expected that at least most of his skin condition is reversible; however, his situation is unique as a known case of high-exposure-dioxin poisoning, with severe effects, in a human. Futher, it is not known what other effects to his body related to the poisoning might surface in future months and years. The actual intake of dioxin in this poisoning is unknown.

See also: Arsenic; Hemlock, Poison; Nerve Agents; Strychnine.

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Nutmeg

Christopher P Holstege

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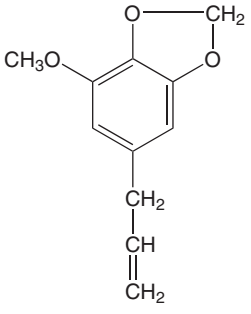
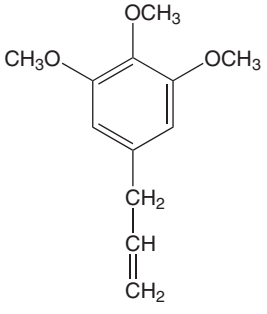
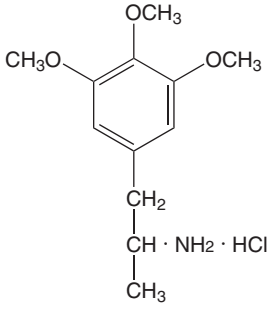
- Chemical Abstracts Service Registry Number: CAS 8008-45-5
- Synonyms: *Myristica fragrans*; Brown slime; Mace; Madashaunda (narcotic fruit); Pala banda; Spice of madness
- Chemical Structures:



Myristicin



Elemicin



Amphetamine corresponding to elemicin

Uses

Nutmeg is used as a spice, as a hallucinogen, and as an herbal remedy for ailments such as rheumatism, diarrhea, anxiety, and excessive flatulence.

Background Information

Nutmeg is the seed of *Myristica fragrans*, an aromatic evergreen tree cultivated in Indonesia and Grenada.

Exposure Routes and Pathways

Nutmeg is ingested whole, in ground or grated form, or as a slurry of water and powder (brown slime). Nutmeg powder is occasionally sniffed.

Toxicokinetics

The volatile oils in nutmeg consist of allylbenzene derivatives and terpines. Myristicin, elemicin, and safrole comprise 80% of the allylbenzenes. Myristicin and elemicin may be biotransformed into MMDA (3-methoxy-4,5-dimethylene-dioxamphetamine) and TMA (3,4,5-trimethoxyamphetamine), respectively, both consisting of a difference of only an amine group added to the side chain. Symptoms occur within 3–8 h, followed by 6–24 h of alternating periods of stupor and delirium. Recovery normally occurs within 24 h but may take several days.

Mechanism of Toxicity

A probable metabolite of myristicin is MMDA and of elemicin is TMA. Both of these metabolites are psychoactive compounds related to amphetamine. Other components of the volatile oil, such as eugenol, isoeugenol, safrol, and linalool, are structurally similar to some serotonin agonists and may contribute to the psychological effects. The terpine hydrocarbons are unlikely contributors to the psychomimetic effects but may increase absorption of the allylbenzenes by irritation of the stomach. Nutmeg has weak monoamine oxidase-inhibiting abilities. Nutmeg inhibits the synthesis and activity of prostaglandin B in the colon, giving an antidiarrheal effect. It also has antiinflammatory properties.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD of nutmeg oil is 2600 mg kg^{-1} in rats, 4620 mg kg^{-1} in mice, and 6000 mg kg^{-1} in hamsters. Fatty degeneration of liver has been noted in dogs and cats.

Human

Nutmeg is abused for its narcotic and hallucinogenic properties. One to three seeds or 5–30 g of the ground nut are used to attain psychogenic effects. One tablespoon of ground nutmeg or one grated nutmeg yields ~7 g. A fatality was reported in an 8 year old who ate two nutmegs. A 55 year old was also suspected to have died from acute nutmeg poisoning and was found to have a blood level of 4.0 μg ml^{-1}. Nutmeg may produce symptoms similar to those of an anticholinergic poisoning. The reported initial neurological effects include giddiness, tingling, euphoria, and hallucinations that may include distortion of time and space, detachment from reality, sensation of separation from one's limbs, and fear of impending death. This is followed by alternating delirium and extreme drowsiness or stupor. However, common unpleasant side effects occur and include headache, nausea, vomiting, abdominal pain, dizziness, chest pain, flushing, tremor, and tachycardia. The blood pressure may slightly increase, but a marked decrease with cyanosis and shock has been reported. Palpitations, agitation, anxiety, dry mouth, chest tightening, and blurred vision were reported in a pregnant woman in her third trimester who ingested one tablespoon of nutmeg. The fetal heartbeat was increased for 12 h. Levels for myristicin and elemicin are not generally available. Myristicin has been isolated from nutmeg using high-performance liquid chromatography. Other laboratory values have been reported to be normal.

Chronic Toxicity (or Exposure)

Human

Chronic nutmeg abuse has been reported to induce psychosis that is reversible after cessation of nutmeg ingestion.

Clinical Management

Treatment should focus on keeping the patient calm while hallucinations are occurring, maintaining blood pressure, and controlling nausea and vomiting. Gastric emptying can be considered if the ingestion was recent. Activated charcoal and a cathartic may be given. Benzodiazepines have been used to decrease anxiety and agitation. Intravenous fluid administration along with antiemetics may be indicated to treat dehydration, nausea, and vomiting.

See also: Amphetamine; Benzodiazepines.

Further Reading

Abernethy MK and Becker LB (1992) Acute nutmeg intoxication. *American Journal of Emergency Medicine* 10: 429–430.

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Occupational Exposure Limits

Andrew Maier

Occupational exposure limits (OELs) provide health and safety professionals an important tool for protecting worker health. OELs provide health and safety guidance to chemical users, inform workers of potential adverse effects of chemical exposure, and provide a scientific basis for evaluating whether existing environmental exposure controls are adequate.

Many organizations around the world develop OELs using approaches that fit the unique needs of the constituencies involved and the mission of the organization. For example, some organizations set health-based guidelines that reflect best scientific judgment regardless of other considerations, while many regulatory organizations evaluate policy and management issues such as implementation costs and technical feasibility as part of the OEL determination. Nevertheless, the general scientific approach used by most organizations is similar and includes a detailed critical review of the epidemiology and toxicology information to identify potential hazards, selection of sensitive adverse effects, dose-response estimation to determine appropriate thresholds, and an evaluation of tenant uncertainties to ensure the desired margin of safety.

There are several general categories of OELs for airborne chemical exposure, which differ primarily on the duration of exposure considered relevant for preventing the effect of concern. The common OEL duration categories include:

- *Time-weighted average (TWA)*: These limits are generally developed to protect from health effects caused by longer-term or chronic exposures (e.g., chronic target organ damage) and are compared against air concentrations measured over full-shift exposure durations (e.g., 8 or 10 h, depending on the organization). Note that methods to adjust OELs for other durations based on toxicokinetic considerations have been developed for cases involving exposures that occur during nonstandard work schedules.
- *Short-term exposure limit (STEL)*: These limits are generally developed for substances that induce effects of concern following fairly brief periods of exposure. For example, many STELs are based on thresholds for the induction of irritant responses or central nervous system depression, or for preventing chronic or irreversible damage due to brief periods of exposure. Many organizations establish STELs as a 15 min TWA air concentration that should not be exceeded during a work shift. Many compounds do not have sufficient data to serve as the basis for developing a STEL. However, some organizations recommend general excursion limits that are a multiple of the full-shift TWA limit (e.g., three times the TWA), as a measure of protection from peak exposures even when no STEL has been established.
- *Ceiling limit*: These limits are generally developed to protect from effects caused very quickly if a threshold concentration is exceeded. For example, ceiling limits are established for many highly potent irritants. The ceiling limit generally refers to the maximum concentrations in air that should not be exceeded at anytime during the work period.

Most published OELs are derived on the basis of preventing adverse effects arising from occupational exposures due to contaminant concentrations in the air. However, dermal exposures may also contribute to the overall body burden. Most OEL-setting organizations have developed qualitative notations to identify those substances for which dermal exposure may contribute significantly to the total body burden. For substances with a skin notation, caution should be used in interpreting the level of protection afforded by the OEL if skin exposure may occur.

Most OEL-setting organizations also establish qualitative notations to indicate the ability of a compound to induce dermal or respiratory sensitization. This approach is used since dose-response thresholds for the induction of these sensitization responses are generally not well understood, and sensitized individuals may respond to very low exposure

concentrations and may not be protected by an OEL based on other toxicity endpoints.

Organizations differ in the approaches used to develop OELs for carcinogens. Some organizations develop OELs using a threshold-based assumption for all compounds, while others do not set health-based limits for carcinogens that are thought to act via linear (e.g., genotoxic) mechanisms. Most organizations do provide some classification approach to identify carcinogens.

Other Types of OELs

Several other types of occupational exposure limits are derived in addition to the OELs derived to protect against airborne chemical exposures in traditional workplace settings.

Dermal contact is the primary route of exposure for many substances that have low vapor pressure and are not aerosolized. For these substances and exposure situations, it can be valuable to develop dermal exposure limits. Few dermal exposure limits have been published by the primary OEL-setting organizations. Nevertheless, the field is maturing with increasing publication of proposed reference values for dermal exposure in the literature.

Most OELs are developed to protect workers from the development of any adverse effects. However, in some cases, guidance on thresholds for effects of greater severity is an important tool – in particular for emergency response applications. There are a number of different organizations that establish acute emergency exposure values. In occupational settings, immediately dangerous to life or health (IDLH) values are often used for setting protective equipment requirements and in emergency planning.

Assessing exposures to physical agents is also important for protecting worker health. Many organizations establish workplace exposure guidelines for physical agents and stresses, including noise, heat or cold, nonionizing and ionizing electromagnetic radiation, and ergonomic stressors such as vibration and repetitive trauma.

Key OEL-Setting Organizations

There are many other organizations that establish workplace exposure guidance. The list in the Relevant Websites section at the end of the article represents those organizations that establish OELs that are frequently cited for international application, but this list is not comprehensive. In addition, many companies develop OELs for their specific products. These company limits are not often published, but they can be requested from product suppliers or be found on company product literature (such as Material Safety Data Sheets).

See also: American Conference of Governmental Industrial Hygienists; American Industrial Hygiene Association; Exposure Criteria; National Institute for Occupational Safety and Health; Occupational Safety and Health Administration; Occupational Toxicology.

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Relevant Websites

http://www.acgih.org – American Conference of Governmental Industrial Hygienists (ACGIH). The Threshold Limit Value (TLV) Chemical Substance Committee of ACGIH develops TLVs®, which are health-based OEL guidelines.

http://www.aiha.org – American Industrial Hygiene Association (AIHA). The Workplace Environmental Exposure Level Committee establishes WEEL Guidelines which are health-based OELs.

http://www.cdc.gov – (US) National Institute for Occupational Safety and Health (NIOSH) develops health-based recommended exposure limits (RELs).

http://www.osha.gov – (US) Occupational Health and Safety Administration (OSHA). OSHA has regulatory authority in the United States for workplace health and safety. The OELs promulgated by OSHA are Permissible Exposure Limits (PELs).

http://europe.osha.eu.int – (EU) Scientific Committee on Occupational Exposure Limits (SCOEL). The EU committee establishes health-based OELs for use by EU member countries. Occupational Exposure Limits: Summary of Information from EU Member States and Other Sources.

http://www.hse.gov.uk – (UK) Health and Safety Executive. The Health and Safety Commission's Advisory Committee on Toxic Substances (ACTS) recommends new OELs or revision of a current OEL value, which can be adopted as enforceable limits by the regulatory authority.

www.hvbg.de – (Germany) The DFG Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work area – the MAK Commission sets health-based OELs for threshold substances (carcinogens that act via genotoxic mechanisms are addressed through a separate process).

Occupational Safety and Health Act, US

Michael A Kamrin

The Occupational Safety and Health Act (OSHAct) is administered and enforced by the Occupational Safety and Health Administration (OSHA). Both OSHA and OSHAct were created in December 1970, the same month the US EPA (Environmental Protection Agency) was created. Unlike the US EPA, OSHA is essentially an enforcement organization and most of its employees are inspectors who perform thousands of workplace inspections per year; it is a division of the Department of Labor. The OSHAct assures, as far as possible, that all working men or women have risk-free working environments; and imposes on employers the obligation to provide employees with workplaces that are free from recognized health and safety hazards and to maintain compliance with specific OSHA standards.

Many states and territories also have their own occupational safety and health plans that have been approved by OSHA and many of these are more stringent than the federal OSHA requirements.

The OSHA Hazard Communication Standard, better known as the "Right-to-Know" law, requires that the hazards of all chemicals produced in or imported into the United States are evaluated and that employers provide their employees with all appropriate hazard information. This involves providing employees with hazard communication/training programs and access to material safety data sheets (MSDSs) and written records. OSHA considers the MSDS the primary vehicle for transmitting detailed hazard information to downstream employers and employees.

Chemical manufacturers and importers must make a "hazard determination" of the chemicals with which they are involved. This involves an assessment of the physicochemical properties of a material (e.g., flammability, explosivity, corrosivity, and reactivity) as well as potential acute and chronic toxicity. However, manufacturers and exporters are not required to conduct additional testing. Typically, the hazard determination is made on the basis of existing company data or information from the published scientific literature.

Worker exposure to chemicals in the workplace is regulated through the promulgation of permissible exposure limits (PELs) that are maximum allowable exposure limits or maximum time-weighted average limits over an 8 h working day. These are complemented by short-term exposure limits. In March 1989, OSHA reduced the PELs for many substances and set new ones for substances previously not regulated; OSHA is still in the process of developing permanent health-based workplace standards. Many of the standards are based on recommendations made in criteria documents prepared by the National Institute for Occupational Safety and Health (NIOSH), although OSHA has its own standards office. Another listing of exposure limits contains the threshold limit values (TLVs) recommended by the American Conference of Governmental Industrial Hygienists (ACGIH). The standards recommended by OSHA, NIOSH, and ACGIH often differ from each other and may be quite controversial. As a result, standards may become mired in hearings and in the courts.

See also: American Conference of Governmental Industrial Hygienists; Carcinogen Classification Schemes; Medical Surveillance; National Institute for Occupational Safety and Health; Occupational Safety and Health Administration; Occupational Toxicology.

Further Reading

DiBerardis LJ (ed.) (1998) *Handbook of Occupational Safety and Health*, 2nd edn. Hoboken, NJ: Wiley-Interscience.

Occupational Toxicology

Elizabeth V Wattenberg

Introduction

The aim of occupational toxicology is to help create a safe workplace. Historically, occupational studies have provided some of the strongest evidence that exposure to xenobiotics (chemicals or other agents that are foreign to the body) in the environment can cause disease in humans. As early as 370 BC, Hippocrates described symptoms of lead poisoning in a metal worker. In 1775, Sir Percival Pott recognized that soot played a role in the high rate of scrotal

cancer among chimney sweeps. In 1977, case studies on pesticide workers indicated that exposure to dibromochloropropane could cause infertility and sterility in men. Such observations have sparked interest in investigating how xenobiotics injure or disrupt biological systems. In turn, results from toxicological studies are used to develop methods to assess workplace exposures and to establish occupational exposure limits.

Occupational toxicology draws from the same framework as other disciplines in toxicology (**Figure 1**). This framework outlines the major physiological steps that can influence the dose–response for a xenobiotic. A dose can be defined as the amount of a xenobiotic a worker is exposed to in an occupational setting, and a response is some overt physiological effect, such as organ damage. The growing field of molecular toxicology aims to refine the characterization of the dose–response, such that the dose reflects the amount of the xenobiotic or active metabolite that reaches a critical target in the body, and the response is an early, subtle change in cells or components of cells that precedes clinical disease.

Developing occupational exposure guidelines involves risk assessment – the evaluation of toxicological and exposure data to determine the health risks presented by using xenobiotics in the workplace. The toxicological characterization of xenobiotics contributes to the risk assessment process by providing information for the dose–response and hazard identification steps (**Figure 2**). Hazard identification describes the types of physiological effects a xenobiotic can cause – for example, reproductive toxicity, cancer, respiratory problems, or allergic reactions. Toxicology may also play an increasingly important role in exposure assessment as advances in molecular toxicology promise to improve the measurement of the dose of a xenobiotic that is absorbed by the body or that hits a critical target tissue. Risk characterization synthesizes the information gathered in the dose–response, hazard identification, and exposure assessment steps for use in risk management decisions. Risk management strategies can range from recommending the use of protective equipment to setting occupational exposure limits, or possibly to eliminating a xenobiotic from the workplace. Making informed decisions regarding worker health requires a clear understanding of health risk information.

Use of Toxicological Data

Although epidemiological data provide the strongest evidence that specific chemicals affect human health, occupational exposure limits and other preventive measures typically are not based on these types of data alone. The number of chemicals to which workers are exposed far exceeds the number of solid epidemiological studies. Therefore, many occupational health standards and protective health measures are based on data from toxicology studies. Consequently, one of the major challenges that faces an occupational toxicologist is how to interpret the results obtained from animal studies and molecular studies for application to humans.

Occupational toxicology relies on information from both traditional toxicology studies and investigations into the molecular mechanisms of xenobiotics. Toxicology studies involve exposing groups of animals to various doses of a xenobiotic and observing the response. These are the types of toxicology studies that have traditionally been used to develop exposure guidelines. Very simply, toxicologists use these studies

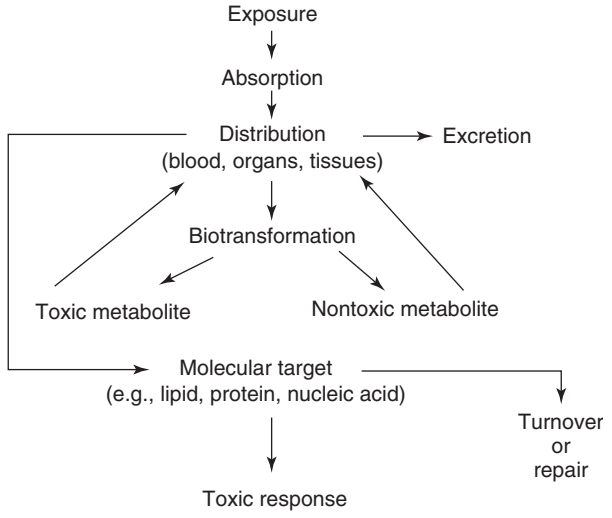

Figure 1 Toxicology framework.

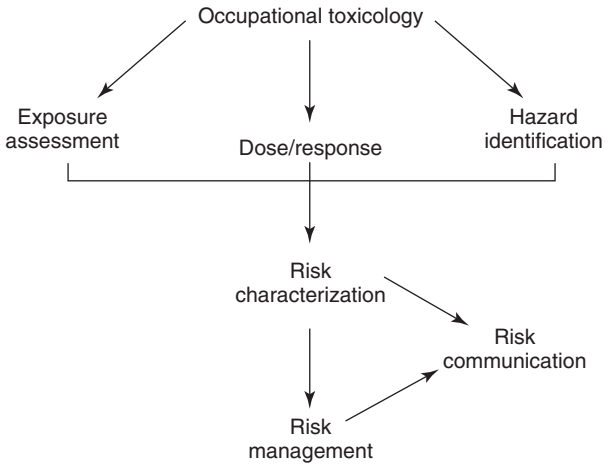

Figure 2 Occupational toxicology and risk assessment.

to determine what dose presents little or no risk of causing a harmful effect in humans. Classic toxicology studies also provide information on the types of effects the xenobiotic can cause, such as cancer, neurotoxicity, or organ damage.

Molecular toxicology investigates the biochemical steps that lead to the physiological response observed in the classic studies. Identifying the critical molecules and cellular processes involved in toxicity can help determine whether the effects seen in animals are likely to occur in humans. For example, studies suggest that the protein α-2μ-globulin plays a key role in the induction of kidney tumors by D-limonene. This protein is synthesized in large quantities by adult male rats, but not by humans, suggesting that although D-limonene causes kidney tumors in male rats, it may not cause the same effect in humans. Characterizing the molecular target of a xenobiotic can also provide clues for the prevention of toxicity or perhaps aid in developing therapies or antidotes for toxic effects. For example, neurotoxic organophosphate insecticides inactivate the enzyme acetylcholinesterase. Pralidoxime acts as an antidote for this type of pesticide poisoning by reactivating this enzyme. Finally, molecular toxicology can help identify biological markers, such as xenobiotics bound to DNA or proteins, altered or unusual macromolecules, or changes in gene expression, which can be used to indicate exposure or preclinical toxic effects.

Exposure

When choosing a study for applications in occupational toxicology, it is important that the exposure protocol be relevant to the exposure scenario in the workplace. The route, duration, and frequency of exposure can have a significant effect on the toxicity of a xenobiotic agent.

The route of exposure determines both the initial physiological barrier faced by the xenobiotic and its initial metabolic fate. Two of the primary defense mechanisms against xenobiotics are barriers, such as skin and membranes, and the biotransformation or breakdown of toxic compounds to nontoxic products. Xenobiotics must elude these defense mechanisms in order to reach the target tissue and cause damage.

Physiological barriers, such as the cells that line the gastrointestinal tract and respiratory tract, and make up the skin, determine the amount and the rate of absorption of specific xenobiotics into the body. Some xenobiotics do act directly at the site of exposure. For example, epoxy resins can cause allergic contact dermatitis, and UV light can cause skin cancer. Other xenobiotics damage tissues that are distant from the site of exposure. For example, many solvents that are inhaled, such as trichloroethylene and perchloroethylene, cause liver damage. These types of xenobiotics reach their targets by being absorbed into the circulatory system.

The toxicological effects observed due to exposure from one route cannot necessarily be used to predict the toxicological effects that would result from exposure via another route. For example, inorganic lead is almost completely absorbed through the lower respiratory tract but does not easily penetrate the skin. Ease of absorption through a particular physiological barrier depends on the physical and chemical characteristics of xenobiotics. In contrast to inorganic lead, organic lead, such as tetraethyl lead, is readily absorbed through the skin. Therefore, the results from a dermal toxicology study on inorganic lead cannot directly predict the dose–response for dermal exposure to organic lead. Likewise, reducing dermal exposure to organic lead would require stricter protective measures than that for inorganic lead.

In addition to presenting the initial physiological barrier to a xenobiotic, the route of exposure also determines the initial metabolic fate of the agent. The liver, which contains the highest concentration of enzymes involved in biotransformation, is the primary site for detoxification. In addition, some xenobiotics can be broken down by the acid pH of the stomach or enzymes present in the gastrointestinal tract. Chemicals that enter the body orally will face both of these defense systems before they are absorbed into the general circulatory system. By contrast, xenobiotics that are absorbed through the respiratory tract or through the skin are transported to the general circulatory system without first passing through the liver. Therefore, they may reach a target tissue before exposure to the detoxifying enzymes in the liver. Injection can be a particularly dangerous route of exposure because the agents bypass all of the barrier properties of the skin and directly enter the bloodstream.

The duration and frequency of exposure can also influence the physiological effect of xenobiotics. The health effects following a short-term, high-dose (acute) exposure to a xenobiotic can differ dramatically from the effects of a long-term, low-dose (chronic) exposure. For example, acute inhalation exposure to vinyl chloride causes respiratory tract irritation, lethargy, and headache. Chronic exposure to vinyl chloride can cause hepatic angiosarcoma. Whereas short-term, low-dose exposure to a specific xenobiotic may not be toxic, prolonged or frequent exposure to the same xenobiotic may deplete detoxifying or repair systems and therefore result in the

accumulation of damaged tissue. Toxicologists interpret short-term animal studies with care because many xenobiotics have latent effects, such that the toxic response is not detected until long after the time of exposure. This also holds true for the interpretation of epidemiological data. For example, latency is well established for carcinogens. Tumors may not appear in animals for weeks or months, and cancer may not appear in humans until decades after exposure. Other xenobiotics, such as neurotoxins, may also have latent effects.

Exposure scenarios differ depending on the type of occupation and the physical workplace. Inhalation is a common industrial route of exposure. Some xenobiotics can be absorbed through the eyes. Many types of work involve dermal exposure to xenobiotics. Medical personnel are at risk of being exposed to a variety of agents through injection. To ensure worker safety, all of the possible pathways of exposure should be examined.

Factors That Affect Toxicity

Both environmental factors and an individual's characteristics can affect the toxicity of a given xenobiotic. For example, exposure to other xenobiotics, diet, age, sex, and genetics can alter the toxicity of a given xenobiotic, in part, by modulating its biotransformation. Biotransformation can detoxify xenobiotics, but this process can also bioactivate xenobiotics – that is, transform a relatively benign parent compound into a more toxic intermediate that can go on to interact with a target and cause damage. Ultimately, the effect of environmental agents on endogenous macromolecules, such as hormones and the enzymes and cofactors involved in biotransformation, determines the fate of a xenobiotic.

In general, biotransformation reactions convert lipophilic (fat-soluble) xenobiotics into compounds that are hydrophilic (water-soluble). As a result, lipophilic xenobiotics can be excreted from the body instead of accumulating or damaging a target tissue. The biotransformation process is broadly divided into phase I and phase II reactions. Phase I reactions prime xenobiotics for phase II reactions by adding or exposing a functional group (e.g., –OH, –SH, $–NH_2$, or –COOH). Phase II reactions take advantage of the functional group produced by the phase I reactions and add a water-soluble molecule to the xenobiotic, making it even more hydrophilic and therefore more readily excreted. Xenobiotics that already have a functional group can also undergo phase II reactions. Biotransformation requires a variety of enzymes and a set of endogenous cofactors. Biotransformation takes place primarily in the liver, which contains a very high level of the enzymes involved in phase I and phase II reactions. Although biotransformation also takes place in other organs, such as the lung, stomach, intestine, skin, and kidneys, the liver generally has a wider variety of metabolic enzymes than the other tissues and therefore can modify a broader range of compounds.

Phase I reactions are primarily catalyzed by a family of enzymes called cytochrome P450s. Like the immune system, which has evolved to combat a broad range of foreign antigens, the cytochrome P450 system has evolved to modify remarkably diverse classes of xenobiotics. Specific isoenzymes of cytochrome P450 catalyze reactions involving particular classes of xenobiotics. For example, CYP1A1 catalyzes the hydroxylation of benzo(*a*)pyrene, and CYP2E1 catalyzes the oxidation of alcohols.

Phase II reactions generally require a transferase type of enzyme and an endogenous cofactor to add a water-soluble molecule to a xenobiotic. Phase II reactions are also called conjugation reactions, and the modified products of these reactions are called conjugates. Like the cytochrome P450s, specific transferase enzymes and their associated cofactors tend to catalyze conjugation reactions with particular structural classes of compounds. For example, the enzyme UDP-glucuronosyl transferase, along with the cofactor UDP glucuronic acid, catalyzes the formation of glucuronide conjugates of aliphatic or aromatic alcohols, carboxylic acid, sulfhydryl compounds, and amines. Glutathione *S*-transferases use the cofactor glutathione to catalyze the formation of conjugates with a number of reactive intermediates, including epoxides.

Some xenobiotics can undergo competing biotransformation reactions. The extent to which a given xenobiotic will be detoxified or bioactivated depends, in part, on the physiological levels of specific enzymes and cofactors required for each biotransformation pathway. For example, nutritional status, sex, age, genetics, and the presence of or previous exposure to other xenobiotics can influence the toxicological fate of xenobiotics by affecting enzyme levels and cofactor pools. If toxicologists understand how these factors modulate toxicity, they can more accurately determine if the results of animal studies can be extrapolated to humans. In addition, this information may be used to identify individuals who might be particularly sensitive to certain types of exposures.

One xenobiotic can affect the toxicity of another by either increasing enzyme activity or depleting essential cofactors. Many of the enzymes involved in biotransformation reactions are inducible, such that

certain compounds can cause an increase in the levels of specific isoenzymes. An increase in the level of an isoenzyme usually results in an increase in the rate of the specific reaction catalyzed by that isoenzyme. Some xenobiotics induce the expression of enzymes that catalyze their own biotransformation. For example, polycyclic aromatic hydrocarbons induce the expression of an isoenzyme of cytochrome P450 that catalyzes the hydroxylation of benzo(*a*)pyrene, and ethanol induces the expression of an isoenzyme that catalyzes the oxidation of ethanol. Xenobiotics can also induce enzymes that catalyze the biotransformation of other compounds. For example, pretreatment of rats with 3-methylcolanthrene increases the biotransformation of aniline. Common inducers of cytochrome P450s include 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), halogenated pesticides, such as DDT, and polychlorinated biphenyls. Natural substances in the diet can also induce some of the enzymes involved in biotransformation, as can steroid hormones. Some of the enzymes involved in phase II reactions are also inducible. For example, 3-methylcholanthrene increases the expression of glutathione-*S*-transferases. Specific isoenzymes of glucuronosyltransferases are also inducible. Finally, an inadequate supply of cofactors can limit phase II reactions. Exposure to xenobiotics can deplete cofactor pools. For example, exposure to high doses of acetaminophen can deplete the supply of phase II cofactors sulfate and glutathione. Nutritional status may also affect cofactor pools.

Because many xenobiotics can modulate biotransformation pathways, it is difficult to predict the health effects or dose–response that would result from exposure to multiple xenobiotics. Mixtures of xenobiotics can cause an additive response, where the mixture acts like one xenobiotic of a dose equal to the sum of the doses of the individual xenobiotics. Xenobiotics can also act synergistically, that is, cause a response that is greater than additive. The actions of different compounds may also be antagonistic such that the response is less than additive. Finally, the components of a mixture may act independently, resulting in no detectable change in response. Few xenobiotics have been tested extensively enough to determine how they might interact with other compounds. Instead, most toxicological studies investigate exposures to a single compound. Where studies on mixtures do exist, they often investigate the interaction of only two compounds. The biological response to two compounds could be altered by the presence of a third. The problem grows more complex as the number of xenobiotics increases. Finally, the type of interaction may depend on the doses of the specific components of a mixture. Clearly, there is no simple formula for accurately predicting the health risk from exposure to mixtures.

Nevertheless, workers are often exposed to more than one xenobiotic. The American Conference of Governmental Industrial Hygienists (ACGIH) has developed exposure limits for airborne substances called threshold limit values (TLVs). To estimate the TLV for a mixture, the ACGIH recommends using a model based on the assumption that when xenobiotics "act upon the same organ system … [i]n the absence of information to the contrary, the effects of the different hazards should be considered as additive" (ACGIH, 2003). This model generates a hazard index (HI) that indicates whether a mixture has exceeded an exposure guideline:

$$\mathrm{HI} = C_1/T_1 + C_2/T_2 + \cdots + C_x/T_x$$

where C_x is the atmospheric concentration of the xth (e.g., first, second) xenobiotic and T_x is the TLV for the xth xenobiotic.

An $\mathrm{HI} \geqslant 1$ indicates that the mixture exceeds the TLV. A separate HI is calculated for each set of xenobiotics in a mixture that causes the same type of toxicological effect. For example, an HI would be calculated for all liver toxicants in the mixture, a separate HI would be calculated for xenobiotics that cause kidney damage, etc. Further refinement of the additivity model or development of an alternative model will require more extensive research.

The ACGIH does recognize that some xenobiotics may act synergistically. For example, exposure to asbestos and cigarette smoking are synergistic for the induction of lung cancer. The ACGIH also recognizes that compounds absorbed through different routes of exposure can act synergistically. For example, drinking alcohol can affect the toxicity of a solvent, like trichloroethylene, that is inhaled. With regard to possible synergistic interactions, the ACGIH maintains that "[s]uch cases at present must be determined individually" (ACGIH, 2003).

The level and activity of specific enzymes involved in biotransformation can differ depending on the species, strain, age, and sex of the test animal. For example, cats cannot carry out glucuronidation reactions, newborn rats have relatively low cytochrome P450 activity, and male rats are more sensitive to carbon tetrachloride toxicity than female rats. These differences are important to consider when interpreting the results from toxicological studies. The observation that age, sex, and genetics can significantly influence biotransformation reactions in animals raises the question of whether these characteristics also affect the biotransformation capacity of humans.

The field of toxicogenetics focuses on the genetic basis for the differences in xenobiotic metabolism. This field has grown out of an interest in identifying individuals who may be particularly sensitive to certain types of drugs or environmental exposures. For example, sensitivity to certain compounds may differ as much as 200-fold among individuals. Individual differences in xenobiotic metabolism result from polymorphisms among the population. That is, some members of the population express different forms of enzymes involved in biotransformation. While these polymorphisms have largely been characterized in terms of drug metabolism, these differences also have implications for exposures to other types of xenobiotics.

One common polymorphism in the United States is for *N*-acetyltransferase, an enzyme involved in phase II reactions. *N*-acetyltransferase catalyzes the acetylation of aromatic amines and hydrazines, and other classes of xenobiotics. People characterized as 'slow acetylators' have relatively low *N*-acetytransferase activity. Consequently, slow acetylators are more sensitive to the toxic effects of certain types of drugs, including sulfa drugs. In addition, a study of workers exposed to benzidine in the dye industry suggested a link between the 'slow' acetylator phenotype and the development of bladder cancer.

Polymorphisms also exist for specific isoenzymes of cytochrome P450. For example, there is a polymorphism within the human population for a cytochrome P450 isoenzyme that catalyzes the 4-hyroxylation of the drug debrisoquine. 'Extensive metabolizers' hydroxylate this drug 10–200 times faster than 'poor metabolizers'. Poor metabolizers express much less of the isoenzyme involved in this reaction than extensive metabolizers. This polymorphism also appears to affect the metabolism of environmental agents. For example, there appears to be an association between the poor-metabolizer phenotype and Parkinson's disease. By contrast, the extensive-metabolizer phenotype may be correlated with an increased risk of developing cancer.

The use of genetic information to identify sensitive individuals raises difficult policy questions. For example, individuals with certain polymorphisms might be sensitive to a xenobiotic at concentrations below the TLV. Although this information could be used to recommend additional protective measures for sensitive workers, there is also concern that the identification of susceptible individuals could result in job discrimination.

Male and female animals can have dramatically different responses to certain xenobiotics. For example, TCDD induces liver tumors in female rats but not in male rats. By contrast, chloroform is a more potent kidney toxin in male mice than in female mice. Female rats are more sensitive to the organophosphate insecticide parathion than male rats. Castration increases the sensitivity of the male rats to parathion. In addition to their role in xenobiotic biotransformation, cytochrome P450s are also involved in the biotransformation and synthesis of endogenous compounds, including fatty acids, prostaglandins, and steroid hormones. Therefore, it should not be surprising that hormones can regulate the activity of specific cytochrome P450 isoenzymes. The question of to what extent men and women differ in their responses to xenobiotic agents is still under investigation. The composition of the work force has changed dramatically in the past few decades, with an increasing number of women holding positions that traditionally had been held by men. Therefore, it is important to consider possible sex-specific differences in toxic responses if results from occupational studies of male workers or other epidemiological data on men are used to set occupational standards that are applied to both men and women.

Biomonitoring: Molecular Targets

One aim of occupational toxicology is to improve exposure assessment. The field of molecular toxicology provides information on the types of molecules and macromolecules that can serve as indicators of exposure. Whereas classic toxicology studies usually examine gross effects, such as changes in body weight, organ damage, or tumor development, molecular toxicology investigates the biochemical mechanisms of action of xenobiotics. In other words, molecular toxicology investigates the molecular or cellular events that eventually lead to the gross effects observed in classic toxicology studies. The results from molecular toxicology studies can be used to develop biological markers, also called biomarkers. Biomarkers are generally defined as 'cellular, biochemical, or molecular alterations' that can be measured in 'biological media such as human tissues, cells, or fluids'. Biomarkers can be applied in occupational settings to measure exposure to xenobiotic agents and to detect an early response that could lead to toxic injury or disease.

Estimating exposure usually requires measuring the amount of the xenobiotic in the air, water, dust, or other media. These types of measurements may be technically difficult or expensive to do. In addition, concentrations of xenobiotics can vary depending on time and location. Therefore, these measurements may not give an accurate estimate of past exposures. Furthermore, monitoring the levels of xenobiotics in the workplace does not necessarily indicate how

much of the xenobiotic has been absorbed into the body or how much reaches the target tissue. One potential use of biomarkers in occupational settings is to refine exposure assessment. Common types of biomarkers include parent compounds or metabolites that can be measured in urine or exhaled breath and metals that can be measured in hair. Development of other types of biomarkers relies on the identification of a molecular target. For example, lead decreases ferrochelatase activity, an enzyme important in heme biosynthesis. As a result, some red blood cells contain zinc–protoporphyrin instead of hemoglobin. Therefore, zinc–protoporphyrin levels in erythrocytes have been used as a biomarker of lead exposure. Likewise, since organophosphate and carbamate pesticides inhibit acetylcholinesterase, measurement of inhibition of choline esterase activity in the blood has been used as a biomarker of exposure to these pesticides. Some xenobiotic agents bind directly to DNA or to proteins. For example, benzo(*a*)pyrene forms DNA adducts and ethylene oxide forms protein adducts. Researchers continue to investigate whether measurement of DNA adducts or protein adducts in easily accessible samples, such as blood, can accurately reflect the interaction of xenobiotics with critical toxicological target tissues such as the lung. Microarray technology can be used to measure global effects on gene expression and protein levels and modifications. Such profiles may be used to assess exposure.

Biomarkers can also be used to detect an early biological response to a xenobiotic agent that precedes serious damage or disease. Examples of precursory responses include mutations in critical genes, changes in hormonal status, and altered gene expression. For example, substantial evidence indicates that carcinogenesis involves the conversion of protooncogenes (normal genes, many of which code for proteins critical for regulating cellular growth control) into oncogenes (genes that can transform normal cells into cancerous cells). Some xenobiotics can cause mutations that convert protooncogenes into oncogenes. These mutations can result in the overproduction of a protein or in the expression of an altered protein, also called an oncogene product. Microarray technology may also reveal early, preclinical responses to chemical exposure and perhaps indicate if workers are at increased risk of developing cancer or other diseases.

A number of parameters must be established in order to validate the use of biomarkers. If a biomarker is used to assess exposure, it is important to determine how long the biomarker persists following exposure. Likewise, the time interval between exposure and biomarker appearance should be determined. In addition, biomarkers often cannot be measured in the target tissue, such as the lung, because the tissue is not easily accessible for sampling. Instead, the biomarker is measured in a surrogate tissue, such as red blood cells. If this is the case, it should be established that the persistence and levels of the biomarker in the surrogate tissue reflect those of the target tissue.

Regulation

The US Congress passed the Occupational Safety and Health Act in 1970. This act created the Occupational Safety and Health Administration (OSHA) in the federal Department of Labor to establish and enforce safety standards for the workplace. OSHA standards are called permissible exposure limits (PELs). Many PELs have been adopted from ACGIH TLVs. TLVs are generally defined as air concentrations of chemicals that most workers can be exposed to for an 8 h workday, 40 h week^{-1} for a working lifetime without suffering adverse effects. TLVs are not guaranteed as safe exposure levels for the entire population. Employers may also institute voluntary exposure limits either because an OSHA standard has not been promulgated for a xenobiotic of concern or because they want to apply an exposure limit that is more protective than either the PEL or the TLV.

Occupational exposure limits are not always based on toxicology alone. The incorporation of toxicological information into the development of occupational policy can depend on economics, technology, and the sociopolitical climate. The history of occupational policies that apply specifically to women illustrates the complex meshing of toxicology and social factors in the development of occupational policy. Although some sex-specific measures were progressive and eventually led to greater occupational safety for both men and women, it has also been argued that other sex-specific measures were instituted to restrict the role of women in the workplace, and that the intent of the policies depended, in part, on whether women were considered a dispensable part of the work force.

For example, in the late nineteenth century, factory inspectors in England recognized lead poisoning as one of the most widespread industrial diseases. In 1882, the Chief Inspector of Factories, Alexander Redgrave, submitted a report that lead to the Factories (Prevention of Lead Poisoning) Act, 1883. In contrast to the Consolidating Act of 1878, which excluded children and young people from working in the white lead industry, Redgrave specifically advised against banning women from this work.

He apparently recognized the economic role of women in industrial society and realized that if women lost their jobs, they would have a difficult time finding other employment. Instead of banning women from the lead industry, he recommended further protective measures that would improve working conditions. Approximately a century later in the United States, new discoveries on the toxicology of lead and a changing work force led to the implementation of just the sort of policy that Redgrave sought to avoid.

The 1970s saw an influx of women entering the US work force. Growing numbers of women began occupying industrial positions that had been traditionally held by men, including positions in companies that manufactured lead batteries. At the same time, there was growing concern that exposure to levels of lead once considered safe could be harmful. For example, toxicological and epidemiological studies suggested that lead was not only a neurotoxin but also a reproductive toxin. As a result of both the influx of women into 'nontraditional' jobs and the toxicological data on lead, some companies instituted so-called fetal protection policies. In contrast to Redgrave's recommendations a century earlier, these policies excluded fertile women from the workplace, regardless of age or intent to have children, rather than institute additional measures to lower exposure to lead. Although toxicological and epidemiological studies also indicated that lead was a reproductive toxin in men, the fetal protection policies did not apply to fertile male employees. Employees from a number of companies sued, and in 1989 the Supreme Court banned fetal protection policies on the grounds that they constitute discrimination. Although OSHA did not lower the PEL for lead, the lead standard was amended in 1991 to include the warning that "Chronic overexposure to lead impairs the reproductive systems of both men and women."

Policymakers develop workplace standards or institute protective measures by considering health risk data along with economics, the available technology, and the sociopolitical climate. The role of occupational toxicology in the development of sound and equitable safety measures is to provide the most accurate interpretation of toxicological and epidemiological data possible.

See also: American Conference of Governmental Industrial Hygienists; Biomarkers, Human Health; Biotransformation; Dose–Response Relationship; Exposure; Hazard Identification; Medical Surveillance; Occupational Safety and Health Administration; Psychological Indices of Toxicity; Risk Assessment, Ecological; Risk Assessment, Human Health.

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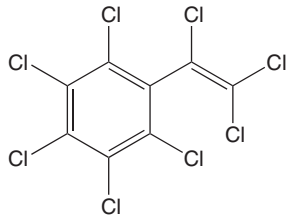
Octachlorostyrene

Alan L Blankenship

- Chemical Abstracts Service Registry Number: CAS 29082-74-4
- Synonyms: Pentachloro(trichloroethenyl)-benzene
- Chemical/Pharmaceutical/Other Class: Octachlorostyrene is a persistent, bioaccumulative, halogenated aromatic compound. Related compounds include other chlorinated styrenes that differ in the number of chlorines (i.e., ranging from 1 to 8)
- Chemical Formula: C_8Cl_8
- Chemical Structure:



Uses

Octachlorostyrene was never commercially produced. Rather, it was historically produced as an inadvertent by-product of high-temperature industrial processes involving chlorine such as the electrolytic production of chlorine gas or magnesium, the refining and degassing of aluminum smelt, and the chlorination and distillation processes involved in niobium and tantalum production. However, recent advances have been made in process technology and pollution prevention practices in some of these industries, such as largely eliminating the electrolytic manufacture of chlorine and aluminum degassing with hexachloroethane, both of which have likely resulted in reductions in known sources of octachlorostyrene.

Exposure Routes and Pathways

Occupational exposure to octachlorostyrene may occur from inhalation of dust and dermal contact in environments where octachlorostyrene is formed as a by-product. The general population may be exposed to octachlorostyrene via consumption of fish and shellfish that contain octachlorostyrene.

Toxicokinetics

In rats, following oral administration, ^{14}C-octachlorostyrene was absorbed and distributed with a rank order of tissue concentrations (greatest to least) of: fat > adrenal glands > skin > lung. After intravenous administration, ~8% of the dose was excreted in feces over the course of 7 days with only a negligible amount in urine. Greater than 90% of the radioactivity in feces was found to be unchanged octachlorostyrene with the remainder being heptachlorostyrene and pentachlorophenyldichloroacetic acid. Approximately 1% of the administered dose was detected as $^{14}CO_2$ in expired air.

Recent data comparing ratios of 4-hydroxy heptachlorostyrene (a metabolite of octachlorostyrene) to octachlorostyrene suggest that there are likely species differences in the ability to metabolize octachlorostyrene. For example, data from an earlier study suggest that polar bears can metabolize octachlorostyrene to 4-hydroxy heptachlorostyrene at a much faster rate than ringed seals.

Mechanism of Toxicity

The mechanism(s) of toxicity and human toxicological properties of octachlorostyrene have not been well characterized. In laboratory animals, histological changes in liver, kidney, and thyroid tissues were observed, but potential impairment in function was not well quantified. In rats, a 50 mg kg^{-1} dose (via intraperitoneal administration) resulted in increased microsomal protein and cytochrome P450 content and also induction of the activities of several enzymes including cytochrome P450 reductase, acetanilide 4-hydroxylase, ethylmorphine *N*-demethylase, and 4-nitroanisole *O*-demethylase. However, there was no induction of benzo(*a*)pyrene hydroxylase activity. Metabolites of octachlorostyrene, such as 4-hydroxy heptachlorostyrene, have been shown to bind to transthyretin in polar bears with a similar degree of affinity as to thyroxine (T_4). This suggests that there is a potential for disruption of T_4 and retinol transport by metabolites of octachlorostyrene in some species.

Acute, Subacute, and Chronic Toxicity (or Exposure)

Animal

In an acute toxicity study with male rats dosed by gavage with single doses of octachlorostyrene at 1300, 1690, 2190, 2850, and 3710 mg kg^{-1}, test animals were sacrificed 14 days later. At all but the lowest dose, there was an increase in liver weight, hepatic microsomal aniline hydroxylase and aminopyrine demethylase activities, serum cholesterol, and uric acid levels.

In a subacute study, both male and female rats were fed diets containing octachlorostyrene at 0.5, 5.0, 50, and 500 mg kg^{-1} for 28 days. Histological changes were observed in the liver and thyroid of rats exposed to doses equal to or greater than 5 mg kg^{-1}. Hepatic microsomal enzyme induction and liver hypertrophy were observed in the two highest dose groups. At 500 mg kg^{-1}, there was an increase in serum cholesterol, total protein, potassium, and sorbitol dehydrogenase.

In a chronic study, weanling Sprague–Dawley rats (20 animals of each sex per exposure group) were fed (*ad libitum*) diets containing 0, 0.005, 0.05, 0.5, 5.0, or 50 mg kg^{-1} octachlorostyrene in diet (fed *ad libitum*) for 12 months. While there was some mortality, it did not appear to be related to treatment. Similarly, tumor incidence was infrequent and appeared unrelated to treatment. However, 5.0 and 50 mg kg^{-1} exposures resulted in kidney effects (e.g., dose-related dilation of proximal tubules and cytoplasmic eosinophilia along with granular casts and proteinaceous losses), and induction of aniline hydroxylase and aminopyrine demethylase activities in hepatic microsomes of both sexes. At the highest exposure level only (50 mg kg^{-1}), there was a

statistically significant increase in relative liver to body weight. The chronic dietary no-observed-adverse-exposure level (NOAEL) from this study was determined to be 0.5 mg kg^{-1}. Corrected for body weight and ingestion rate, the actual dose for the NOAEL is 0.031 and 0.044 mg kg^{-1} day^{-1}, respectively, for males and females.

Human

The effects of octachlorostyrene exposure on humans are not well known. Most of the available human data are from monitoring studies in which tissue residue concentrations have been determined for occupational and nonoccupational populations, including consumers of seafood. As discussed earlier, potential human exposure pathways for octachlorostyrene are through ingestion (especially of contaminated fish), inhalation, and absorption through the skin. Occupational exposure has been shown to result in a greater than 70-fold increase in mean concentrations of octachlorostyrene in the blood in foundry workers (mean of controls, 0.7 ng g^{-1}, lipid; mean of exposed, 54.6 ng g^{-1}, lipid) who use hexachloroethane as a degassing agent for aluminum. Octachlorostyrene has also been detected in the blood of humans ingesting contaminated fish at levels generally ranging up to a few parts per billion (nanograms per gram), and in the breast milk of nonoccupationally exposed women at levels generally less than 1 ng g^{-1}. However, the toxicological relevance of these exposure levels in humans is not known.

Environmental Fate

In aquatic systems, octachlorostyrene is expected to adsorb to suspended solids and sediments based on its K_{oc} value ranging from 200 000 to 10 000 000. Octachlorostyrene has been detected in water at concentrations as high as 7.2 ng l^{-1} but levels typically are well below 1 ng l^{-1}. While there is the potential for volatilization from aquatic systems based on an estimated Henry's law constant of 2.3×10^{-4} atm m^3 mol^{-1}, volatilization is likely attenuated by adsorption to particles. Bioaccumulation by aquatic organisms is likely based on a bioconcentration factor that is estimated to range from 8100 to 33 000. Field estimates of bioaccumulation factors range up to 1 400 000 (from water to rainbow trout in Lake Ontario). Mean concentrations in Lake Ontario sediments and rainbow trout were 13.6 ng g^{-1} (dry weight) and 2.6 ng g^{-1} (wet weight), respectively. The highest concentrations found in fish as part of the National Study of Chemical Residues in Fish (conducted by the US Environmental Protection Agency (EPA)) were from Bayou D'Inde, Louisiana (138 ng g^{-1}), Freeport, Texas (65.3 ng g^{-1}), River Rouge, Michigan (50.7 ng g^{-1}), and Olcott, New York (49.6 ng g^{-1}). Temporal studies, while limited, have indicated a substantial decline in concentrations of octachlorostyrene since the 1970s.

In terrestrial systems, octachlorostyrene is expected to bind to soil particles. In the atmosphere, octachlorostyrene (in the vapor phase) is degraded by reactions with photochemically produced hydroxyl radicals. Octachlorostyrene weakly absorbs ultraviolet light between 295 and 310 nm with slow photolysis. Major transformation products of photolysis include heptachlorostyrene and two isomers of hexachlorostyrene, while minor transformation products of photolysis include pentachlorostyrene and tetrachlorostyrene.

Ecotoxicology

Among its potential adverse effects, octachlorostyrene has the potential to interfere with metabolism in fish and to inhibit photosynthesis in algae. EPA has determined that 'aquatic toxicity values indicate that octachlorostyrene is toxic at relatively low concentrations and thus is highly toxic to aquatic organisms'. Metabolites of octachlorostyrene, such as 4-hydroxy heptachlorostyrene, may have the potential to disrupt T_4 and retinol transport by binding to transthyretin in some species. Bioaccumulation into higher trophic level species has been documented to occur with several species including herring gulls, double-crested cormorants, black-crowned night herons, beluga whales, polar bears, and ringed seals. However, the toxicological significance of the concentrations found in wildlife is not clear at this time.

See also: Styrene.

Further Reading

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Octane

Stephen R Clough

- Chemical Abstracts Service Registry Number: CAS 11-65-9
- Synonyms: *n*-Octane (UN1262, DOT); Oktan (Polish); Oktanen (Dutch); Ottani (Italian)
- Chemical/Pharmaceutical/Other Class: Aliphatic hydrocarbon
- Chemical Formula: C_8H_{18}

Uses

It is used as a solvent and raw material for organic synthesis reactions and is a very important chemical in the petroleum industry. It is also widely used in the rubber and paper processing industries. Isooctane, along with other *n*- and isoparaffins, are used in the blending of fuels to achieve desired antiknock properties. A total of 17 isomers of octane are known to exist; isooctane (2,2,4-trimethylpentane) is a principal ingredient of gasoline.

Exposure Routes and Pathways

Because octane can exist as a liquid or vapor at normal temperature and pressure, exposure could occur by either dermal contact or inhalation (1 ppm air = 4.67 mg m^{-3}); oral exposure would most likely be either incidental or accidental. Isooctane, an octane isomer, can comprise up to 1% of the total hydrocarbons emitted from the exhaust of diesel and gasoline engines.

Toxicokinetics

After absorption, octane is most likely converted to a hydroxy derivative (e.g., alcohol) via the cytochrome P450 oxidase system.

Mechanism of Toxicity

The molecular mechanism of toxicity is not known, although based on its solvent properties a direct physical alteration or disruption of cellular membrane structures and organelles is suspect.

Acute and Short-Term Toxicity (or Exposure)

Animal

Octane has been shown to have narcotic effects in both mice and rats after acute exposure at high concentrations. One study estimated a 4 h LC_{50} in rats of 118 000 mg m^{-3} (25 260 ppm). The lowest concentration to cause an effect on righting reflexes in mice was 35 mg l^{-1} (7490 ppm), while complete loss was seen at 50 mg l^{-1} (10 700 ppm). For 2,5-dimethylhexane (an octane isomer), the narcotic concentration in mice was 70–80 mg l^{-1} (14 980–17 120 ppm), and the effects were less severe than those seen for octane. In rats, oral administration of isooctane caused moderate toxicity, and pulmonary lesions were observed following aspiration of octane into the lungs. None of the branched octane isomers are known to have neurotoxic properties; some types of soil-dwelling bacteria can exist using branched chain octanes as the sole carbon sources.

Human

Octane is moderately toxic if taken orally and more toxic than the lower molecular weight analogs by this route. It is similar in potency to heptane (especially with regard to narcotic effects) but is apparently without the associated neurotoxic signs of heptane and hexane. If it is aspirated into the lungs, it may cause rapid death due to cardiac arrest, respiratory paralysis, and asphyxia. At high air concentrations (generally between 5000 and 13 700 ppm for 30 min) it will have an acute narcotic effect but no adverse effects are apparent in humans at concentrations below 500 ppm.

Clinical Management

Persons who are exposed to high air concentrations should vacate or be removed from the source of the gas and seek fresh air. Upon oral ingestion, persons should not be induced to vomit as pulmonary aspiration may occur, resulting in severe narcosis and/or death.

Ecotoxicology

Young Coho salmon showed no significant mortality in water containing <100 mg l^{-1} octane. No significant mortality of the eggs of the Pacific oyster is seen at concentrations <3500 mg l^{-1}. An EC_{50} of 120 μg l^{-1} was calculated based on effects on the feeding behavior of a test population of blue mussels.

Other Hazards

Extreme care must be taken to keep areas of expected high concentration free from ignition sources; for example, sparks from static electricity. Only explosion-proof equipment should be used in these areas. The lower and upper explosive limits for octane are 1% and 4.7%, respectively.

Exposure Standards and Guidelines

The permissible exposure limit (time-weighted average, TWA) for octane is 500 ppm (2335 mg m^{-3}) while the American Conference of Governmental Industrial Hygienists recommends a TWA threshold limit value of 300 ppm (1400 mg m^{-3}). National Institute for Occupational Safety and Health has recommended a maximum human exposure level of 75 ppm for octane and 350 mg m^{-3} for a mixture of C5–C8 hydrocarbons.

Miscellaneous

Octane is a colorless, highly flammable liquid that is lighter than water. It has an odor that can be detected at 400 ppm. It occurs in natural gas but is principally derived from crude oil.

See also: Gasoline; Petroleum Distillates; Petroleum Hydrocarbons.

Ocular Toxicology *See* Eye Irritancy Testing; Sensory Organs.

OECD *See* Organisation for Economic Cooperation and Development.

Oil, Crude

Michael J Sullivan

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- Chemical Name: Petroleum
- Representative Chemicals: Aliphatic, aromatic, paraffinic hydrocarbons; Naphthenic hydrocarbons; Asphaltic hydrocarbons; Trace metals
- Chemical Abstracts Service Registry Number: CAS 8002-05-9
- Synonyms: Petroleum; Naphtha; Petrol; Rock oil
- Chemical/Pharmaceutical/Other Class: Petroleum hydrocarbons

Uses

The separation of the components of crude oil into useable products is known as refining. Each of the crude oil fractions finds its way into consumer products. A typical list of fractions is: gasoline, kerosene and fuel oil, gas oil, wax distillate, and bottoms or asphaltics. Refineries must be designed to handle the type of crude oil they are going to process. For example, if a crude oil is highly paraffinic in nature, it will yield a lower amount of gasoline fuel by distillation. Highly paraffinic oils may be processed into lubricating stock. The chemical fraction consisting of chemicals with the largest carbon numbers, the asphaltic fraction, is used as roof or road tar.

Background Information

Crude oil is a complex mixture of chemicals. The relative composition of these chemicals will be different in crude oil from different sources. However, the overall composition (i.e., the chemicals present) remains fairly consistent between sources. The chemical classes present in crude oil include paraffinic hydrocarbons, long-chain straight or branched carbon-based chemicals and naphthenic hydrocarbons, multiple-ringed carbon-based chemicals. Also present will be low percentages of sulfur, nitrogen, and oxygen compounds, and trace quantities of many other elements.

Regulatory agencies have classified crude oil into categories that are useful to help understand how the oil will behave if released into the environment. These categories are summarized in **Table 1**.

Table 1 Categories of crude oil

Category	*Name*	*Description*
Class A	Light, volatile oils	Highly fluid Strong odor Spread rapidly High volatiles Penetrates soil Flammable
Class B	Nonsticky oils	Adheres to surface Waxy feel Can be washed away Mild volatiles Nonpenetrating
Class C	Heavy, sticky oils	Tarry/sticky Adheres to surfaces Cannot be washed away Nonpenetrating Low volatiles
Class D	Nonfluid oils	Black/brown solid Nonpenetrating Cannot be washed away Melts upon heating Nonvolatile

Exposure Routes and Pathways

At any one time worldwide, up to 1 000 000 workers are employed in crude oil exploration, production, and refining. Workers employed in these fields can be exposed to crude oil. Activities associated with exposure include drilling, pumping, and transportation of crude oil as well as the cleaning and maintenance of the equipment used in these activities.

Exposure to crude oil can occur through both direct contact with the material and contact with environmental media contaminated with crude oil. The primary route of exposure in the environment to crude oil is direct dermal contact with liquid oil. In the workplace, the primary route of exposure is also dermal contact. Inhalation exposure to crude oil could occur through the production of oil mists or through inhalation of the volatile fraction of the crude oil. Exposure to crude oil through contact with environmental media also constitutes significant routes of exposure. This can occur at spill sites, in former oil fields developed into other uses, or areas of natural oil seeps. Dermal contact or incidental ingestion of soil contaminated with crude oil, as well as the inhalation of dust from crude oil-contaminated soil are common at crude oil contaminated sites. Both human and animal exposures can occur when crude oil is released into the aquatic environment.

Toxicokinetics

Exposure to crude oil is a concern for the organ of contact. For dermal exposure the concern is for the skin. For inhalation exposure, the concern is for the respiratory system. Therefore, absorption and distribution kinetics are not well studied because of these site of contact concerns. However, it would be expected that individual chemicals present in crude oil can be absorbed and would have biological fate appropriate for that chemical or chemical class (e.g., polynuclear aromatic hydrocarbons).

Mechanism of Toxicity

The concern for both dermal and inhalation exposures is the site of contact and effects on that tissue. The mechanism of crude oil toxicity is mediated through its irritant effects which after sufficient exposure duration and concentration result in tissue hyperplasia. Chronic hyperplasia leads to subsequent loss of tissue integrity and damage and in some animal models of cancer. It has been suggested that at exposures below levels that cause chronic irritation, other long-term effects would not be expected.

Acute and Short-Term Toxicity (or Exposure)

Crude oil contains many chemicals considered toxic and the effects of these individual chemicals should be evaluated if exposure is possible. These chemicals are aromatic solvents including benzene, aliphatic chemicals including hexane, and naphthenic chemicals including the polynuclear aromatic hydrocarbons.

Animal

Both eye and dermal irritation have been noted in animal testing. Systemic effects have not been noted and oral toxicity is low. Dermal irritation has been reported in test animals at 24 h doses of 100 mg.

Human

The acute effect of crude oil on humans is narcosis. The effect is reversible even after exposure to high concentrations. Inhalation of vapors can produce pneumonitis.

Chronic Toxicity (or Exposure)

Crude oil has low chronic toxicity. Exposures insufficient to cause tissue irritation may also not be sufficient to cause other, more-serious chronic effects.

Animal

Dermal application of crude oil to the shaved backs of animals has produced limited adverse effects (see section Carcinogenicity). A dose of 25 mg, three times per week for 105 weeks produced dermal irritation at the site of application. No systemic effects were noted. Some limited evidence of developmental toxicity of crude oil has been reported but only at doses that were high enough to cause significant maternal toxicity.

Samples of crude oil have been tested for carcinogenicity in animals. Crude oil samples from single sources or composites of several sources were tested by dermal application of mice. Crude oil from single sources produced both benign and malignant tumors. Some composites have produced a low incidence of skin carcinomas whereas other composites have not. When crude oil fractions were applied to the skin of mice, skin tumors were also produced. In the rabbits tested, a single crude oil source produced skin papillomas in rabbits in one experiment but no effects were seen in rabbits using other sources. In all studies where crude oil was reported to be associated with skin cancer, there was significant damage to the skin and effects including drying, cracking, irritation, and hyperkeratosis. In summary, there is limited evidence for carcinogenicity in experimental animals.

Human

Adverse effects of crude oil on the skin have been reported in petroleum workers. These effects include dryness, pigmentation, hyperkeratosis, warts, and eczema.

Epidemiology studies of workers exposed to crude oil have been performed in petroleum producing, pipeline, and production operations. In a retrospective cohort mortality study, deaths from all types of cancers were low with decreases in lung and testicular cancer related deaths. Thyroid cancer-related deaths were increased. In a case–control study, an elevated risk for lung cancer was observed among older men. These workers were also exposed to welding fumes and paints, and smoking was not controlled. In another case–control study, an excess risk for testicular cancer was observed among petroleum and natural gas extraction workers; however, this isolated finding is inconsistent with other studies. Another case–control study exposure to crude oil was related to rectal and lung cancers. However, the authors noted that the study numbers were small and the finding may have been confounded by lifestyle factors.

In the summary, there is inadequate evidence for carcinogenicity in humans. Overall, crude oil is not classifiable as to its carcinogenicity to humans. Therefore, it is placed in the International Agency for Research on Cancer (IARC) classification Group 3.

In Vitro Toxicity Data

Crude oil did not increase the number of sister chromatid exchanges in cultured human lymphocytes. However, in studies of mice treated *in vivo* crude oil did cause an increase in the number of sister chromatid exchanges at the highest dose tested. No effects were observed in bone-marrow cells or sperm. Sister chromatid exchanges were caused by the aromatic fraction of crude oil in cultured mammalian cells. Crude oil extracts did not induce mutation in bacteria. However, the neutral fractions of crude oil which contain aromatic or polycyclic aromatic compounds generally had mutagenic activity in bacteria.

Environmental Fate

When released to the environment, crude oil undergoes the process of 'aging'. This occurs by both abiotic processes (volatilization and oxidation) and biotic processes including biodegradation. Often the abiotic aging will occur before the biotic one. The chemical-specific properties will determine how an individual chemical or chemical class fares during this aging. For example, small volatile compounds would be expected to be lost first from both land and water releases. Large paraffinic compounds would be expected to be somewhat resistant to aging. The asphaltic compounds would be the residual material and have limited exposure opportunities due to their properties. Terrestrial releases of crude oil do not lead to bioaccumulation in terrestrial organisms.

In aquatic environments, the heavier and less volatile/soluble compounds in crude oil will adsorb to suspended solids and subsequently settle in the sediments. Some heavy fractions with high density may sink into the sediment. This happens after the initial removal of the smaller and more volatile chemicals by either dissolution or volatilization. This is followed by biodegradation of those crude oil constituents that can serve as a food source for bacteria. Biodegradation is a significant mechanism for removal of hydrocarbons released into the environment. However, this generally occurs on the order of months and years. It is not believed that there is significant bioaccumulation of petroleum hydrocarbons in aquatic organisms.

Exposure Standards and Guidelines

No exposure standards for crude oil are available. Occupational exposures to oil mists are a concern. Both the Occupational Safety and Health

Administration (OSHA) permissible exposure limit (PEL) and the American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV) are $5\,\mu g\,m^3$ for oil mists.

Long-term animal studies of dermal exposure to crude oil can be used to set a no observed adverse effect level (NOAEL) that can be used to predict safe human exposure levels for both dermal and systemic effects. A reference dose of $0.04\,mg\,kg^{-1}\,day^{-1}$ has been suggested for exposures to crude oil. The individual aliphatic and aromatic fractions of crude oil have also been evaluated for toxicity and sufficient information exists to set references doses for these fractions. An understanding of the exposure to the individual fractions is necessary to use this process. The use of the reference dose for either crude oil as a whole or the individual fractions is preferable to evaluating only the toxic constituents in crude oil. This latter strategy is commonly employed in risk assessment however; it ignores the hydrocarbon matrix within which these toxic chemicals are found. This hydrocarbon matrix affects the exposure to these toxic constituents which is not accounted for in typical exposure assessments.

See also: Petroleum Distillates; Petroleum Hydrocarbons.

Further Reading

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Total Petroleum Hydrocarbon Criteria Working Group (TPHCWG) (1999) *Human Health Risk-Based Evaluation of Petroleum Release Sites: Implementing the Working Group Approach*, vol. 5. Amherst, MA: Amherst Scientific Publishers.

Oil, Lubricating

Michael J Sullivan

- Representative Chemicals: Aliphatic, aromatic, paraffinic hydrocarbons; Naphthenic oil
- Synonyms: Lub oil; Crankcase oil; Motor oil
- Chemical/Pharmaceutical/Other Class: Petroleum hydrocarbons

Uses

Lubricating oil is used to lubricate the parts of motors. The purpose is to reduce friction between parts, remove heat (i.e., act as a coolant), and act as a sealing liquid. Oils can also be used in machining and applications where friction protection and heat removal are needed.

It is also worth noting that both paraffinic and naphthenic oils are used as food additives and in cosmetics. Two chemical classes (paraffin waxes, CAS 8002-74-2, and petrolatum, CAS 8009-03-8), are considered Generally Recognized as Safe food ingredients by the Food and Drug Administration.

Background Information

Lubricating oil is a generic name for a wide range of products that are characterized by hundreds of base chemicals and additives. The most common lubricating oils are crude oil distillate fractions although both synthetic and plant-based lubricating oils are used. This article focuses on petroleum-based lubricating oils.

Lubricating oils are composed of 80–90% petroleum hydrocarbon distillate with 10–20% additives to impart specific properties to the oil. The petroleum hydrocarbon distillate generally consists of paraffinic or naphthenic compounds, whose properties are listed in **Table 1**.

Unused lubricating oil changes under the use conditions of heat and friction and, if appropriate,

Table 1 Properties of paraffinic and naphthenic oils

Paraffinic oil	*Property*	*Naphthenic oil*
Long carbon chains	Chemical structure	Multiple carbon rings
High	Resistance to oxidation[a]	Medium
High	Pour point[b]	Low
High	Viscosity[c]	Low
Low	Volatility[d]	High
Low	Specific gravity[e]	High

[a] Measure of stability/chemical breakdown.
[b] Lowest temperature at which oil will pour.
[c] Resistance to flow/shear.
[d] Property of transition to vapor state.
[e] Density related to water.

exposure to exhaust gases of internal combustion engines. Used lubricating oil or used crankcase oil generally have higher concentrations of polynuclear aromatic hydrocarbons than unused oils. Used oils are not specifically addressed in this article but would be considered to be more toxic because of increased presence of toxic constituents.

Environmental releases of unused lubricating oil have been reported. The largest releases have been associated with lubricating oil manufacture and storage. These oils, stored in large above-ground tanks, have been the subject of large releases similar to other petroleum hydrocarbon materials (e.g., fuels). Soil and groundwater beneath these tanks can become contaminated with concentrations ranging up to saturation limits.

Exposure Routes and Pathways

Exposure to lubricating oil can occur through both direct contact with the material and contact with environmental media containing the oil. The primary route of exposure in the environment is direct, through dermal contact with the liquid oil. In the workplace, the primary route of exposure is the inhalation of oil mists generated during machine lubricant use. Exposure to oil mists in the occupational environment is of sufficient concern that an exposure standard has been set. Secondary exposure to lubricating oil occurs through contact with environmental media. These consist of exposure to contaminated soil via dermal contact, incidental ingestion, or inhalation of dust particulates. When lubricating oil is released to water, exposure can occur through ingestion and dermal contact with contaminated water.

Toxicokinetics

Exposure to lubricating oil is a concern for the organ of contact. For dermal exposure the concern is for the skin. For inhalation exposure, the concern is for the respiratory system. Therefore, absorption and distribution kinetics are not well studied because of these sites of contact concerns. However, it would be expected that individual chemicals present in lubricating oil can be absorbed and would have biological fate appropriate for that chemical or chemical class (e.g., polynuclear aromatic hydrocarbons).

Mechanism of Toxicity

The concern for both dermal and inhalation exposures is the site of contact and effects on that tissue. The mechanism of lubricating oil toxicity is mediated through its irritant effects, which after sufficient exposure duration and concentration result in tissue hyperplasia. Chronic hyperplasia leads to subsequent loss of tissue integrity and damage and in some animal models cancer.

Acute and Short-Term Toxicity (or Exposure)

Lubricating oils have low acute toxicity. Their sublethal acute effects are generally limited to irritation of those tissues in contact with the oil.

Animal

Low animal toxicity has been demonstrated in various studies. For example, a published oral LD_{50} for a mouse is $22\,g\,kg^{-1}$. Dermal irritation has been reported in rabbits at a total dose of 100 mg for 24 h.

Human

Low human acute toxicity would be expected due to the use of oils in food and cosmetic products. However, exposure to oils can cause irritation of the eyes, skin, and respiratory tract.

Chronic Toxicity (or Exposure)

Lubricating oils have low chronic toxicity although chronic exposures to levels that cause irritation can lead to other effects.

Animal

A 90 day study of various oils was performed in rats exposed with doses from 2 to $2000\,mg\,kg^{-1}$ in their feed. No treatment-related effects were reported in rats exposed to lubricating oil (paraffinic and naphthenic) with carbon ranges above C30. Rats fed oils with lower molecular weight fractions, carbon ranges C15–C30, showed histological changes in the liver and lymph nodes, which were noted at doses $20\,mg\,kg^{-1}$ and higher. Females were more sensitive than males.

Human

Low human chronic toxicity would be expected due to the use of oils in food and cosmetic products.

Carcinogenicity

Various lubricating oils have been tested for their carcinogenicity in animals using dermal application. Many studies have reported an increase in the number of tumors and this is associated with chronic skin irritation and hyperplasia. At doses where these skin effects were not noted, there were no

increases in tumor incidence rates. It has been suggested that unused lubricating oils with low polynuclear aromatic hydrocarbon content are not carcinogenic. This may be due to 'matrix' effects of the hydrocarbon base material. It has also been suggested that at exposures below those associated with dermal irritation and hyperplasia, these lubricating oils are not carcinogenic.

A single listing in Registry of Toxic Effects of Chemical Substances (RTECS) for lubricating oil suggest tumorgenic potential in humans exposed via inhalation to $5\,mg\,m^{-3}$ for 5 years. However, this reference was not reviewed and is not consistent with other reported chronic human exposures.

In Vitro Toxicity Data

A number of *in vitro* assays for mutagenicity have been performed on a variety of lubricating oils and their fractions. With the exception of oils with high polynuclear aromatic hydrocarbon content, the results of the studies on lubricating oil are negative.

Clinical Management

The clinical signs of overexposure to lubricating oil either by dermal contact or inhalation of mists would be irritation and inflammation of the contact tissues. This irritation is reversible and effective management is cessation of exposure by change of activities and effective use of dermal and/or pulmonary personal protective equipment.

Environmental Fate

Since lubricating oils contain a wide range of chemicals, the environmental fate of a release of lubricating oil is dependent on the chemical make-up of that oil. Generally, the chemicals in lubricating oil have low water solubility and high binding constants to organic carbon. Their low water solubility limits the potential for impacting deep groundwater yet these oils cause surface sheens and visible contamination when released to surface waters. When released to soil, the oils would not be expected to migrate far from the point of release although once bound to soil, these chemicals can spread as contaminated soil is spread.

Exposure Standards and Guidelines

Occupational exposures to oil mists are a concern. Both the Occupational Safety and Health Administration permissible exposure limit and the American Conference of Governmental Industrial Hygienists threshold limit value are $5\,\mu g\,m^{-3}$ for oil mists.

The toxicity studies of lubricating oil in animals suggest that no-observed-adverse-effect levels (NOAELs) can be set and used to predict safe human exposure levels. Lubricating oils with carbon ranges above C30 have shown NOAELs $\sim 2000\,mg\,kg^{-1}$ for a 90 day study. Oils with smaller carbon numbers have reported NOAELs $\sim 20\,mg\,kg^{-1}$. These values would result in oral reference doses in the range of $0.2–20\,mg\,kg^{-1}\,day^{-1}$ using appropriate safety factors.

Miscellaneous

The evaluation of petroleum hydrocarbon toxicity and it application to human health risk assessment is complicated. Strategies range from considering only the known toxic constituents (e.g., benzene or naphthalene) and ignoring the paraffinic and naphthenic fractions (i.e., not evaluating the hydrocarbon base material) to only evaluating the hydrocarbon base material. Given the uncertainty inherent in both of these strategies, an alternative would be to evaluate both the hydrocarbon base material and the toxic constituents. This strategy however suffers from the potential to double count the toxicity of those toxic constituents that are in both evaluation processes.

See also: Fuel Oils; Oil, Crude.

Further Reading

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Oleander

Fermin Barrueto Jr.

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- SYNONYMS: *Nerium oleander*; *Nerium indicum*; *Nerium odorum*; Common oleander; Rose-bay; Yellow oleander, *Thevetia peruviana*, Rose laurel

Uses

Oleander is used as an ornamental shrub along road sides and in gardens. The plant is also used in rodenticides, insecticides, and homeopathic remedies. In some countries, particularly Sri Lanka, this shrub has become a notorious method of suicide.

Background Information

Oleander is in the Apocynaceae (dogbane) plant family. The large ornamental evergreen shrub may grow 20–25 ft in height. Leaves are long and narrow, with pointed tips. During the summer months large clusters of white, pink, or red flowers appear at the ends of the branches.

Exposure Routes and Pathways

Common exposure pathways include ingestion of plant parts and inhalation from the burning of oleander bushes. Homeopathic extracts, insecticides, and rodenticide extracts are other available sources.

Mechanism of Toxicity

Common oleander contains at least five cardiac glycosides and the toxicity is identical to digitoxin poisoning where there are two main mechanisms of toxicity. First, these cardioactive steroids (like digoxin) increase the vagal tone, which leads to bradycardia but can lead to further AV nodal dysfunction and heart block. Second, these cardiac glycosides inhibit the Na^+,K^+-ATPase enzyme system. This causes a disturbance in the sodium gradient causing increased intracellular sodium and extracellular potassium. The excess intracellular sodium leaves the cell in exchange for Ca^{2+} through an antiport system. Sodium exits the cell and there is a subsequent increase in intracellular calcium. This calcium binds the ryanodyne receptor on the sarcoplasmic reticulum leading to a larger efflux of calcium to bind the myosin–actin filaments responsible for muscle contraction. Due to the increased intracellular sodium, there is an increase in the resting membrane potential leading to myocardial excitability. This is why the most common dysrhythmia seen in digoxin toxicity is premature ventricular contractions, though any dysrhythmia can be seen except a supraventricular tachycardia.

Chronic Toxicity (or Exposure)

Animal

Animals have the same potential for toxicity as humans. Cases have been documented in cows of oleander ingestion, which have shown similar effects to those that appear in humans.

Human

The range of toxicity is dependent on how it is ingested, what part of the plant is ingested, and the presence of any comorbidity. Significant oleander poisoning closely resembles digitoxin poisoning and can be treated as such. Gastrointestinal and cardiac symptoms predominate. Within the first several hours, nausea, vomiting, and abdominal pain are characteristically present. Cardiotoxic effects, such as conduction abnormalities, ventricular dysrhythmias, and asystole, can be present. Poisoned patients can present with bradycardia; first-, second-, and third-degree heart block; normotensive or hypotension; and hyperkalemia.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. With significant recent ingestions, decontamination with activated charcoal may be considered. Continuous cardiac monitoring and serial potassium levels should be performed. Atropine is useful in managing bradycardia and varying degrees of heart block. Low-dose phenytoin improves atrioventricular conduction and can terminate heart block, though with digoxin-specific Fab this has fallen out of favor. Ventricular dysrhythmia can be managed with phenytoin and/or lidocaine but is best managed with digoxin-specific Fab. Intravenous glucose and insulin and sodium bicarbonate can be used in life-threatening hyperkalemia though the most effective treatment is administration of digoxin-specific Fab. For patients who have persistent severe cardiovascular disease, an electrical pacemaker should be considered after administration

of digoxin-specific Fab. Digoxin-specific Fab, which is used to treat severe digitalis glycoside poisoning, has been demonstrated to be effective in the management of oleander poisoning and decreases morbidity and mortality.

See also: Digitalis Glycosides; Plants, Poisonous.

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Olfaction *See* Sensory Organs.

Opium

Christopher P Holstege

- Chemical Abstracts Service Registry Number: CAS 8008-60-4
- Synonyms: Crude opium; Gum opium; Powdered opium; Raw opium; Standardized opium powder
- Chemical/Pharmaceutical/Other Class: Opiate agonist

Uses

Opium is used for analgesia, sedative–hypnotic narcosis, antiperistalsis, and in the treatment of neonatal withdrawal. It is also a drug of abuse.

Background Information

Opium is the air-dried milky exudate obtained by incising the unripe capsules of *Papaver somniferum*.

Exposure Routes and Pathways

Opium can be solubilized and given parenterally. Tinctures and suspensions are available for oral administration and suppositories for rectal administration. In addition, it can be insufflated as a powder.

Toxicokinetics

Opium contains several alkaloids, including no less than 10% anhydrous morphine and small amounts of codeine and papverine. After oral administration, morphine is absorbed from the gastrointestinal tract. The drug is rapidly metabolized after oral administration and plasma concentrations of unconjugated morphine are lower than those achieved after parenteral administration. Activity following parenteral administration of concentrated opium alkaloids is similar to parenterally administered morphine. Peak analgesia occurs within 60 min and can be maintained for up to 7 h. Rectal adsorption is erratic. Morphine is distributed throughout the body. Approximately 35% is protein bound. The volume of distribution is 3–4 $l kg^{-1}$. Opium preparations are metabolized in the liver. The major pathway for the metabolism of morphine is conjugation with glucuronic acid to form both active and inactive products. Morphine-6-glucuronide is a major metabolite of morphine that is twice as potent as morphine. In patients with renal failure, an accumulation of morphine-6-glucuronide may cause toxicity in the absence of significant morphine levels. A small percentage of morphine is excreted unchanged. Nearly

all morphine is metabolized and eliminated by the kidneys, with the major metabolite being morphine-3-glucuronide.

Mechanism of Toxicity

Morphine, the major active principle of powdered opium, is responsible for the action of opium, although other alkaloids contribute to it. Morphine's toxicity is a result of its extensive effect on the central nervous system (CNS), mainly that of a descending depression. Opiates interact with stereospecific and saturable binding sites primarily located in the CNS. Interaction with these receptors mimics the actions of endogenous enkephalins and endorphins. Their action also appears to involve an alteration in the release of neurotransmitters, such as the inhibition of acetylcholine, norepinephrine, and dopamine.

Acute and Short-Term Toxicity (or Exposure)

Animal

Dogs act similarly to humans – symptoms may include drowsiness, ataxia, vomiting, respiratory depression, miosis, coma, and hypotension. Opiates and their derivatives have an excitatory effect on the CNS of cats and horses. Naloxone may be used at $0.02\,mg\,kg^{-1}$ if needed.

Human

Symptoms of toxicity may occur in varying degrees in nontolerant individuals who receive greater than a therapeutic dosage. The primary insult is respiratory depression from direct depression of the CNS. This state may then progress to apnea or respiratory arrest. Pulmonary edema is a common complication. Therapeutically, opium results in analgesia. In toxic doses, CNS depression ensues and can progress to coma. Miosis is frequent, but in an acidotic or asphyxiated state the pupils may be dilated. Opium can cause hypotension and bradycardia. Hypothermia may also develop if there is peripheral vasodilation. Laboratory analysis does not dictate treatment but can confirm the presence of opiates.

Chronic Toxicity (or Exposure)

Human

Opiates have a high potential for abuse. Chronic users may develop tolerance, thus necessitating larger doses for the desired effect. Abrupt cessation can cause withdrawal, yielding restlessness, vomiting, and diarrhea.

Clinical Management

In patients presenting with opium toxicity, the airway should be patent and adequate ventilation assured. If the patient has either inadequate ventilation or a poor gag reflex, then the patient may be at risk for subsequent CO_2 narcosis, worsening acidosis, and/or aspiration. If necessary, endotracheal tube intubation should be performed. The initial treatment of hypotension consists of intravenous fluids. Close monitoring of the patient's pulmonary exam should be performed to assure that pulmonary edema does not develop as fluids are infused. The patient should be placed on continuous cardiac monitoring with pulse oximetry. Frequent neurological checks should be made. Gastrointestinal decontamination should be considered only after initial supportive care has been provided and airway control has been assured. Activated charcoal ($1\,g\,kg^{-1}$) may be administered to patients who have ingested opium and present early. Syrup of ipecac is contraindicated after overdose with the opium due to the potential for rapid clinical deterioration. Gastric lavage should be avoided.

Naloxone may be of benefit in reversing the neurological and respiratory depressant effects of opium. A dose of 0.4–2.0 mg is given intravenously slowly, titrated to resumption of adequate respirations, and can be repeated as needed. The therapeutic effect of naloxone may be of shorter duration than that of opium activity; therefore, it is imperative that opium intoxicated patients who demonstrated improvement after naloxone be closely monitored for resedation. Vital sign measurements and neurological checks should be monitored frequently until resolution.

See also: Codeine; Drugs of Abuse; Morphine.

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Organochlorine Insecticides

Benny L Blaylock

Organochlorine insecticides are chlorinated hydrocarbon compounds that fall into three basic structure classifications: aryl (aromatic), carbocyclic, and heterocyclic.

They may be differentiated from other chlorinated hydrocarbon compounds (e.g., solvents) by molecular weight. Organochlorine insecticides, by virtue of their cyclic structure, have molecular weights ranging from 291 to 545, whereas chlorinated hydrocarbon solvents and fumigants have molecular weights that generally are less than 236.

Organochlorine insecticides may be divided into three broad groups: dichlorodiphenylethanes, such as DDT and methoxychlor; cyclodienes, such as chlordane and dieldrin; and hexachlorocyclohexanes, such as lindane. Mirex and chlordecone, however, are organochlorine insecticides whose caged structures do not fit well into the previous groups.

The first organochlorine synthesized was DDT. Although it was first synthesized by Zeidler in 1874, it was not produced or used for many years. Mueller rediscovered DDT in 1939 and won the Nobel Prize for his efforts in 1948. The first major uses for DDT were vector control of typhus and malaria and control of lice and other pests during World War II.

Other organochlorine compounds were synthesized and came into general use during the late 1940s, thus introducing the synthetic insecticide era. From the 1940s through the 1960s, organochlorine insecticides were used extensively to control insect pests in both agricultural and domestic settings and for vector control of malaria, typhus, and other diseases affecting human health. Head and body lice are still treated effectively today with lindane (although the pyrethroids are now more commonly used).

Low volatility, lipid solubility, and environmental persistence are characteristic of organochlorine insecticides. Initially, these properties helped make organochlorines very useful and effective. However, starting in the 1960s and 1970s, environmental problems began to emerge as a result of environmental persistence of these insecticides. Bioaccumulation in the food chain and the resulting toxicity led to decreased use of these insecticides and eventually to their general ban in the United States and Europe. By 1973, DDT use had ceased in the United States. By 1988, chlordane and heptachlor were no longer produced for use in the United States. Biologically, the organochlorine insecticides are generally nervous system stimulants, although there are distinct differences in the activities of the individual chemicals. The mode of action for organochlorine insecticides in general is alteration of enzymatic and electrophysiological properties of nerve cell membranes. Ion flow is altered by inhibiting Na^+, K^+, and Ca^{2+} adenosine triphosphatases that pump ions across neuronal membranes. The Na^+ channel activation is normal but its closing is prolonged. Additionally, the cyclodiene group inhibits the uptake of Cl^- ions by γ-aminobutyric acid. These activities inhibit the repolarization of neurons after excitation. The nerve remains partially depolarized and extremely sensitive to complete depolarization by very small stimuli.

All organochlorine insecticides may be absorbed through the gastrointestinal tract, respiratory tract, and skin, although there is variation among classes. Organochlorine insecticides are, in general, very lipophilic and tend to accumulate in the mammalian system in adipose tissue and/or organs with high fat content. Biotransformation of organochlorine insecticides is slow. Metabolism is by liver microsomal P450 enzymes to hydroxyl derivatives by dechlorination, conversion to stable epoxides, and/or *O*-dealkylation and hydroxylation, depending on the class. Excretion of the parent compound is usually in bile or through the intestinal wall. In either case, final elimination is usually in the feces. Urinary excretion after glutathione conjugation is also an important route of excretion. Organochlorine insecticides have been found in both cows' milk and human milk. In the liver, most organochlorine insecticides induce cellular hypertrophy, granule margination and the production of lipospheres containing fat droplets. Focal necrosis is observed with high doses. Nodules of hypertrophied hepatocytes appear in the centrilobular area with a loss of lobular architecture.

Although generally negative in mutagenicity tests, organochlorine insecticides are associated with liver tumors in rodents. Whether this is a direct carcinogenic effect or due to promotion of spontaneous tumorigenic events is not currently known. Though conclusive proof of carcinogenicity in humans is lacking but tumor potential, based on animal data, it cannot be totally discounted in humans.

Endocrine disruption has recently become a significant concern for organochlorine insecticides in both human and environmental health. Many organochlorine chemicals, including cyclodiene insecticides, mirex, and toxaphene as well as PCBs and PCDDs have been shown to have endocrine disrupting properties.

Symptomatology includes paresthesia, ataxia, nausea, vomiting, fatigue, and, in more acute cases, tremor, convulsions, coma, respiratory arrest, and death.

Clinical management is generally symptomatic. Convulsions are usually controlled using diazepam, pentobarbital, or phenobarbital. In some instances, treatment with activated charcoal is effective in increasing the excretion of the pesticide. Cholestyramine has been proven effective in chlordecone poisoning. In severe cases, mechanical maintenance of cardiac function and respiration is necessary.

See also: Chlordane; DDT (Dichlorodiphenyltrichloroethane); Dieldrin; Lindane; Methoxychlor.

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Organophosphate Poisoning, Delayed Neurotoxicity

Rudy J Richardson

The term 'delayed neurotoxicity' may be used to describe any type of toxicity to the nervous system involving a delay between the precipitating chemical exposure and the appearance of neurological signs or symptoms. However, this designation usually refers to organophosphorus (OP) compound-induced delayed neurotoxicity (or delayed neuropathy) (OPIDN), also known as OP compound-induced delayed polyneuropathy (OPIDP).

The particular syndrome of OPIDN is produced by certain organic compounds of pentacovalent phosphorus. The less common and relatively unstable organic compounds of trivalent phosphorus, such as triphenyl phosphite, can produce a different spatial–temporal pattern of neurodegeneration, which is distinct from OPIDN.

The underlying pathology in OPIDN involves bilaterally symmetrical degeneration of sensory and motor axons in distal regions of peripheral nerves and spinal cord tracts. Generally, the longest, largest diameter fibers tend to be preferentially affected. The most prominent lesions are often found in the dorsal columns of the cervical spinal cord, especially in the fasciculus gracilis. Injury to this tract results in specific sensory deficits, including loss of recognition of limb position (proprioception) and vibration sensitivity. Pathogenesis studies indicate that the primary lesion in OPIDN is in the axon rather than the myelin sheath or the cell body of the neuron, and that demyelination occurs secondarily to axonal degeneration. The process has been likened to a 'chemical transection' of the axon, with subsequent Wallerian-type degeneration, as opposed to a 'dying back' of the axon following an insult to the cell body as once hypothesized.

Signs and symptoms of axonopathy appear after a delay of at least 8 days following absorption of an effective dose of an OPIDN-producing (neuropathic) OP compound and will consist of abnormal sensations (paresthesias) in the extremities, including numbness and tingling. There may also be pain, particularly in the calves of the legs. Distal reflexes may be absent or attenuated. The feet and lower legs are usually affected predominantly and before involvement of the hands and arms, but severe cases will involve the upper and lower limbs in a 'glove and stocking' distribution. Incoordination of movement (ataxia) develops at about the same time as the sensory disturbances and may progress to partial flaccid paralysis (paresis) after ~10–21 days. Recovery from severe disease is usually poor, and there is no specific treatment. Over a period of months to years, flaccidity may be replaced by spasticity, reflecting regeneration of peripheral nerve injury with residual damage to descending upper motor neuron pathways in the spinal cord.

Because of the ubiquity of OP compounds and the serious and often irreversible nature of OPIDN, much effort has been expended to develop ways to identify the OP compounds that pose a genuine risk of causing this condition. Consequently, although the pathogenic mechanism remains unknown, human OPIDN is now an extremely rare disease, with a worldwide incidence of only about two cases per year, usually from intentional ingestion of massive

doses of OP compounds in attempted suicides. Sporadic episodes of OPIDN affecting domestic animals and livestock also occur, largely from misapplication of OP compounds used directly on the animals for control of insect or arachnid pests. Most of the ~30 000 human cases that occurred between 1930 and 1960 arose from contamination of cooking oil or beverages with tri-*o*-cresyl phosphate (TOCP; also known as tri-*o*-tolyl phosphate, TOTP). Over half of the cases of OPIDN have been attributed to consumption of an alcoholic extract of Jamaica Ginger ('Ginger Jake') that had been adulterated with solvents containing TOCP. Ginger Jake was used as a source of alcohol during Prohibition in the United States. The resulting paralysis became known as 'Jake Leg' or 'Jake Walk'. Awareness of OPIDN coupled with the advent of improved methods for assessing the relative potential of OP compounds to produce the disease has led to the virtual elimination of human cases. Nevertheless, neuropathic OP compounds and OPIDN continue to be active fields of study. This apparent paradox arises from the importance of OP chemistry in diverse applications, the threat of neuropathic OP compounds as agents of terrorism or warfare, and the promise of neuropathic OP compounds as tools in neurological research.

Experimental studies have identified the adult chicken as the species of choice for testing OP compounds for their potential to cause OPIDN. Hens of greater than 8 months of age are now used in routine testing. Other species in addition to humans and chickens that are known to be susceptible to single doses of neuropathic OP compounds include certain nonhuman primates, water buffalo, cattle, swine, sheep, dogs, and cats. Rats and mice have been considered resistant to the clinical manifestations of OPIDN. However, recent studies have shown that histopathological lesions, particularly in the spinal cord, can be produced in these species by compounds known to cause OPIDN in the adult hen. The apparent resistance of rodents to OPIDN may be due, at least in part, to the fact that relatively young (less than 3 months of age) animals have been used in most studies. Generally, the young of a given species are much more resistant to OPIDN than adults are. For example, chicks younger than ~50 days of age will not develop OPIDN after a single dose of a neuropathic OP compound. Moreover, chicks are resistant to repeated doses if they are younger than ~14 days of age. Species and age differences in susceptibility to OPIDN have been attributed to long axons in large animals and robust repair of neural injury in young animals.

The complete mechanism of OPIDN has not been elucidated. However, there is good evidence that the disease is initiated by a concerted two-step reaction involving inhibition and aging of a critical amount of a protein called neuropathy target esterase (neurotoxic esterase, NTE) in target neural tissues. The net result of the aging step is the rapid formation of a

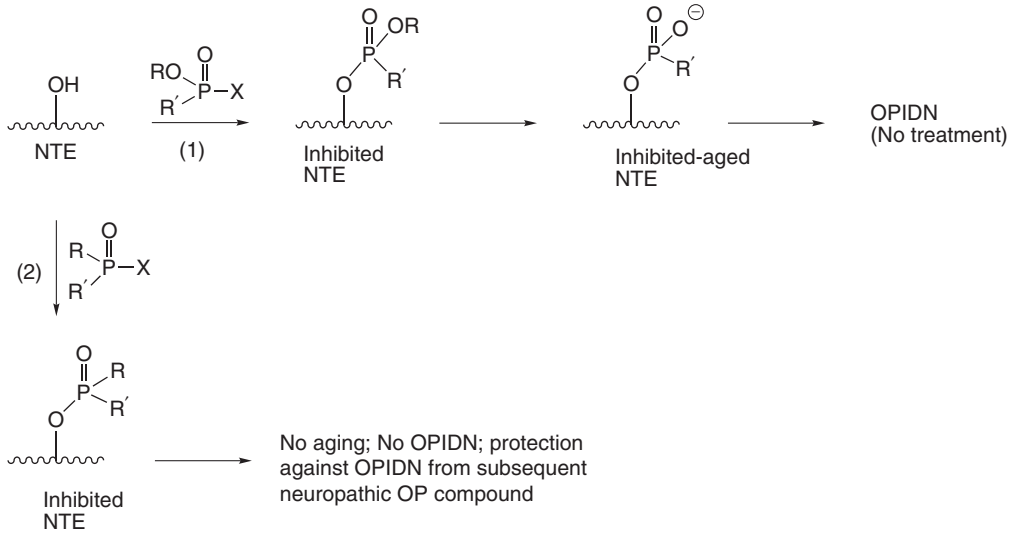

Figure 1 Inhibition and aging of NTE are required for initiation of OPIDN. NTE is represented by a wavy line containing the active site serine hydroxyl group. Pathway (1) shows inhibition by a phosphonate, which undergoes rapid aging to yield a negatively charged phosphonyl adduct. OPIDN follows within 8–21 days and is not treatable. Pathway (2) shows inhibition by a phosphinate, which cannot undergo aging. The neutral phosphinylated adduct does not trigger OPIDN; however, it confers protection against subsequently administered neuropathic (ageable) NTE inhibitors. For each type of inhibitor, R and R′ may be substituted or unsubstituted alkyl or aryl groups. X is the primary leaving group that is displaced by the serine hydroxyl of NTE and may be, for example, substituted or unsubstituted alkoxy, aryloxy, or fluorine.

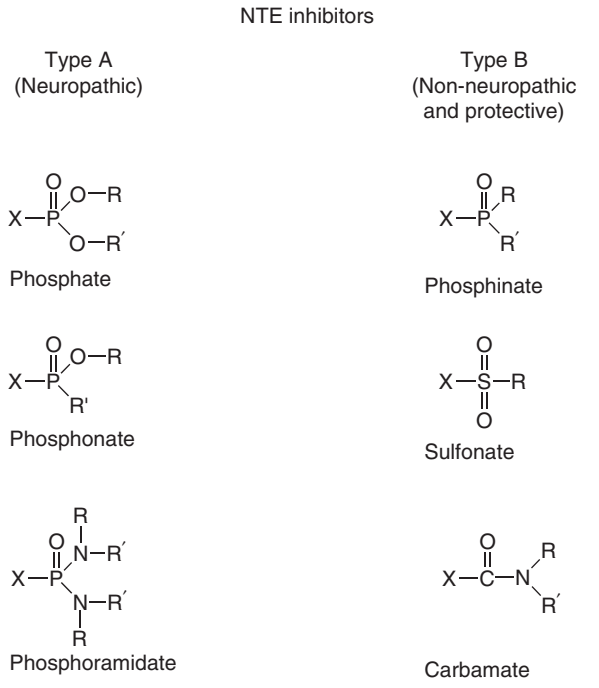

Figure 2 NTE inhibitors. For each type of inhibitor, R and R′ may be substituted or unsubstituted alkyl or aryl groups. X is the primary leaving group that is displaced by the serine hydroxyl of NTE and may be, for example, substituted or unsubstituted alkoxy or aryloxy. Fluorine can be a leaving group for the OP NTE inhibitors and is the most common leaving group for sulfonate NTE inhibitors. Type A inhibitors are neuropathic and include certain phosphates, phosphonates, and phosphoramidates. Mixtures of subtypes are possible. Phosphonates are intrinsically asymmetric and enantiomers may have different inhibitory and/or aging properties. Some phosphoramidates may have one or more hydrogen atoms as R-groups. Type B inhibitors are not neuropathic, but pretreatment protects against OPIDN from subsequent exposure to Type A inhibitors. Type B inhibitors include certain phosphinates, sulfonates, and carbamates.

negatively charged species in the active site of the enzyme (**Figure 1**). Such a reaction can take place with OP inhibitors of NTE such as phosphates, phosphonates, or phosphoramidates, which have an ester or amide group in addition to the leaving group (**Figure 2**). Phosphates and phosphonates undergo aging by net loss of an R-group. Phosphoramidates having only a single R-group attached to the phosphoramidate nitrogen appear to age by loss of the phosphoramidate proton rather than by loss of an R-group. Compounds that do not inhibit NTE do not cause OPIDN, even if they belong to a structural class capable of undergoing the aging reaction. For example, although paraoxon belongs to the phosphate class of OP compounds, it does not produce OPIDN because it is a poor inhibitor of NTE.

NTE inhibition and aging transpire within minutes to hours following absorption of an effective dose of a neuropathic OP compound. Thus, events that remain to be elucidated contribute to the delay of 8–21 days between exposure and the initial signs of ataxia and paresis. However, if inhibition but no aging occurs by dosing with an NTE inhibitor that is incapable of generating a negative charge at the active site, no OPIDN ensues. Furthermore, an animal whose NTE is inhibited with a nonaging compound is protected against a subsequent dose of an OP compound that would be neuropathic in a naive animal (**Figure 1**). Nonaging inhibitors of NTE include representatives from the phosphinate class of OP compounds, certain carbamates, and sulfonyl fluorides, such as phenylmethanesulfonyl fluoride (PMSF) (**Figure 2**).

The threshold of NTE inhibition in target neural tissue that correlates with the development of OPIDN after a single dose of a neuropathic OP compound is ~70%. For many compounds, inhibition measured in brain is paralleled in spinal cord and peripheral nerve, and brain values are often used in screening tests in hens to assess relative neuropathic potency. Repeated dosing also appears to require that a high point of inhibition be reached before OPIDN will develop. The threshold appears to be the same as for acute dosing for some compounds, but for some others, the critical level of inhibition may be as low as 50%. With repeated dosing, there still appears to be a delay of ~8–21 days between the time inhibition exceeds the threshold value and the appearance of signs of OPIDN.

NTE has also been found in circulating lymphocytes and platelets, where its inhibition has found some use as a biomarker of exposure to neuropathic OP compounds. There is a reasonably good correlation between inhibition of NTE in leukocytes and brain when the measurements are carried out within 24 h of an acute exposure. However, a good correlation might not be found later (even by 48 h) or under conditions of repeated exposures. Nevertheless, leukocytes provide an accessible source of NTE for detection of inhibition by neuropathic OP compounds. Currently, there is considerable interest in using protein mass spectrometry to detect OP adducts on NTE as sensitive and specific biomarkers of exposure to neuropathic OP compounds.

It is important to realize that OPIDN depends on a particular type of chemical modification of NTE rather than mere inhibition of its enzymatic activity. Inhibition of NTE is a necessary, but not sufficient, condition for OPIDN. Aging of the inhibited enzyme results in a complete change in the toxicological outcome. Whereas inhibition without aging results in no clinically apparent injury, suprathreshold inhibition with aging triggers an inexorable neurodegenerative process leading to evident disease. The

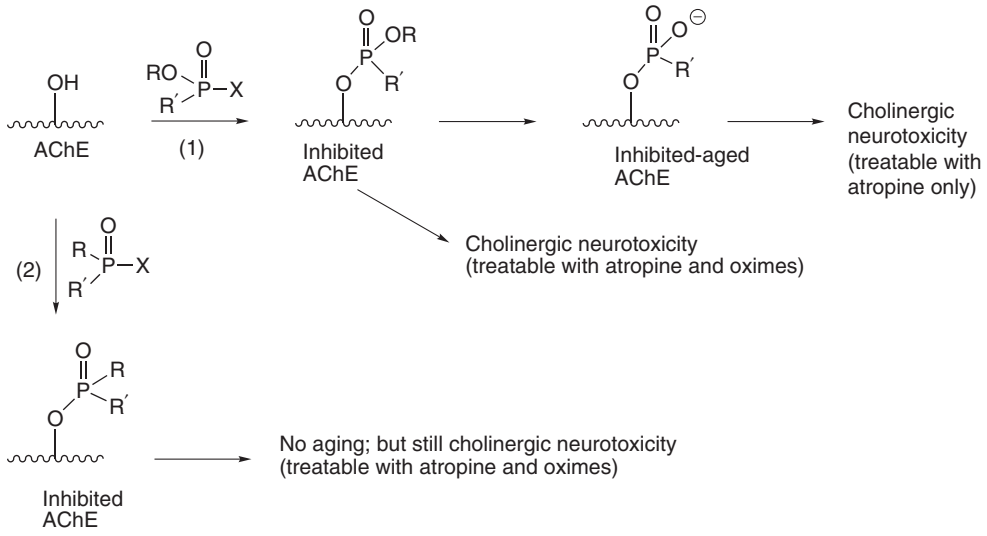

Figure 3 Inhibition of AChE is sufficient for cholinergic neurotoxicity. AChE is represented by a wavy line containing the active site serine hydroxyl group. Pathway (1) shows inhibition by a phosphonate leading directly to cholinergic toxicity, treatable by both atropine (acetylcholine antagonist) and oximes (AChE reactivators). If aging occurs, the type of toxicity does not change, but oxime reactivators are no longer effective. Pathway (2) shows inhibition by a phosphinate, which cannot undergo aging. Cholinergic toxicity still occurs and is treatable by both atropine and oximes. R and R′ may be substituted or unsubstituted alkyl or aryl groups. X is the primary leaving group that is displaced by the serine hydroxyl of AChE and may be, for example, substituted or unsubstituted alkoxy, aryloxy, or fluorine. Neither inhibition nor aging of inhibited AChE can produce OPIDN – this requires inhibition and aging of NTE (see **Figure 1**).

situation with NTE is completely different from that with acetylcholinesterase (AChE). Inhibition of a sufficient amount of AChE will produce cholinergic toxicity, regardless of whether or not aging of inhibited AChE occurs (**Figure 3**). Aging of inhibited AChE does not alter the type of toxic response, but it does change the options available for therapy against cholinergic toxicity. For example, oximes such as pralidoxime methiodide (2-PAM) are used to reactivate inhibited AChE, but these agents are ineffective if aging of the enzyme has occurred. Moreover, oximes do not appear to affect the clinical course of OPIDN following administration of a neuropathic OP compound, except to allow survival of an otherwise lethal dose of a compound that also has cholinergic toxicity.

In a homologous series of OP compounds, increasing potency for AChE inhibition and cholinergic toxicity correlates with decreasing potency for NTE inhibition and OPIDN. The relative inhibitory potency (RIP) of an OP compound or its active metabolite for NTE versus AChE *in vitro* can be used as a convenient index of the probable neuropathic potential of the compound. A commonly used measure of inhibitory potency is the IC_{50}, the concentration required to inhibit 50% of the enzyme activity under a standardized set of reaction conditions and time of incubation of the inhibitor with the enzyme preparation. A better measure of inhibitory potency is the bimolecular rate constant of inhibition, k_i. When pseudo-first-order kinetics are observed, it is valid to use the relationship, $IC_{50} = 0.693/k_i t$, where t is the time of preincubation of the inhibitor with the enzyme. Comparisons of AChE/NTE k_i ratios or NTE/AChE I_{50} ratios *in vitro* (RIPs) with toxicity data *in vivo* have shown that values less than 1 indicate that the dose required to produce OPIDN is less than the median lethal dose (LD_{50}). In contrast, RIP values greater than 1 correspond to doses greater than the LD_{50} being required to produce OPIDN. The higher the RIP, the safer the compound with respect to its capacity to produce OPIDN. Thus, insecticidal OP compounds will generally be much more potent inhibitors of AChE than NTE and will not produce OPIDN except at doses that would require treatment for cholinergic toxicity. On the other hand, compounds can be made that are better inhibitors of NTE than AChE. If such compounds can also undergo aging, not only will they produce OPIDN; they will do so at doses that elicit little or no cholinergic toxicity.

Marginal or subclinical OPIDN can be potentiated to full-blown disease by subsequent treatment with nonaging inhibitors of NTE. The phenomenon is called 'promotion' by some authors, which is an appropriate term if the initial insult is undetectable. Potentiation was initially a surprising finding, especially in view of the fact that reversing the order of dosing of the nonaging and aging NTE inhibitors affords protection against OPIDN. However, it now

appears that the outcome of many types of neural injuries can be exacerbated by dosing with nonaging NTE inhibitors as well as with inhibitors of other serine esterases or proteases. The apparent indifference to the method of producing the initial lesion suggests a general mode of action for potentiation, such as interference with regeneration and repair. With respect to understanding the potentiation of OPIDN and its practical significance, more data are needed to answer the following questions: What is the extent to which the NTE inhibition threshold for initiation may be lowered by potentiators? What are the potencies of potentiators at a given dose level of initiator? What are the potencies of initiators at a given dose level of potentiator? What are the structure–activity relationships of potentiators? What is/are the mechanism(s) of action of the effect?

Although the physiological function of NTE is currently unknown, knocking out the gene is embryonic-lethal in mice, indicating an essential role in development. NTE is an integral membrane protein concentrated in the endoplasmic reticulum. It contains a domain with homology to cyclic nucleotide-binding regions in other proteins, implying a regulatory or signaling function. Mutation of a homologous protein called SWS in *Drosophila* results in a spongiform neurodegenerative disease, suggesting that NTE might be linked to neurological or neurodevelopmental disorders. Thus far, the goal of using cell expression systems to produce full-length NTE for study has proved to be elusive. However, it has been possible to generate the NTE catalytic domain, called NEST. The enzymological properties of this truncated NTE are similar to those of the full-length protein, and it is being used to examine mechanisms of inhibition and aging by neuropathic OP compounds. A recent intriguing finding is that NEST mediates an ionic conductance across liposome membranes, which is selectively disrupted by aging inhibitors of the enzyme. Certainly, much work remains to be done to elucidate the normal and pathogenic roles of NTE, but the accomplishments thus far are proving to be useful in a wide range of fields, from toxicological risk assessment to developmental neurobiology.

Acknowledgment

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See also: Acetylcholine; A-Esterases; Cholinesterase Inhibition; Neurotoxicity; Organophosphate Poisoning, Intermediate Syndrome; Organophosphates.

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Organophosphate Poisoning, Intermediate Syndrome

Ramesh C Gupta

Organophosphate (OP) insecticide-induced intermediate syndrome (IMS) was reported for the first time in human patients in Sri Lanka in 1987. Thereafter, this syndrome has been diagnosed in OP-poisoned patients in South Africa (1989), Turkey (1990), Belgium (1992), United States (1992), Venezuela (1998), France (2000), and elsewhere. IMS is usually observed in patients who have ingested a massive dose of an OP insecticide either accidentally or in a suicidal attempt. A similar syndrome has also been observed in dogs and cats poisoned maliciously or accidentally with massive doses of certain OPs.

IMS is clearly a separate clinical entity from acute cholinergic crisis and delayed neuropathy. The acute cholinergic crisis usually emerges within a few minutes to a few hours and is due to acetylcholinesterase (AChE) inhibition resulting in acetylcholine accumulation at the synapses in the nervous system and at the neuromuscular junctions. Patients acutely poisoned with OPs exhibit muscle fasciculations,

convulsions, seizures, salivation, lacrimation, tracheobronchial secretion, and diarrhea due to overstimulation of muscarinic and nicotinic receptors within the peripheral and central nervous systems. Delayed neuropathy, commonly referred to as organophosphate-induced delayed neurotoxicity (OPIDN), a neurological manifestation of some OPs, usually occurs ~2 or 3 weeks after exposure. OPIDN occurs due to the inactivation of neurotoxic or neuropathy target esterase (NTE) and is characterized by predominately sensory, motor, distal, and symmetrical polyneuropathy.

IMS in OP-poisoned patients appears 24–96 h after an apparently well-treated acute cholinergic crisis phase. By definition, OP-poisoned patients should completely recover from the cholinergic crisis and then develop a syndrome. Clinically, IMS is characterized by acute paralysis and weakness in the territories of several cranial motor nerves, neck flexors, facial, extraocular, palatal, nuchal, proximal limb, and respiratory muscles 24–96 h after poisoning. Generalized weakness, depressed deep tendon reflexes, ptosis (drooping of the upper eyelids due to paralysis of the third cranial nerve), and diplopia (double vision of an object) are also evident. These symptoms may last for several days or weeks depending on the OP involved. Despite severe AChE inhibition, muscle fasciculations and muscarinic receptor-associated hypersecretory activities are absent.

OP compounds that are known to cause IMS are listed in **Table 1**. These compounds in general are highly lipid soluble, and in some cases, the metabolites of these OP compounds have a long-lasting half-life. Other contributing factors for IMS include the chemical structure of OP compounds, impairment of systemic functions (cardiovascular, hepatic, renal), and the time elapsed between ingestion of an OP and treatment.

Based on electromyographic (EMG) findings from OP-poisoned patients and experimental studies on laboratory animals, scientists have found that the defect in IMS is at the neuromuscular endplate and postsynaptic level, but the effects of neural and central components in producing muscular weakness have not been ruled out. EMG findings in the early stages revealed marked decrements at low rates of repetitive nerve stimulation and increments at a high rate, suggesting diverse types of impaired neuromuscular transmission. IMS seems to be due to persistent AChE inhibition at the endplate, presumably leading to combined pre- and postsynaptic impairment of neuromuscular transmission.

Table 1 Organophosphates known to cause intermediate syndrome in humans

Bromophos	Fenthion	Monocrotophos
Chlorpyrifos	Malathion	Omethoate
Diazinon	Merphos	Parathion
Dicrotophos	Methamidophos	Phosmet
Dimethoate	Methylparathion	Trichlorfon

Note: Exposure to a single or combination of these OP compounds can produce IMS.

Perhaps there are gradations of IMS and genetic or environmental factors which influence its onset. Some OPs may have a higher affinity for nicotinic acetylcholine receptors or selectively distribute to muscle, producing a neuromuscular dysfunction that is longer lasting than at muscarinic sites. There may also be differences in the onset of IMS, which would depend on OP distribution or metabolism to the active metabolite. Perhaps the lesions produced at the neuromuscular junction are more permanent than the muscarinic lesions. Currently, very little is known about the type of damage at the motor endplate or about risk factors associated with IMS. Thus, more detailed laboratory and clinical tests are necessary to determine the exogenous and endogenous factors contributing to its development.

Some investigators suggest that the IMS may result from inadequate oxime therapy, while others suggest that prolonged oxime therapy by continuous infusion has no role in the routine management of OP intoxication. The undisputed fact remains, that in patients with IMS, a long-lasting inhibition of AChE occurs due to the persistence of the OP or its active metabolite in the body. This appears to be a trigger for nicotinic receptor overstimulation and toxicity at the neuromuscular junction, leading to weakness and paralysis of respiratory and other muscles. Also in IMS patients, both clinical signs and AChE are unresponsive to atropine or oxime therapy. Signs and symptoms of IMS usually persist for 1–3 weeks, followed by a complete recovery. IMS makes the management of OP poisoning more complicated since the clinician has to observe the patients for an additional 3 or 4 days for a possible respiratory arrest. In such cases, urgent respiratory support is absolutely necessary.

Confirmatory tests for IMS include persistent inhibition of AChE, decreasing response on electromyography, and necrotizing myopathy on muscle biopsy. In most instances, recovery from IMS is complete, though a few deaths have been reported due to severe respiratory insufficiency. Only a few IMS patients have developed delayed neuropathy. The risk of death in IMS is as dangerous as it is in the cholinergic crisis phase. Due to development of severe respiratory distress, tracheostomy is recommended for ventilatory support. Patients may remain on a respirator for 7 days or more. In conclusion, prolonged inhibition of

cholinesterases and dysfunction of the neuromuscular junction are the pathophysiological hallmarks of IMS. Because of the risk of appearance of an IMS, a prolonged clinical medical supervision seems necessary after recovery from the cholinergic crisis. The administration of atropine sulfate and pralidoxime should be continued for a long period, even if efficiency of these drugs on the development of the IMS is limited. Evidently, a drug protocol that can clearly benefit the patients of IMS remains to be established.

See also: Cholinesterase Inhibition; Neurotoxicity; Organophosphate Poisoning, Delayed Neurotoxicity; Organophosphates; Pesticides.

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Organophosphates

Marion Ehrich

The term ‘organophosphates’ (organophosphorus compounds and organophosphorus esters) generally refers to chemicals that are organic derivatives of phosphoric acid. These compounds contain both phosphorus and carbon atoms, which may be linked directly or through another atom such as oxygen. Each phosphorus atom has three such linkages, and the terms ‘phosphate’, ‘phosphonate’, and ‘phosphinate’ refer to the number of linkages made through oxygen atoms (three, two, and one, respectively). Phosphorus linkages to carbon can also be made through nitrogen atoms (‘phosphoroamidates’ if one such linkage; ‘phosphorodiamidates’ if two), or through sulfur atoms (‘phosphorothiolates’ if one such linkage; ‘phosphorodithiolates’ if two). Some organophosphates have a phosphorus–fluoride linkage (‘phosphorofluoridates’). As derivatives of phosphoric acid, four atoms are directly attached to the phosphorus atoms – three by single bonds, as described previously, and one represented as a double bond. The doubly bonded linkage to the phosphorus atom of organophosphates is with an oxygen atom or with a sulfur atom. The nature of the atom linked with a double bond to the phosphorus atom also affects the chemical nomenclature of the compound, with ‘thio’ used in the chemical name to identify the S-containing phosphorothionate compounds. It is generally recognized that these phosphorothionate compounds are not active toxicants but rather protoxicants. Oxidation, which results in exchange of the doubly bonded sulfur for a doubly bonded oxygen, is necessary for conversion of the protoxicant phosphorothionate compounds to active neurotoxicants.

Organophosphates first gained notoriety during World War II, when they were synthesized by German chemists for use as highly toxic nerve gases. The potential for their use as chemical warfare agents continues today. However, other less volatile, less toxic organophosphates have since been synthesized, and these are used as insecticides, defoliants, herbicides, therapeutic agents in human and veterinary medicine (e.g., as ophthalmic agents and antiparasitics, respectively), flame retardants, fuel additives, lubricants, and plasticizers. They are in especially widespread use as insecticides, with formulations prepared for use in homes and gardens, on pets and livestock, and on crops and fields. Their popularity as insecticides is based on their effectiveness and on their biodegradability.

Examples of organophosphates include the insecticides malathion, parathion, diazinon, fenthion, azinphos methyl, terbufos, dichlorvos, and chlorpyrifos; the nerve gases soman, sarin, tabun, and VX; the ophthalmic agent echothiophate; the anthelmintic trichlorfon; tricresyl phosphate-containing

industrial chemicals; and the herbicides/defoliants DEF and merphos.

Most organophosphates are lipid soluble and all can be hydrolyzed, a reaction that results in detoxification. Some of them are gases or volatile liquids. These chemical properties contribute to their usefulness and to their toxicity by affecting their absorption and metabolism. Lipid-soluble substances more readily pass membrane barriers than compounds that are less lipid soluble, resulting in more complete absorption regardless of the route by which humans or animals are exposed. This lipid solubility aids the passage of organophosphate insecticides through the chitin exoskeleton of insects. Due to their lipid solubility, however, most organophosphates can pass through the skin of humans and animals as well, although the extent of absorption and the time in which it occurs varies from compound to compound. Volatility means that exposure to some organophosphates can be by inhalation, with absorption via the lungs. Organophosphates are esters, which means that they can be split into their component acid and an alcohol by the addition of water across the ester bond. This degradatory reaction (hydrolysis) occurs when organophosphates are in the presence of water and, in particular, in the presence of enzymes (esterases) that catalyze this reaction. That organophosphates are subject to hydrolysis increases the probability that they will biodegrade in the environment and that they will not accumulate in the environment or in the bodies of exposed subjects.

Organophosphate insecticides inhibit neural acetylcholinesterase, an enzyme responsible for the degradation of the neurotransmitter acetylcholine. This is the means by which they are effective as insecticides and the means by which they are toxic to humans and animals. Acetylcholine is a neurotransmitter found in the brain, spinal cord, and peripheral nervous system. In the peripheral nervous system, it is the neurotransmitter at effector organs of the parasympathetic nervous system, at ganglia of the autonomic nervous system (both sympathetic and parasympathetic ganglia), and at junctions between nerves and skeletal muscles. The presence of excess acetylcholine, due to inhibition of acetylcholinesterase, at muscarinic receptors (which are at the effector organs of the parasympathetic nervous system) results in clinical signs that include blurred vision due to pupil constriction, tearing, breathing difficulty due to excessive respiratory secretions, vomiting and diarrhea due to increased activity of the gastrointestinal tract, increased frequency of urination, and slowing of the heart rate. The presence of excess acetylcholine, due to inhibition of acetylcholinesterase, at nicotinic receptors found in autonomic ganglia can result in exaggeration of the effects seen by stimulation of parasympathetic muscarinic receptors and, in addition, can cause hypertension and tachycardia due to concurrent stimulation of the sympathetic nervous system. The presence of excess acetylcholine at nicotinic receptors of neuromuscular junctions of skeletal muscles can result in muscle twitching, tremors, and cramps. This may be followed by muscle weakness and flaccid paralysis. Excess acetylcholine in the brain and spinal cord (central nervous system) may cause anxiety, restlessness, emotional instability, confusion, ataxia, weakness, convulsions, and/or coma. Death is usually due to respiratory failure, which may be the result of a combination of effects in the peripheral and central nervous systems. Some organophosphates are very toxic; others are not. Toxicity resulting from organophosphate exposure is dependent on the chemical structure, lipid solubility, formulation and formulation vehicle, dosage, and the absorption, distribution, metabolism, and excretion of the substance to which subjects are exposed.

The acetylcholinesterase enzyme contains two sites for binding of acetylcholine, its natural substrate. Organophosphates combine with one of these sites, called the esteratic site, preventing the attachment of acetylcholine. Treatment of acetylcholinesterase inhibition is directed toward protecting the acetylcholine receptor from excess neurotransmitter and toward removal of the organophosphate from the inhibited enzyme. Atropine competes with acetylcholine for the muscarinic receptors at which it acts in the parasympathetic nervous system; this drug is used to reduce symptoms associated with overstimulation of those receptors with the acetylcholine that accumulates as acetylcholinesterase is inhibited. Symptomatic treatment of organophosphate toxicity may also include diazepam, a tranquilizer and anticonvulsant. Oxime drugs (e.g., pralidoxime or 2-PAM) attach to the organophosphate itself, removing it from acetylcholinesterase. Oximes must be given in a relatively short time frame after exposures occur, however. Although initially reversible, with time the attachment between the organophosphate and the enzyme can become irreversible, a condition generally referred to as 'aging'. Once aging has occurred, treatment of toxicity with oximes is ineffective. Time is needed for synthesis of new acetylcholinesterase molecules before enzyme activities return to preexposure levels.

Prevention of organophosphate toxicity is aimed at protecting the acetylcholine receptor and/or acetylcholinesterase itself. Atropine can be used to prevent as well as to treat organophosphate poisonings. In addition, use of 'reversible' inhibitors of acetylcholinesterase has been used to prevent organophosphate

toxicity. The rationale behind use of such compounds (carbamates such as physostigmine) is that they occupy the site at which organophosphates could bind to acetylcholinesterase and, consequently, provide time for the organophosphate to be metabolized and excreted before sites become free for occupancy on acetylcholinesterase. Carbamates may actually be given after exposure to organophosphates, with the assumption made that absorption will take some time, so the carbamates can be used to occupy sites on acetylcholinesterase until the danger of further absorption of organophosphates is past. After sufficient time, the carbamate is withdrawn and the carbamylated enzyme is given time to spontaneously reactivate. Experimental therapies for prevention and/or treatment of organophosphate toxicosis with potential for use in extenuating circumstances (e.g., nerve gas exposure) include administration of enzymes responsible for organophosphate hydrolysis.

Esterases other than neural acetylcholinesterase may also be inhibited by organophosphates, although this is dependent on the compound and the species of animals exposed. These esterases include acetylcholinesterase of mammalian erythrocytes; pseudocholinesterases found primarily in nonneural sites, such as the liver and plasma; neurotoxic esterase (also known as neuropathy target esterase) found primarily in the nervous system; and carboxylesterases (also known as aliesterases), which are relatively nonspecific enzymes found in many cells, including those of the nervous system and liver. That organophosphates can inhibit esterases other than neural acetylcholinesterase provides an opportunity to monitor exposure using red blood cell acetylcholinesterase and/or serum pseudocholinesterase as markers. Organophosphate-induced signs of toxicity generally only occur after significant inhibition of neural acetylcholinesterase or significant inhibition and aging of neurotoxic esterase; inhibition of pseudocholinesterases or carboxylesterases causes no apparent clinical signs. The organophosphates that inhibit neurotoxic esterase do not include commonly used insecticides; toxicity that follows inhibition of this enzyme differs considerably from that caused by inhibition of acetylcholinesterase. Weeks after exposure, humans and certain species of animals develop progressive degenerative changes that can be seen on microscopic examination of peripheral nerves and/or the spinal cord. This organophosphate-induced delayed neuropathy can result in incoordination, ataxia, and paralysis. Specific treatments for this disorder have not been developed.

The capability of organophosphates to inhibit pseudocholinesterases and carboxylesterases without causing clinical signs provides a mechanism by which serial exposures to nontoxic dosages can result in toxicity. These enzymes provide sites additional to those on acetylcholinesterase at which organophosphates can attach, but once inhibited, they may not be available when humans or animals are exposed to organophosphates for a second time. Thus, more organophosphate is available to attach to acetylcholinesterase, resulting in toxicity at dosages that would not be toxic without prior exposure. Instead of the potentiation of toxicity seen with subsequent dosing as described previously, however, multiple administrations of low dosages of organophosphates over a sufficient period of time may result in tolerance as receptors for acetylcholine become desensitized or downregulated. Metabolism of a compound may also increase with repeated exposure.

Due to their widespread use, especially as insecticides, exposure to organophosphates may be intentional, accidental, or environmental. Once exposure occurs, regardless whether the route is dermal, oral, or by inhalation, absorption is likely because organophosphates have considerable lipid solubility. This property also increases the potential that they will be generally distributed throughout the body, and will easily pass into the nervous system, where they exert their toxic effects. Many organophosphates, especially the insecticides, enter the body as S-containing phosphorothionate protoxicants. These protoxicants are readily activated to esterase inhibitors by mixed function oxidase enzymes of the liver. Organophosphates are metabolized by a variety of esterases, including those that they may inhibit (B-esterases such as pseudocholinesterases and carboxylesterases) and those that they do not inhibit (arylesterases or A-esterases, also known as organophosphorus acid anhydrases, phosphohydrolases, or phosphorotriesterase hydrolases); metabolites may be excreted in urine, feces, and milk. Organophosphates are readily hydrolyzed in aqueous solutions with high pH; therefore, soapy water is useful for decontamination of skin and clothing.

Although the primary mechanism of toxicity associated with exposure to organophosphates has to do with inhibition of acetylcholinesterase, signs can still occur after the cholinergic crisis has resolved. Such signs include the delayed neuropathy that can occur weeks after inhibition of neurotoxic esterase, an intermediate paralytic syndrome that may occur several days after severe acetylcholinesterase inhibition, muscle damage that may begin during the cholinergic crisis and which may develop into a myopathy that can reverse within weeks of exposure, and cardiotoxicities that may be part of or occur after the acute syndrome. Residual neurobehavioral effects may remain following recovery

from significant acetylcholinesterase inhibition. In addition, there have been reports of effects that may be a consequence of repeated low-dose exposures at doses that are insufficient to cause clinical evidence of acetylcholinesterase inhibition in humans. The effects that have been reported to appear include anxiety, confusion, impairment of judgment, visual disturbances, behavioral changes, memory deficits, and incoordination. Certain organophosphates have also been reported to have immunotoxic or teratogenic effects.

Species differences in susceptibility to organophosphate toxicities are notable. For example, significant neurotoxic effects that remain after recovery of acetylcholinesterase activity have not been reported for studies performed in animals, but a number of reports suggest that a variety of long-lasting behavioral and functional changes could occur in some humans. Species differences in clinical manifestations of organophosphate-induced delayed neuropathy are also notable. Although locomotor difficulties occur in humans, hens, cats, sheep, cattle, and a variety of other species, they are not obvious in the rodent species commonly used for toxicity testing (rats and mice). Other species differences may be related to the pharmacokinetics (absorption, distribution, and clearance) of organophosphates. For example, insects, due to their small size and to their chitin exoskeleton (which does not provide an impediment to organophosphate insecticide absorption), are much more likely to succumb to organophosphate toxicity than are other animal species. Absorption also contributes to species differences among mammals. Cats are more likely to absorb organophosphates after exposure by the dermal route, and the propensity of this species to groom also increases the likelihood of oral exposure even when the original exposure was by the dermal route. Metabolic differences among species are a significant factor associated with species differences. Avians, for example, are more susceptible to organophosphate toxicity because they have less capability to hydrolyze these chemicals by enzymes that are not inhibited by the organophosphates.

Pesticide assessment guidelines under the Federal Insecticide, Fungicide, and Rodenticide Act stipulate that organophosphates proposed for use as insecticides be tested both for their capability to cause acute toxicities due to inhibition of acetylcholinesterase and for their potential to cause inhibition of neurotoxic esterase and subsequent delayed neuropathy. Testing could be performed in laboratory rodents because they, like all species, are susceptible to acetylcholinesterase inhibition, but rodents do not develop notable ataxia, and neuropathological manifestations are very restricted if these species are exposed to organophosphates that cause delayed neuropathy. Testing for the toxicity of organophosphates includes, therefore, adult hens as the animal model for organophosphate-induced delayed neuropathy. Inhibition of neurotoxic esterase, ataxia, and neuropathy are detectable in hens, and the relationship between dosages causing acetylcholinesterase inhibition and delayed neuropathy can be determined.

See also: Anticholinergics; Behavioral Toxicology; Carbamate Pesticides; Carboxylesterases; Cholinesterase Inhibition; Federal Insecticide, Fungicide, and Rodenticide Act, US; Nerve Agents; Neurotoxicity; Organophosphate Poisoning, Delayed Neurotoxicity; Organophosphate Poisoning, Intermediate Syndrome; Psychological Indices of Toxicity.

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Organotins*

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The organotins comprise a large class of organometallic compounds containing one or more tin atoms bound covalently to one or more carbon-containing moieties (R groups), which can be either alkyl or aryl groups. Because tin may assume either a $+2$ or $+4$ valence state, up to four R groups may replace inorganic anions, yielding mono-, di-, tri-, and tetra-substituted organotin compounds. As of 1982, 259 organotin compounds were described in the *CRC Handbook of Chemistry and Physics*, a number that far exceeds the number of organically substituted forms of any other metal, including mercury, lead, arsenic, and germanium. This large number of organotin compounds attests to their broad utility as industrial catalysts and stabilizers, as preservatives in construction material, and as agricultural and marine pesticides. More than 1000 patents have been granted for organotin stabilizer formulations alone, and annual world production has reached nearly 50 000 metric tons. Major uses of organotins include stabilization of polyvinyl chloride (PVC) plastics during heated polymerization and catalysis of the polymerization of urethane foams; a variety of monomethyl, dimethyl, dibutyl, and dioctyl tin derivatives are used for these purposes. Pesticidal usage relies primarily on trialkyl and triaryl tins, which provide effective rodent repellents, fungicides, insecticides, and molluscicides; bis(tri-*n*-butyl tin) oxide (TBTO) is used as a preservative for wood, paper, leather, and textiles.

Exposure to organotins may occur occupationally, during manufacturing processes, or during application of biocidal agents containing the compounds. The general public may also receive exposure from this latter route (e.g., during spray application of paints and wood preservatives). In addition, significant leaching of tin stabilizers from plastic packaging into foodstuffs has been demonstrated; thus, the US Food and Drug Administration closely regulates the use of organotins in the manufacture of these materials. Plastic tubing used for medical procedures may also contain organotin stabilizers, as well as PVC piping used in domestic water systems, leachates of which may gain entry directly into the body. Finally, inorganic tin may be biomethylated by microflora in marine and estuarine sediments, leading to human exposure via consumption of seafood as well.

In contrast to the low toxicity of inorganic tin salts, the toxic properties of organotins have been recognized since their discovery in the latter part of the nineteenth century. Investigation of the mammalian toxicology of organotins was spurred by several case reports of severe reactions to inhalation of trimethyltin vapors, and by the 'Stalinon' disaster in France in 1954. In this latter episode, preparations of diethyltin diiodide, designed for the treatment of skin disorders, were contaminated with triethyltin iodide. More than 100 people died and a similar number were disabled after taking this medication.

Subsequent studies of the toxicity of organotins have provided several generalizations based on the chemical characteristics of the compounds. First, organotins primarily affect the skin, immune and nervous systems; the liver and kidneys are less sensitive. Recent evidence indicates potent endocrine-disrupting effects in both invertebrates and mammals. Second, the toxicity of organotins tends to increase with the number of organic ligands. For example, trialkyl forms appear to be the most toxic of the alkyl tins, and tetraalkyl tins are metabolically dealkylated to trialkyl forms. Third, toxicity tends to decrease with increasing size and complexity of the R group because of reduced penetration into target organs with increasing chain length, and because tetraalkyl compounds with large alkyl groups are less readily dealkylated than those with small alkyl groups. For example, dioctyltin and triphenyltin are poorly absorbed from the mammalian gut, whereas trimethyltin and triethyltin are absorbed with high efficiency. Dioctyltin dichloride (DOTC) appears to be an exception, in that it causes a severe yet reversible immunotoxicity in rats, but not in mice, guinea pigs, quail, or chickens. Further, these effects are not observed when the carbon chain extends beyond eight, suggesting that the observed immunotoxicity may be specific to DOTC in rats. Fourth, the anionic component of the organotin contributes to the toxicity of the compound by affecting its volatility and solubility in water; those compounds more volatile and soluble will naturally gain access to target organs in preference to those less so. Fifth, among the highly toxic trialkyltins, the primary target of toxicity appears to be the nervous system for trimethyltin and triethyltin, whereas the immune, reproductive, renal, and hepatic systems are more sensitive to tripropyltin

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and tributyltin. The only aryltin with documented toxicity is triphenyltin, whose actions resemble those of tributyltin.

Mechanism of Toxicity

Organotin compounds exert a number of cellular, biochemical, and molecular effects, and toxicity is primarily influenced by bioavailability of the toxicant, route of exposure, and the level and time course of exposure. Both di- and trialkyltin compounds are potent inhibitors of mitochondrial oxidative phosphorylation. Tetra-substituted organotins have no direct selective action on mitochondria, but are converted *in vivo* to toxic tri-substituted species. Perturbation of calcium homeostasis has also been shown to be involved in the cytotoxic action of tributyltin. The effects of elevated calcium concentrations include thymocyte killing and stimulation of apoptosis. Trimethyltin, triphenyltin, and dibutyltin are less potent in altering calcium homeostasis in thymocytes; however, increased free intracellular Ca^{2+} in the neuron synapses appear to be involved in the neurotoxicity of both trimethyltin and triethyltin. Tri-substituted organotins also interact with various intracellular enzymes that may result in toxicity, mainly cytochrome P450-dependent monooxygenases, which serve an important role in detoxifying xenobiotics. Tributyltin and triphenyltin exert selective effects upon different components of this enzyme system, and significant species differences occur. Lastly, the reproductive effects of tributyltin appear to involve another P450-dependent system: inhibition of aromatase, an enzyme responsible for the conversion of testosterone to estradiol, has been implicated as the primary pathway by which the aromatase inhibitors tributyltin and triphenyltin cause endocrine disruption. Moreover, this mechanism seems to be conserved across a wide phylogenetic range, as tributyltin and triphenyltin have been shown to inhibit cytochrome enzymes in gastropods, fish, rats, and human tissue.

Effects on Humans and Experimental Mammals

All of the common organotins with low molecular weight are potent irritants to the skin and mucous membranes, and even brief contact can cause severe chemical burns and dermatitis. Dermal effects represent the most immediate hazard for workers handling these compounds, though these effects are reversible over time. Despite high acute toxicity, no organotins appear to be carcinogenic. Indeed, some evidence exists for a potential therapeutic role for organotin compounds in the diagnosis and treatment of tumors.

Next to skin, the immune system appears to be the organ system most sensitive to di- and tri-substituted organotins in mammals. Toxic effects have been reported in thymus, lymph, and spleen, with the thymus being the most sensitive target. For example, TBTO in the diet of rats has been shown to cause atrophy of the thymus and lymphoid organs, to deplete stores of iron in the spleen, to reduce hormonal activity of the pituitary–thyroid axis, and, of greater biological consequence, to decrease resistance to bacterial and parasitic infections. Many of these effects occur at lower doses of tributyltin and dioctyltin in neonatal rats than in adults. In adult rats, thymic atrophy also follows from dietary exposure to diphenyltin and triphenyltin, and to dialkyltins whose toxicity decreases with increasing chain length. Mice appear to be far less sensitive than rats and other species to the thymolytic effects of ingested organotins. This resistance is likely due to differences in the uptake and elimination of the compounds, because it is not observed after systemic injections of dialkyltins. The reversibility of the immunotoxic effects of organotins has not been well studied; existing data indicate that thymic atrophy after oral exposure to dibutyltin and dioctyltin recovers after termination of exposure, though more slowly in rats dosed perinatally than in adult rats.

The neurotoxicity of trialkyltins has been well studied, using primarily trimethyltin (TMT) and triethyltin (TET). These highly toxic compounds are not used commercially, but have proven to be useful tools for the study of the nervous system and its response to organotins. TMT is readily absorbed by any route and readily penetrates the central nervous system (CNS), where it destroys neurons, and for unknown reasons targets large pyramidal neurons. Damage is most prominent in the hippocampus but can also be detected in many regions of the CNS and spinal cord. Because the affected cells die, many functional changes caused by TMT (including emotional disorders, cognitive dysfunction, and hearing loss) are persistent. TET is also neurotoxic; however, its primary target cells are the neuroglia, which generate the myelin sheath surrounding the axons of large neurons in the CNS. Thus, TET toxicity is characterized by cerebral edema and demyelination instead of neuronal cell loss. Because these effects are slowly reversible, recovery after TET intoxication is more likely than after TMT intoxication. In addition to their CNS effects, both TMT and TET have been shown to produce peripheral neuron degeneration and central chromatolysis of specific neuron groups.

Hepatic effects of alkyltins involve injury to the bile duct, hepatocellular necrosis, and changes in the activity of some enzyme systems. The effects of dibutyltin on biliary function appear to occur only in species with common hepatic and pancreatic bile ducts (e.g., rats, mice, and hamsters). Species with separate pancreatic and hepatic bile ducts, including rabbits, guinea pigs, hens, and cats are not similarly affected, suggesting that humans, who also have separate systems, would not be affected in this manner. Damage to hepatocytes appears to involve a combination of secondary effects, including bile duct injury and direct toxicity to the cells. Changes have also been reported in the activity of enzymes involved in heme synthesis and metabolism of xenobiotics, including both cytochromes and mixed-function oxidases.

Organotins also exert hematological effects, including microcytic anemia and inhibition of platelet aggregation. The anemia appears to involve both interference with the synthesis of hemoglobin, perhaps via inhibiting iron uptake, and direct hemolytic effects. The inhibition of platelet aggregation has been attributed to tin-induced loss of serotonin from the cells.

While not yet studied thoroughly, reproductive effects of organotins have also been reported. Tributyltin has been shown to cause preimplantation embryonic loss in female rats, and to reduce testis weight, spermatid and sperm counts in male rats. However, most of the studies of reproductive and endocrine effects of organotins have involved invertebrates studied in an ecological context.

Ecological Effects

Unlike TMT and TET, tributyltin (TBT) and triphenyltin (TPT) are used commercially and are among the most harmful pollutants in aquatic ecosystems. TBT gained widespread application as a biocide in antifouling paints on ships and in wood protection. TPT, although sometimes employed as a cotoxicant with TBT in antifouling preparations, is mainly applied as an agricultural fungicide, entering the aquatic environment through runoff and atmospheric deposition. Although regulation of TBT and TPT has resulted in decreased contamination, complete removal from the environment can occur only through biodegradation, photolysis by sunlight, sedimentation, flux, and biological uptake. Little is known about the exact mechanisms contributing to TBT and TPT degradation in the environment, but only a limited number of microbes are capable of degrading TBT. As such, the potential consequences of biological uptake are of global concern. The endocrine disrupting effects of TBT are most evident in marine organisms; shellfish develop ambiguous genitalia and display increased androgen levels and decreased estrogen levels. To date, over 100 gastropod species have been adversely affected by TBT, in addition to both marine and freshwater fish. TPT residues have also been reported in freshwater and marine gastropods and fish; however, the toxic action of TPT has not been fully delineated. In addition to the direct toxic effects of organotins, exposure to TBT has been shown to exacerbate effects of infectious diseases, with TBT-exposed oysters succumbing to parasitic infection at levels markedly below those causing mortality in an unexposed group. Thus, organotins, like other anthropogenic stressors, may contribute to disease outbreaks by creating populations of immunosuppressed hosts.

See also: Pesticides; Tin.

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Ototoxicity

Michael J Sullivan

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Introduction

Ototoxicity is defined as affects on the organs of the inner ear. Oto means ear. The organs of the inner ear supply two key functions to the body: hearing and balance. Chemicals that cause ototoxicity can affect the cochlea (hearing) or the vestibular system (balance). Ototoxicity of the cochlea or the Organ of Corti may also be termed cochleotoxicity. Ototoxicity of the vestibular system may also be termed vestibulotoxicity. However, generally the term ototoxicity is used in this field of study. Both of these functions may also be impaired by effects on the vestibulocochlear nerve, the nerve sending balance/hearing information from the inner ear to the brain. Effects on either hearing or balance can be caused by effects on the brains itself. Effects on nerves or the brain would be classified as neurotoxic.

Figure 1 shows both a schematic of the inner ear (both cochlear and vestibular organs) and a histology cross section. Several turns of the cochlea can be seen rising from the middle ear in turns of decreasing diameter. Within the cochlea is the Organ of Corti with the functional cellular units of hearing, the hair cells. Damage to and permanent loss of hair cells by either chemicals or noise results in permanent hearing loss.

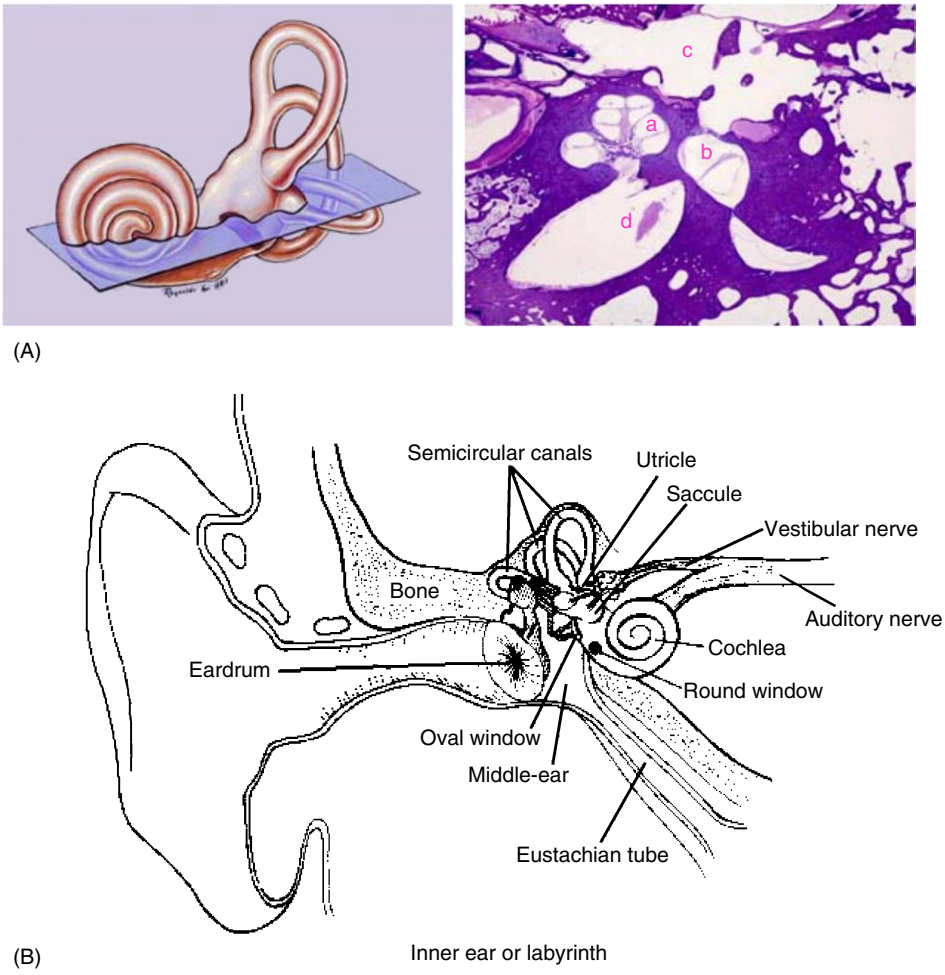

Figure 1 (A) Drawing of the inner ear to illustrate the plane of the microscopic section shown in (B). (B) Horizontal slice of the inner ear prepared for microscopic study: a, cochlea; b, vestibule (balance organs); c, middle ear space; d, portion of the hearing nerve in the internal auditory canal.

Symptoms of Ototoxicity

Ototoxicity may be manifest in many ways depending on the agent involved. Ototoxic effects may be severe or mild, permanent or temporary, or affect hearing or balance or both. An understanding of how the particular ototoxic agent causes the effects would be needed before an effective testing regime could be developed.

Determining effects on hearing may be complicated. Early signs of hearing loss may be the symptom of tinnitus or ringing sound in the ears. This can be caused by both direct effects on the cochlea or the vestibulocochlear nerve. Hearing loss is often manifested by the loss of changes in sensitivity to specific frequencies. Some ototoxic agents affect high frequencies (e.g., aminoglycoside antibiotics) and others affect middle-frequencies (e.g., toluene). Noise, although a physical agent is also ototoxic and can affect those frequencies that are characteristic of the sound pattern.

Testing for Ototoxic Effects

Estimates of how many people suffer from ototoxicity are not available. This is because in humans the early signs of ototoxicity will often not be reported. The slight ringing in the ears may be thought to be temporary or not noticeable. The initial loss of sensitivity to certain sound frequencies may be so slight that it would not be reported to a physician. The symptoms of high frequency hearing loss or loss of balance can also be incorrectly attributed to aging. For these reasons, ototoxicity can often remain undiagnosed.

In some cases, the ototoxicity may be manifest as a sudden onset of tinnitus or significant hearing loss. These symptoms should immediately be reported to a physician because both medicinal and industrial chemicals can be the cause. It would be advisable to complete a hearing test to determine a hearing baseline for that individual. A regular hearing monitoring program could be used to determine if hearing is worsening. If the patient is under a treatment regime that includes ototoxic agents, the use of these agents may need to be terminated before permanent hearing loss results.

Ototoxicity of the vestibular system will also be overlooked in patients. The loss of balance may manifest itself either sporadically (e.g., only a certain movement axis affected) or the symptoms my be mild and worsening slowly. There are tests available to determine if the vestibular system has been affected. These include both physical tests (e.g., balance tests) and electrosensory tests.

Testing regimes in animals are available to evaluate the potential ototoxic effects of chemicals. These tests range from the gross testing of animal reaction/reflexes to sudden noise (even different frequencies) to the more sensitive electrosensory tests. In treated animals, the cochlea may be harvested at the time of necropsy and the Organ of Corti examined for evidence of damage.

Damage to Tissues Caused by Ototoxic Agents

As mentioned previously, the effects of ototoxic agents can be on the cochlea or vestibular organs directly or on the vestibulocochlear nerve. Each of these types of damage will manifest itself in changes in hearing or balance. It is often these functional changes that are noticed and measured. However, when the effect of the ototoxic agent is directly on the cochlea or vestibular organs, there are pathological changes that can be found. Just like the harvesting and processing of other tissues, for example, liver, the organs of the inner ear can be harvested and examined microscopically.

In the cochlea, it is the Organ of Corti that is examined. Like some other organs, the Organ of Corti has a three-dimensional structure that is related to function. This organ, contained within the cochlea (named because of the spiraling shell shape) spirals from the oval window to the apex in turns of decreasing diameter. It is the distance from the oval window that determines the specific hearing function, that is, the frequency of sound detected. High-frequency sound with short wavelengths is detected by portions of the Organ of Corti close to the oval window. Low-frequency sound with long wavelengths is detected by portions of the Organ of Corti most distant from the oval window. The pathology of where the damage has occurred in the cochlea should match the functional hearing loss observed.

The functional unit in both organs of the inner ear is the hair cell. They are composed of long cylindrical cells that have hair-like features at one end. When physical energy in either the cochlea (caused by sound) or in the vestibular organ (caused by movement) causes the hair to bend, an electrical signal is sent to the brain that is interpreted as either hearing or movement. These hair cells are the target for some ototoxic agent. When hair cells die they are not replaced and function is lost. Maps of the cochlear hair cells, termed cytocochleograms, can be constructed to show where hair cells damage has occurred.

Agents that Cause Ototoxicity

Many classes of agents can cause ototoxicity. These classes include:

- physical,
- antibiotics,
- diuretics,
- industrial solvents/environmental chemicals, and
- chemotherapy for cancer.

Table 1 lists many of the agents that cause ototoxicity.

When new drugs or chemicals are being introduced, the battery of testing generally does not include testing for either form of ototoxicity. In some cases if there is a structural similarity between an existing ototoxic agent and the drug/chemical being tested, ototoxic testing may be requested. Generally, drugs and chemicals are only found to be ototoxic after a sufficient amount of use or exposure has accumulated in the population and these effects begin to be reported. This, of course, can take years before the extent of ototoxicity is known.

It is also possible that there can be exposure to multiple ototoxic agents simultaneously. For example, multiple antibiotics can be prescribed and the ototoxic effects could be additive. A patient may be on diuretics and receiving chemotherapy or antibiotic therapy. In these cases is has been observed that the effects of these two agents is synergistic, that is, the effects of the combined treatment is greater than the expected effect of each agent individually. Exposure of workers to industrial chemicals can also occur in a noisy occupational environment. Studies of the interaction of ototoxic solvents and noise have shown additive effects related to both functional changes and hair cell loss.

Table 1 List of ototoxic agents

Physical
Noise
Antibiotics
Streptomycin
Gentamicin
Tobramycin
Netilmycin
Neomycin
Erythromycin
Kanamycin
Vancomycin
Diuretics
Acetazolamide
Bumetanide
Furosemide
Ethacrynic acid
Industrial solvents and environmental chemicals
Butyl nitrite
Carbon disulfide
Lead
Mercury
Tin
Trichloroethylene
Carbon monoxide
Hexane
Manganese
Styrene
Toluene
Xylene
Chemotherapy for cancer
Cisplatin
Vincristine
Other
Aspirin

Reversibility of Ototoxicity

The reversibility of ototoxic effects is dependent on the dose, the duration, and the ototoxic agent. For aspirin, which can cause tinnitus, the effects can be transient and end soon after exposure ends. For the aminoglycoside antibiotics gentamicin, kanamycin, netilmycin, and tobramycin, the severity of hearing loss varies. With these agents hearing loss begins at the higher frequencies and with sufficient dosing cochlear hair loss is significant and hearing loss at selected frequencies permanent. Even when dosing is done carefully to balance the beneficial antibiotic effects and the harmful ototoxic effects it is estimated the chances of hearing recovery are low and thought to be $\sim$10%. The onset of hearing loss with diuretics is usually immediate and if symptoms are noticed and exposure stopped, the effects are reversible. The industrial solvent toluene has been found to cause hair cell loss in the middle frequency range. With only mild hair cell loss any functional loss of hearing would not be noticed. However, higher exposures leading to significant and permanent loss of hair cells (which are not replaced) could lead to permanent middle-range hearing loss.

See also: Acetylsalicylic Acid; Butyl Nitrite; Cancer Chemotherapeutic Agents; Carbon Disulfide; Cisplatin; Lead; Mercury; Tin; Trichloroethylene.

Further Reading

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Otto Fuel II

Richard D Phillips

- Chemical Abstracts Service Registry Number: CAS 106602-80-6
- Chemical Formulas: A mixture of propylene glycol dinitrate, $C_3H_6N_2O_6$; 2-Nitro-diphenylamine, $C_{12}H_{10}N_2O_2$; and Dibutyl sebacate, $C_{18}H_{34}O_4$

Uses

Otto Fuel II is a distinct-smelling, reddish-orange, oily liquid that the US Navy uses as a fuel for torpedo and other weapons systems. It is a mixture of three synthetic substances: mostly propylene glycol dinitrate (~75%), dibutyl sebacate (~23%), and 2-nitrodiphenylamine (~2%).

Propylene glycol dinitrate is the explosive part of Otto Fuel II. It is a colorless liquid with an unpleasant odor. Other names for propylene glycol dinitrate are PGDN, 1,2-propylene glycol dinitrate, and 1,2-propanediol dinitrate.

Dibutyl sebacate is a clear liquid. It is most often used for making plastics, many of which are used for packaging food. It is also used to enhance flavor in foods such as ice cream, candy, baked goods, and nonalcoholic drinks. Some consumer products, such as shaving creams, also contain dibutyl sebacate. Other names for dibutyl sebacate are decanedioic acid, dibutyl ester, sebacic acid, dibutyl ester, and dibutyl decanedioate.

2-Nitrodiphenylamine is a solid. Otto Fuel II contains 2-nitrodiphenylamine to control the explosion of propylene glycol dinitrate. It is also used as a solvent dye. Other names for 2-nitrodiphenylamine are 2-nitrobenzamine, 2-nitro-*N*-phenyl, 2-nitro-*N*-phenylaniline, and Sudan Yellow 1339.

Exposure Routes and Pathways

Exposure to Otto Fuel II may occur by the inhalation, oral, or dermal routes. Inhalation exposures to Otto Fuel II would consist primarily of inhalation exposure to propylene glycol dinitrate. Oral exposure to Otto Fuel II is possible through consumption of contaminated water. It is likely that significant ingestion of dibutyl sebacate may occur as a result of its civilian use in food packaging materials and as a flavor enhancer in ice cream, candy, baked goods, and nonalcoholic beverages. Dermal exposure to Otto Fuel II and its components is likely through contact with the fuel. Limited information was located regarding the degradation of the components in the environment, but the available data indicate that degradation would occur fairly rapidly (i.e., within days).

Humans are most likely to be exposed to Otto Fuel II or its components in areas where it is used as a torpedo fuel or where it is manufactured.

Toxicokinetics

There is evidence that the only volatile compound of Otto Fuel II, propylene glycol dinitrate, is absorbed following exposure. Also, there is evidence for dermal or oral absorption from toxicity studies but no definitive toxicokinetic work has been done.

The predominant metabolite of propylene glycol dinitrate was nitrate with propylene glycol 2-mononitrate. Information regarding the metabolism of the other two components is not available.

Animals given propylene glycol dinitrate subcutaneously rapidly excreted unmetabolized propylene glycol dinitrate and its metabolites in urine. Inorganic nitrate was the major metabolite excreted in urine, accounting for ~56% of the nitrate in the injected dose.

Mechanism of Toxicity

The mechanism of toxicity for Otto Fuel II is related to its major component, propylene glycol dinitrate. Propylene glycol dinitrate is an organic nitrate and shares many of the cardiovascular properties of therapeutic nitrates such as nitroglycerin for its vasodilating capacity. One of the earliest consequences of overexposure to propylene glycol dinitrate (or to Otto Fuel II) is a vasodilation of the cerebral vessels,

which is believed to be the major factor in the development of the typical 'trinitrotoluene' headache. Should the overexposure be more severe, the relaxation of the vascular smooth muscle can result in a fall in blood pressure followed by a compensatory vasoconstriction. However, a decrease in the magnitude of the vasodilating effect has been observed after repeated exposure to organic nitrates. Although the exact mechanism of initiation and maintenance of tolerance to organic nitrates is not known, several possibilities have been suggested, including depletion of sulfhydryl groups at the receptor sites, reduced availability or activity of the active intermediate *S*-nitrosothiol, and altered pharmacokinetics leading to decreased nitrate concentration in vascular tissues. Massive overexposure to propylene glycol dinitrate can produce toxic levels of methemoglobin. This property is shared by many organic and inorganic nitrates and also by aromatic amines, 2-nitrodiphenylamine among them.

Acute and Short-Term Toxicity (or Exposure)

Animal

An oral LD_{50} value of 2000 mg kg^{-1} in rats has been reported for Otto Fuel II. The oral LD_{50}s for the components of Otto Fuel II indicate relatively low toxicity. Oral LD_{50} values ranging from 250 to $\sim$2000 mg kg^{-1} have been reported for propylene glycol dinitrate in rats.

Exposure to propylene glycol dinitrate at concentrations up to 200 ppm for 4 h was tolerated in rats without signs of toxicity. Rats, guinea pigs, and dogs exposed continuously for 90 days at 35 ppm showed no treatment-related deaths.

Elevated methemoglobin levels were observed in a number of acute-, intermediate-, and chronic-duration exposure studies in laboratory animals exposed to propylene glycol dinitrate.

Continuous (24 h day^{-1}) exposure of rats, guinea pigs, dogs, and monkeys to propylene glycol dinitrate for 90 days resulted in elevated methemoglobin levels during exposure and histopathologic evidence of hemolysis in all four species. At concentrations as low as 10 ppm, hemosiderin deposits (indicating phagocytosis of oxidized hemoglobin released from hemolyzed red cells) were observed in kidneys and livers from dogs and in kidneys from some rats. At 16 ppm, hemosiderin deposits were observed in the liver of dogs and monkeys; at 35 ppm, in addition to the liver and kidneys, heavy hemosiderin deposits were observed in the spleens of these animals. At 35 ppm, all four species exhibited elevated methemoglobin levels.

Degenerative changes in the liver and kidneys were also observed in rats, guinea pigs, dogs, and monkeys exposed to propylene glycol dinitrate for 90 days.

Although no information was located regarding reproductive performance of Otto Fuel II or its individual components, limited data are available regarding effects of propylene glycol dinitrate on the gross and microscopic structure of reproductive organs and/or revealed no treatment-related effects.

Human

Exposure to Otto Fuel II is likely to cause eye and respiratory irritation in humans exposed to significant levels. In addition, headaches of presumed vascular origin are possible based on experience by torpedo maintenance workers. In experimental exposure to volunteers to propylene glycol dinitrate vapor, headaches were reported by some subjects at concentrations of 0.2 ppm for up to 8 h.

Studies designed to assess the neurological effects of Otto Fuel II and its component, propylene glycol dinitrate, in humans have indicated that an alteration of central nervous system activity may result from occupational exposures. In one study, workers were given tests of balance and oculomotor performance before and after a torpedo maintenance procedure. The maintenance procedures were $\sim$30–60 min in duration, and propylene glycol dinitrate concentrations measured in the work area ranged from 0 to 0.22 ppm. Subjects exposed to 0.2 ppm propylene glycol dinitrate for 1–8 h were observed to have altered visual evoked responses. With repeated 7.5–8 h exposures to 0.2 ppm, the change in the visual evoked response was observed to increase in magnitude indicating a cumulative effect. Exposure to 0.5 ppm for 8 h resulted in nausea, dizziness, and more markedly altered visual evoked responses. At the highest concentration tested, 1.5 ppm, subjects experienced coordination deficits and altered visual evoked responses and coordination.

Chronic Toxicity (or Exposure)

Animal

No chronic studies on Otto Fuel II are available. There is insufficient information to conclude whether or not Otto Fuel II or its components are carcinogenic.

In Vitro Toxicity Data

The US Navy concluded that Otto Fuel II assayed at toxic levels did not cause a significant increase in the

frequency of sister chromatid exchange in mouse lymphoma cells in the presence or absence of rat liver microsomes (S9). However, in the mouse lymphoma cell forward mutation assay, increased mutation frequencies and the number of mutant colonies were observed, indicating that at severely cytotoxic levels, the US Navy concluded that Otto Fuel II was mutagenic in the mammalian cell line. Otto Fuel II was not mutagenic in *Saccharomyces cerevisiae* D4 and several histidine-requiring mutant strains of *Salmonella typhimurium*.

Clinical Management

Based on currently available information, the constituent of Otto Fuel II that presents the main health concern is propylene glycol dinitrate. Exposure to propylene glycol dinitrate occurs primarily by inhalation or through dermal absorption. In an acute exposure situation, general recommendations include removing the exposed person from the source of exposure. Dermal absorption may be reduced by removing contaminated clothing, blotting any excess liquid material on the skin with an absorbent material, and washing the skin with copious amounts of water and mild soap. Contaminated eyes should be flushed with water or normal saline. If ingestion QJ;of Otto Fuel II or propylene glycol dinitrate has occurred, absorption from the gastrointestinal tract may be limited by administering water or milk for dilution and activated charcoal to adsorb the material.

Environmental Fate

The limited data located on the environmental fate of Otto Fuel II components indicate that propylene glycol dinitrate is removed from water primarily by volatilization. Neither 2-nitrodiphenylamine nor dibutyl sebacate is volatile or soluble enough for the partitioning to air or water to be important fate processes. The data on biodegradation of propylene glycol dinitrate and 2-nitrodiphenylamine are mixed. Some experiments indicate these compounds are readily degraded and others indicate limited biodegradation. A bioconcentration factor has been calculated only for 2-nitrodiphenylamine. It indicates that this chemical does not bioconcentrate in aquatic organisms or biomagnify in the food chain.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Otto Fuel II and Its Components.

Oxalates

Eric M Silberhorn

- Representative Chemicals: Oxalic acid; Calcium oxalate; Sodium oxalate
- Chemical Abstracts Service Registry Number: Oxalic acid (CAS 144-62-7)
- Synonyms: Ethanedioic acid; Ethane-1,2-dioic acid
- Chemical Formula: $C_2H_2O_4$

Uses

Oxalic acid is used in the manufacture of dyes, inks, bleaches, paint removers, varnishes, wood and metal cleaners, dextrin, cream of tartar, celluloid, tartaric acid, purified methyl alcohol, glycerol, and stable hydrogen cyanide. It is used in the following industries: photographic, ceramic, metallurgic, rubber, leather, engraving, pharmaceutical, paper, and lithographic.

Background Information

Oxalates, typically in the form of calcium oxalate, are present in a variety of common poisonous plants including caladium (*Caladium* spp.), dumbcane (*Dieffenbachia* spp.), elephant's ear (*Colocasia* spp.), Jack-in-the-pulpit (*Arisaema triphyllum*), *Philodendrum*, pothos (*Scindapsus aureus*), rhubarb (*Rheum rhaponticum*), sorrel (*Rumex crispus*), and skunk cabbage (*Symplocarpus foetidus*). It occurs in plants in a partly insoluble form as acid oxalate and free oxalic acid, and in a partly insoluble form as calcium oxalate. When present, oxalates usually occur in all parts of the plant, although in rhubarb they are found primarily in the leaves and much lower in the stalks, which are therefore edible. Cooking does not make rhubarb leaves edible.

Oxalic acid and its salts (i.e., calcium, sodium) are a product of normal human metabolism. Calcium oxalate is the major constituent of kidney stones. Oxalates may also be produced by several common molds. For example, *Penicillium* and *Aspergillus*

molds can convert sugar into calcium oxalate at very high yields.

Exposure Routes and Pathways

Exposure to oxalates may occur through consumption of certain plants and foods in which they are naturally present (see above). Exposure may also occur through contact with, or inhalation of, commercial products (e.g., bleaches, cleaners) or as a result of accidental ingestion or contact with some commercial antifreeze products that contain ethylene glycol, which is metabolized *in vivo* to oxalates.

Toxicokinetics

Oxalic acid is poorly absorbed with a bioavailability of 2–5%. It is excreted unchanged in the urine. Normal urinary oxalic acid excretion ranges from 8 to 40 mg day^{-1}.

The soluble oxalates found in rhubarb and most other poisonous plants are readily absorbed from the gastrointestinal tract and lead to systemic formation of calcium oxalate.

Oxalic acid, and ultimately calcium oxalate, may also be formed *in vivo* as a result of the normal metabolism and biotransformation of ethylene glycol and several other compounds (e.g., ascorbic acid, glycerol, xylitol, glycolaldehyde, glycolic acid, glycoxylic acid).

Mechanism of Toxicity

Oxalic acid may have a direct corrosive effect on the eyes, skin, and digestive tract after contact. However, once absorbed (or produced as a result of the metabolism of other compounds), oxalic acid and other soluble oxalates react with calcium in the plasma to form insoluble calcium oxalate. Systemic formation of calcium oxalate may produce hypocalcemia directly. Precipitation of calcium oxalate in the renal system (proximal tubules of the kidney) may lead to local necrosis of the tubular epithelium, producing kidney dysfunction and electrolyte imbalance. Precipitation of calcium oxalate may also occur in the blood vessels, heart, lungs, and liver leading to local effects.

Acute and Short-Term Toxicity (or Exposure)

Animal

The toxic dose of oxalic acid to the dog and cat is ~1 and 0.2 g, respectively.

Human

The oral lethal dose of oxalic acid for adults is 15–30 g, although the ingestion of as little as 5 g has caused death.

Oxalic acid exposure typically produces immediate irritation and local effects on the skin, eyes, and mucosal membranes of the gastrointestinal tract (if ingested) or respiratory tract (if inhaled). Slightly delayed effects may occur on the respiratory system and kidneys.

Early symptoms of rhubarb and other plant poisonings may include mucosal irritation of the gastrointestinal tract, including signs such as sore throat, nausea, vomiting, anorexia, diarrhea, and abdominal pain; however, these symptoms are not always present in rhubarb poisoning. Kidney dysfunction and electrolyte imbalance occur subsequently and may be severe enough to cause death. In severe cases, anuria, oliguria, proteinuria, hematuria, and oxaluria are present. Hypocalcemia may produce paresthesias, tetany, hyper-reflexia, muscle twitches, and muscle cramps.

Chronic Toxicity (or Exposure)

Animal

A reproduction and fertility assessment of oxalic acid in Swiss CD-1 mice has been conducted with administration through drinking water (0.05%, 0.10%, and 0.20%; equivalent to ~89, 162, and 275 mg kg^{-1} day^{-1}). In a preliminary dose-range-finding study, water consumption was reduced in the middle and high dose groups by ~25%, but produced no adverse clinical signs in adults. In definitive studies, effects on reproductive parameters (e.g., live pups/litter, pup weight, abnormal sperm forms) were found at the 0.2% exposure level in both F_0 and F_1 mice, leading the investigators to conclude that oxalic acid is a reproductive toxicant at concentrations that reduce parental water consumption, but that cause few other somatic effects.

Human

Chronic occupational exposure to oxalic acid fumes has been associated with headache, vomiting, pain of the lower back, anemia, and fatigue. Chronic exposure may also lead to chronic inflammation of the upper respiratory tract and hypocalcemia. Prolonged contact of oxalic acid with the hands or feet may produce dermatitis, localized pain, cyanosis, and possibly gangrenous changes as a result of localized vascular damage.

In Vitro Toxicity Data

Sodium oxalate acts as a uremic toxin, inhibiting endothelial cell replication and migration *in vitro* at concentrations greater than $30\,\mu mol\,l^{-1}$. The inhibitory effect was fully reversible upon removal of oxalate, but only if exposure was limited to 5 days or less. In *Salmonella* mutagenicity testing using the standard National Toxicology Program (NTP) protocol, oxalic acid produced negative results.

Clinical Management

For exposure to oxalic acid, the exposed area should be decontaminated and tissue injury treated as for other strong acids. If on skin or in eyes, there should be thorough rinsing with water for at least 15 min. If ingested, emesis should not be initiated. Rather immediately there should be dilution with milk or water and calcium gluconate or lactate administered ($150\,mg\,kg^{-1}$ orally). For dilute oxalic acid ingestions, activated charcoal should be administered (adult: 60–100 g; child: 30–60 g). This may not be advisable in concentrated ingestions due to possible necessity for endoscopy. An electrocardiogram and serum calcium monitoring are required. Renal failure may require hemodialysis.

For ingestion of oxalates from plants, treat similarly as for oxalic acid.

Environmental Fate

Oxalic acid will readily degrade in aquatic ecosystems and is expected to also degrade in soil. Under typical environmental conditions (pH 5–9), oxalic acid will exist as the oxalate ion in soil and water (pK_{a1} and pK_{a2} values are 1.25 and 4.28, respectively).

Exposure Standards and Guidelines

The Occupational Safety and Health Administration permissible exposure limit, time-weighted average (PEL–TWA) and short-term exposure limit (STEL) for oxalic acid in air are 1 and $5\,mg\,m^{-3}$, respectively. The American Conference of Governmental Industrial Hygienists PEL–TWA and STEL for oxalic acid in air are also 1 and $5\,mg\,m^{-3}$, respectively. The National Institute for Occupational Safety and Health IDHL (immediate danger to life or health) is $500\,mg\,m^{-3}$.

See also: Ethylene Glycol; Kidney; Plants, Poisonous.

Further Reading

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Oxidative Stress

Kartik Shankar and Harihara M Mehendale

Cellular Events Leading to Oxidative Stress

Oxygen is critical to sustenance of life in all anaerobes and higher organisms. However, catalytic reactions involving oxygen can also result in the formation of several free radicals. Formation of the superoxide ($O_2^{\bullet}$) and the hydroxyl ($^{\bullet}OH$) radicals normally occurs during various biochemical processes, which are duly kept in check by cellular mechanisms that are designed to quench free radicals. However, certain toxicants cause a perturbation in this balance by either causing an increase in the formation of oxidative free radicals (the bipyridyl herbicide, diquat) or inhibition of the cellular antioxidant defense mechanisms (buthionine sulfoxime). Others like the well-known model hepatotoxin carbon tetrachloride, after being bioactivated to a reactive trichloromethyl free radical initiate a 'run-away' process of peroxidation of cellular membrane lipids.

Several enzyme systems are also responsible for producing oxygen free radicals. Several examples of reactions catalyzed by the P450 and flavin monooxygenases lead to the generation of reactive oxygen species (ROS). Microsomal P450s are capable of undergoing futile cycling in the absence of substrate to produce ROS. The cytochrome P450, CYP2E1 is notorious in this regard and has been described as a 'leaky' enzyme. CYP2E1, and from recent reports CYP4A enzymes are a major source of hydrogen peroxide and NADPH-dependent lipid peroxidation. Other enzyme systems also produce oxidative reactive intermediates: the cyclooxygeneases, nitric oxide synthases, and prostaglandin synthases.

Cellular Defenses against Oxidative Stress

Since several processes can potentially result in a prooxidant state detrimental to cellular homeostasis, cells have developed a wide range of antioxidant defense mechanisms to mitigate oxyradicals. Cellular glutathione is one of the most ubiquitous of these antioxidant mechanisms. Glutathione is a tripeptide (L-γ-glutamyl-L-cysteinyl-glycine) that is present in varying concentrations (0.5–10 mM) in different cell types. Most of the cellular glutathione exists in the reduced form (GSH, ~95%) while less than 5% is present as oxidized gluthathione disulfide (GSSG). Four enzymes are critical in maintaining appropriate levels of GSH in the cell. Gammaglutamyl cysteine is the enzyme that transfers a cysteine moiety in the final and rate-controlling step of GSH biosynthesis. Formation of glutathione conjugates of electrophilic compounds is mediated by a glutathione-*S*-transferases, a superfamily of enzymes that are distributed in both the cytosol and microsomal compartments of cells. The reduced glutathione pool in the cell can be oxidized via glutathione peroxidase, an enzyme that releases two molecules of hydrogen peroxide in the process. Conversely, GSSG can be 'regenerated' to the reduced form by glutathione reductase using NADPH as the proton donor.

Superoxide dismutases are enzymes that catalyze the dismutation of superoxide radicals to hydrogen peroxide and molecular oxygen. Superoxide dismutases either have Cu^{2+} and Zn^{2+} (cytosolic isoform) or Mn^{2+} (mitochondrial isoform). Reduction of hydrogen peroxides in cells is accomplished via catalases to water and oxygen. Cellular organelles called peroxisomes contain large quantities of catalase. In addition, NADPH:quinone oxidoreductases (DT-diaphorase) also act as antioxidant enzymes by catalyzing two-electron reduction of quinones.

Oxidative (Redox) Status as a Signaling Event

Since an extreme pro-oxidant status of a cell can lead to a progressive loss of cellular function and eventual death, cells have developed mechanisms to detect changes in the redox status of cells. These 'switches' are used to regulate and turn on the antioxidant machinery of the cell. These signals are largely mediated by redox-sensitive transcription factors. Although a large number of very extensively studied transcription factors are now know to respond to redox status, nuclear factor-kappaB (NF-κB) deserves special mention. NF-κB usually resides in the cytoplasm bound to an inhibitor protein, IκB. Changes in redox status of a cell (increase in hydrogen peroxide) will activate enzymes like MAPK kinases, which will phosphorlyate and hence cleave the inhibitory protein from NF-κB. The free NF-κB now translocates into the nucleus and binds to particular DNA sequences on specific genes called consensus sequences. Binding of NF-κB to response elements on genes alters the transcription of those genes. Several genes including other transcription factors, which may exert a similar transcriptional control over gene expression, are also induced or repressed; hence creating a cascade of signaling events. Several genes regulated by NF-κB are involved in upregulating antioxidant defense mechanisms, including catalase. NF-κB also exerts significant effects on other signaling events that decide cell proliferation and survival. It must be noted that NF-κB is only one of numerous transcription factors regulated by redox status. AP-1 and nrf2 are other notable transcription factors.

Endogenous and Therapeutic Antioxidants

Antioxidant compounds have a useful place in preventing or reducing peroxidative damage to cellular macromolecules either due to an offending xenobiotic or altered pathological state (diabetes). Several vitamins, both lipid and water soluble, possess antioxidant properties. Ascorbic acid (vitamin C) is a water-soluble compound capable of one-electron reduction of several free radicals. Vitamin E and other related tocopherols, on the other hand, are lipid soluble and effectively prevent peroxidation of polyunsaturated lipids present in membranes. Carotenoids such as β-carotene (vitamin A) inactivate singlet oxygen molecules. Other synthetic compounds such as promethazine, diethyldithiocarbamate, ubiquinol, butylated hydroxyanisole, *N*-acetylcysteine (NAC), among others have shown to protect against oxidative and reactive intermediate injury in experimental studies. Indeed, NAC is the standard treatment for liver injury in humans following toxic ingestion of acetaminophen. The mechanism of NAC action is thought to be a precursor for increasing GSH stores and prevents reactive intermediate induced damage. Recent studies also suggest that improving status via GSH may also improve prognosis via enhancing liver tissue repair.

See also: Ascorbic Acid; Cytochrome P-450; Diquat; Glutathione; Mechanisms of Toxicity.

Further Reading

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Oxygen

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 7782-44-7
- Synonym: LOX
- Chemical/Pharmaceutical/Other Class: Elemental gas
- Chemical Formula: O_2
- Chemical Structure: O═O

Uses

Oxygen produces flame for welding and lighting. It is also used in the production of fuels. Therapeutically, it is used to relieve hypoxia and as a component of the gas mixture for respiratory support.

Background Information

Oxygen is essential for life in appropriate ranges of pressure and concentration. Normal air consists of 20.04% oxygen.

Exposure Routes and Pathways

Exposure route is inhalation. Industrial exposures to high oxygen pressure are uncommon. Caison workers and tunnel makers may be exposed to pressures high enough to cause lung damage. Some potential risks for intoxication with oxygen also exist for drivers and persons living or working in closed compartments, where the air is reconditioned by the addition of pure oxygen (e.g., submarines and spacecraft), should the regulation system malfunction.

Toxicokinetics

Oxygen is absorbed almost entirely through the lungs, but may be taken up through mucous membranes of the gastrointestinal tract, the middle ear, and the accessory sinuses. It diffuses through the lining of the lung alveoli into the blood capillaries, is dissolved in the blood plasma, diffuses into the red blood cells, and is bound to the hemoglobin that they contain. Toxicity occurs at elevated pressures (e.g., deep sea diving). The latent period is 2 h at 3 atm and 30 min at 4 atm. It rapidly equilibrates with external atmosphere.

Mechanism of Toxicity

The partial reduction of molecular oxygen in biological systems produces the cytotoxic intermediates superoxide, hydrogen peroxide, and hydroxyl radical. The superoxide radical plays a significant role in a number of pathophysiologic states including oxygen toxicity, radiation damage, phagocyte-mediated inflammation, and postischemic injury. Oxygen radical scavengers such as superoxide dismutase and catalase protect the body against normal levels of oxygen-free radicals.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animal studies have shown the importance of considering potential chemical interactions in the production of oxygen-related toxicity. For example, oxygen tolerance can occur in mice with pre-exposure to one concentration of oxygen mitigating later exposure to 100% oxygen by modifying cellular and enzymatic composition of the lung. Further, damage of the alveolar zone in mice by the antioxidant butylated hydroxytoluene (BHT) can be greatly enhanced by subsequent exposure to oxygen concentration which, otherwise, would have little if any demonstrable effect. The synergistic interaction between BHT and oxygen results in a resulting interstitial pulmonary fibrosis. Based on these types of data, it can be seen that acute or chronic lung disease may be caused not only by one agent, but very likely in many instances by the interaction of several agents.

Human

Convulsions have occurred in man after oxygen has been breathed for 45 min at 4 atm; after 1–3 h at 1 atm, neuromuscular coordination and power of attentions were adversely affected. To study the early changes in the lower respiratory tract in persons exposed to hyperoxia usually considered safe, normal subjects were evaluated by bronchoalveolar lavage before and immediately after ~17 h of breathing more than 95% oxygen. A significant alveolar-capillary 'leak' was observed, as detected by the presence of increased plasma albumin and transferrin in lavage fluid. Some of the effects of exposure to 17 h of more than 95% oxygen are reversible; however, hyperoxia for this length of time lowers the structural or functional barriers that normally prevent alveolar-capillary 'leak' and induces processes that can culminate in fibrosis of the alveolar wall.

Chronic Toxicity (or Exposure)

Animal

Dogs inhaling 100% oxygen at atmospheric pressure had adverse effects beginning after 36 h, with death in 60 h. Inhaling 90% oxygen required double the exposure time for similar results. Animal studies have found eye effects similar to those noted below from prolonged exposure to a high concentration of oxygen.

Human

Concentrations of over 60% cause respiratory/pulmonary irritation, reduce vital capacity, and cause substernal distress. Oxygen poisoning causes nervousness, muscular twitch, hilarity, convulsions, or unconsciousness. Severe retinal damage in adults is rare during hyperoxia; however, one case was an individual with myasthenia gravis who developed irreversible retinal atrophy after breathing 80% oxygen for 150 days. The retinal vasculature was markedly constricted with no blood flowing through both eyes.

In Vitro Toxicity Data

Hyperoxia was toxic to cultured human pulmonary endothelial cells, with impairment of replicative function (expressed as growth impairment index), monitored by cell number determination and tritiated thymidine incorporation.

Clinical Management

In cases of pulmonary irritation, the oxygen concentration should be reduced to 60% or less. With oxygen poisoning, the oxygen concentration should be reduced to 200 mm kg^{-1}.

Exposure Standards and Guidelines

The human TC_{Lo} is 100 ppm per 14 h exposure.

See also: Ozone; Respiratory Tract.

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Oxygenates *See* Fuel Oxygenates.

Ozone

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 10028-15-6
- Synonym: Triatomic oxygen
- Chemical/Pharmaceutical/Other Class: Elemental gas
- Chemical Formula: O_3

Uses

Ozone is used for water treatment for taste and odor control, in the deodorization of air and sewage gases, as a mold and bacteria inhibitor in cold storage, in the oxidation of furnace carbon black for ink black manufacture, for bleaching flour, oils, paper pulp, starch, sugar, textiles, and waxes, for aging liquor and wood, and for processing some perfumes, vanillin, and camphor. It is also used in the treatment of industrial wastes, in the rapid drying of varnishes and printing inks, and in the deodorizing of feathers.

Exposure Routes and Pathways

Inhalation and exposure to mucous membranes are possible exposure routes. Ozone is formed locally in air from lightning and equipment such as photocopiers and residential electronic air cleaners, and in the outer layers of the atmosphere by the action of solar ultraviolet radiation on the oxygen in the air. It is a significant component of photochemical smog, and is produced when nitrogen oxides and hydrocarbons from motor vehicle emissions and other sources react with oxygen and sunlight. Ozone also reacts with limonene from consumer products and other sources, and this leads to formation of chemicals (e.g., formaldehyde, formic acid, and autooxidation products of limonene) capable of producing sensory irritation, bronchoconstriction, and pulmonary irritation.

Toxicokinetics

Ozone rapidly oxidizes thiol-containing compounds and unsaturated fatty acids.

Mechanism of Toxicity

The biochemical mechanism of pulmonary injury is due to the formation of reactive free-radical intermediates from oxidization of thiol-containing compounds and unsaturated fatty acids. The primary site of injury is the lung, and the injury is characterized by pulmonary congestion, edema, and hemorrhage.

Acute and Short-Term Toxicity (or Exposure)

Animal

The LC_{50} in rats is 4.8 ppm. No increase in chromosomal aberration levels was seen in hamsters that inhaled ozone, although small increases in chromatid aberration levels were seen. Pregnant rats exposed to up to 1.97 ppm during parts or all of organogenesis had no defects in their offspring.

Human

Short-duration exposures can lead to dryness of the throat and mucous membranes of the nose and eyes. In studies with exercising subjects, acute ozone exposure produces a variety of reversible symptoms, including cough, shortness of breath, and pain on deep inspiration. Alterations in lung function and an influx of inflammatory cells into the lungs have also been observed. Mild to moderate exposure produces upper respiratory tract symptoms and eye irritation (e.g., lacrimation, burning of the eyes and throat, nonproductive cough, headache, substernal soreness, bronchial irritation, and an acrid taste and smell). More severe exposures, such as that seen in an industrial setting, may produce significant respiratory distress with dyspnea, cyanosis, and pulmonary edema. Chest X-rays show increased bronchovascular markings and bilateral lung densities. Symptoms resolve over 1–2 weeks although fatigue, headache, and exertional dyspnea may persist for several months. Ozone may exacerbate the small airway impairment of smoking adults.

Chronic Toxicity (or Exposure)

Animal

Ozone produces cell injury and connective tissue alterations in the lungs. Rats exposed for 6 h day^{-1} to a combination of 0.8 ppm of ozone and 14.4 ppm of nitrogen dioxide began to demonstrate respiratory insufficiency and severe weight loss $\sim$7–10 weeks after the initiation of exposure. About half of the rats died between days 55 and 78 of exposure; no overt ill effects were observed in animals exposed to filtered air, to ozone alone, or to nitrogen dioxide. The biochemical findings (e.g., increased lung content of DNA, protein, and collagen) were consistent with extensive breakdown and remodeling of the lung parenchyma and its associated vasculature. Histopathologic evaluation showed severe fibrosis, alveolar collapse, honeycombing, macrophage and mast cell accumulation, vascular smooth muscle hypertrophy, and other indications of severe progressive interstitial pulmonary fibrosis and end-stage lung disease. This animal model of progressive pulmonary fibrosis was judged to resemble the final stages of human idiopathic pulmonary fibrosis.

In 2 year and lifetime inhalation studies, there was no evidence of carcinogenic activity of ozone in male or female rats exposed to 0.12, 0.5, or 1.0 ppm. There was equivocal evidence of carcinogenic activity of ozone in male mice based on increased incidences of alveolar/bronchiolar adenoma or carcinoma. There was some evidence of

carcinogenic activity of ozone in female mice based on increased incidences of alveolar/bronchiolar adenoma or carcinoma. However, oxygen and ozone both have been found to enhance or to inhibit the development of tumors in mouse lung under various exposure conditions. As a general rule, preexposure to the oxidant, before administration of a carcinogen, or exposure to high levels for a comparatively short time immediately following carcinogen administration favors development of tumors. On the other hand, prolonged exposure begun after a certain time following carcinogen exposure inhibits tumor development. The paradoxical effects of the two oxidants depend on experimental design; results can be tentatively explained in terms of oxidant-induced cell proliferation or by oxidant-mediated cytotoxicity.

Human

Ozone can aggravate asthma and increase susceptibility to respiratory diseases such as pneumonia and bronchitis. The TC_{Lo} is 50 ppm. Pulmonary symptoms at low levels (60–200 ppm) include substernal pain, cough, dry throat, wheezing, and dyspnea. The American Conference of Governmental Industrial Hygienists (ACGIH) lists ozone as A4 (not classifiable as a human carcinogen).

In Vitro Toxicity Data

In a study of *in vitro* transformation, ozone (6 ppm for 10 min) acted in an additive fashion with ultraviolet light (4 J m^2) to produce enhanced levels of transformation in hamster embryo cells and mouse C3H/10T-1/2 cells. Mouse C3H10T1/2 cells were exposed to 5 or 1 ppm ozone for 5 min. Some of the cell cultures were exposed to gamma rays immediately before or after ozone treatment. Following 6 weeks in culture, transformation was scored using morphological criteria. Ozone (at 5 ppm) and radiation acted as independent carcinogens, and when the cells were first exposed to radiation, transformation was enhanced in a synergistic manner.

Clinical Management

The victim should be removed from exposure and monitored for respiratory distress.

Ecotoxicology

Ground-level ozone interferes with the ability of plants to produce and store food. This makes them more susceptible to disease, insects, other pollutants, and harsh weather. Ozone damages the leaves of trees and other plants. Ozone and the chemicals that react to form it can be carried long distances from their origins, thus causing air pollution over wide regions.

Exposure Standards and Guidelines

The ACGIH threshold limit value, 8 h time-weighted average (TWA), is 0.05 ppm for heavy work, 0.08 ppm for moderate work, and 0.1 ppm for light work; 0.20 ppm is the level for heavy, moderate, or light workloads of $\leqslant$2 h. The (US) Occupational Safety and Health Administration permissible exposure limit, 8 h TWA, is 0.1 ppm. The (US) National Institute for Occupational Safety and Health (NIOSH) recommended exposure ceiling value is 0.1 ppm, and the NIOSH immediately dangerous to life or health value is 5 ppm. In 1997, the US Environmental Protection Agency (EPA) announced new stricter national ambient air quality standards for ground-level ozone, the primary constituent of smog. After a lengthy scientific review process, including extensive external scientific review, EPA determined that these changes were necessary to protect public health and the environment. The new standard is intended to be more protective of the health of children and adults who play and work outdoors in the summer. In establishing the 8 h standard, EPA set the standard at 0.08 ppm as an average over an 8 h period. Areas in the United States would have until 2010 to meet the new standard.

See also: Pollution, Air; Pollution, Air Indoor; Pollution, Water.

Further Reading

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Leikauf GD and Prews DR (2001) Ozone. In: Bingham E, Cohrssen B, and Powell CH (eds.) *Patty's Toxicology*, 5th edn., vol. 3, pp. 676–688. New York: Wiley.

Uysal N and Schapira RM (2003) Effects of ozone on lung function and lung diseases. *Current Opinion in Pulmonary Medicine* 9: 144–150.

Relevant Website

http://www.oma.org – OMA Ground Level Ozone Position paper (from the Ontario Medical Association).

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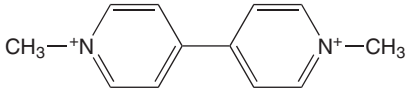
Paraoxon Detoxification *See* A-Esterases.

Paraquat

Kevin N Baer

- Chemical Abstracts Service Registry Number: CAS 1910-42-5 (dichloride)
- Synonyms: Paraquat dichloride; Methyl viologen; 1,1′-Dimethyl-4,4′-bipyridinium ion; Gramoxone
- Chemical/Pharmaceutical/Other Class: Quaternary nitrogen (bis-pyridinium) compound
- Chemical Structure:



Uses

Paraquat is used as a broad-spectrum herbicide on weeds and grasses in agricultural and nonagricultural areas. It is used as a desiccant on tomatoes, cotton, beans, soybeans, potatoes, sunflowers, and sugar cane to aid in harvesting and to induce resin soaking on pine trees.

Exposure Routes and Pathways

Accidental or intentional ingestion is the most common route of exposure. Poisonings from inhalation and dermal exposure have also occurred.

Toxicokinetics

Paraquat has low but rapid gastrointestinal absorption (5–10%) and low skin absorption. Peak plasma concentrations occur in less than 2 h following ingestion. Generally, paraquat is not metabolized to any large extent. In animal studies, metabolites have been detected in urine, possibly resulting from the action of intestinal microflora. Paraquat is actively transported to alveolar cells, where it is reduced to form highly reactive free radicals. The volume of distribution is large and has been estimated at $2.75\,l\,kg^{-1}$. Paraquat tends to attain higher and more prolonged levels in the lung. Clearance is rapid by the kidneys with 80–90% of the dose excreted in the urine after 6 h. The terminal half-life increases from 12 to 120 h or longer as renal failure begins.

Mechanism of Toxicity

Paraquat produces lung damage by all routes of exposure. Progressive and generalized proliferation of fibrous connective tissue is observed in the pulmonary alveoli where paraquat is selectively concentrated. The mechanisms of action result from a metabolically catalyzed single electron oxidation/reduction reaction resulting in NADPH depletion and the generation of oxygen free radicals. For example, superoxide radicals are formed and attack unsaturated lipids of cell membranes. This in turn produces lipid free radicals, resulting in membrane damage and loss of the functional integrity of the cell. In some animal models, paraquat leads to damage of dopaminergic cells in the subtantia nigra similar to that seen in Parkinson's disease.

Acute and Short-Term Toxicity (or Exposure)

Animal

Paraquat is highly toxic by inhalation. Particle sizes used in agricultural practices (400–800 μm) limits lower airway deposition, thereby lessening inhalation hazard. Paraquat is moderately toxic by the oral route and only slightly toxic by the dermal route. Paraquat causes moderate to severe eye irritation and minimal dermal irritation. The oral and dermal LD_{50}

values reported in rats and mice range from about 20 to 150 mg kg^{-1}.

Human

Paraquat may result in severe toxicity to all organ systems and death within 24 h after ingestion, inhalation, and dermal exposure. The initial symptoms after ingestion are burning in the mouth and throat with vomiting and diarrhea and subsequent fluid and electrolyte loss. Depending on the dose (>60 ml), esophageal perforation, renal failure, cardiac arrhythmias, convulsions, and coma can occur. Early death is usually due to hepatic and renal toxicities. The lethal dose in humans is estimated to be ~40 mg kg^{-1}.

Chronic Toxicity (or Exposure)

Animal

Subchronic exposure to paraquat led to pulmonary damage. A rabbit dermal toxicity study noted scabbing and inflammation when tested at the two highest doses (2.6 and 6.0 mg kg^{-1} group). Inhalation of small particles (<2μm in diameter) resulted in pulmonary changes and lesions in the larynx. Chronic exposure in dogs led to chronic pneumonitis. Paraquat did not appear to be carcinogenic in two long-term studies in rats and in mice. In developmental toxicity studies, paraquat caused delayed or retarded ossification in the forelimb and hindlimb digits and posterior portion of the skullat maternally toxic dosages only. Paraquat does not appear to influence reproduction.

Human

Survivors of the initial poisoning or from poisonings from as little as 10–15 ml of the concentrate often develop a progressive pulmonary fibrosis associated with dyspnea and pulmonary edema several days or weeks after exposure. As a result, death is due to asphyxia.

In Vitro Toxicity Data

Paraquat did not demonstrate mutagenic capacity.

Clinical Management

No specific treatment is known. All cases of paraquat exposure should be managed as a potentially fatal exposure. Basic life-support measures should be instituted; however, the administration of supplemental oxygen is not advised. Treatment must be instituted early, within 10 h after ingestion. Treatment involves removal of paraquat from the alimentary tract by gastric lavage and cathartics, prevention of further absorption by Fuller's earth (30% w/v), and removal of absorbed paraquat by hemodialysis or hemoperfusion. Use of various drugs, such as D-propranolol, prednisone, and vitamins E and C, has provided little benefit.

Signs of toxicity in animals are similar to those in humans. Paraquat has been shown to be mutagenic, carcinogenic, and teratogenic in experimental animals.

Environmental Fate

Paraquat is relatively immobile in soil. Paraquat resists hydrolysis, photodegradation in water, and microbial degradation under both aerobic and anaerobic conditions. Dissipation is primarily by adsorption to organic material and clay particles. As paraquat persists in clay soils, it may be found in surface water from erosion. Since it binds so strongly to clay particles, paraquat is not generally a groundwater concern.

Ecotoxicology

Paraquat is practically nontoxic to honey bees, only slightly toxic to fish, and moderately toxic to terrestrial animals. Hazard for birds and mammals is generally short lived after paraquat application.

Exposure Standards and Guidelines

The reference dose for paraquat is 0.0045 mg kg^{-1} day^{-1}. The acceptable daily intake for paraquat is 0.004 mg kg^{-1} day^{-1} while the threshold limit value is 0.1 mg m^{-3}.

See also: Lipid Peroxidation; Pesticides; Pollution, Water; Respiratory Tract.

Further Reading

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Lock EA and Wilks MF (2001) Paraquat. In: Krieger R (ed.) *Handbook of Pesticide Toxicology*, 2nd edn., pp. 1559–1604. San Diego: Academic Press.

Parathion

Jason R Richardson

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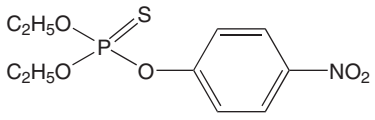
- Chemical Abstracts Service Registry Number: CAS 56-38-2
- Synonyms: Bayer E-605; Corthion; *O*,*O*-Diethyl-*O*-(*p*-nitrophenyl) phosphorothioate; Diethyl 4-nitrophenyl phosphorothionate; DNTP; DDPP; Ethyl parathion; AC 3422; Alkron; Alleron; Aphamite; Corothion; E-605; ENT 15108; Etilon; Fosferno 50; Niran; Nitrostigine; Orthophos; Panthion; Paramar; Paraphos; Parathene; Parawet; Pethion; Phoskil; Rhodiatox; Soprathion; Stathion; Sulphos; Thiophos
- Chemical/Pharmaceutical/Other Class: Organophosphorus insecticide
- Chemical Structure:



Uses

Parathion is an effective insecticide for a range of insect pests. It has nonsystemic, contact, stomach, and fumigant actions. Because of its high acute toxicity, parathion is no longer approved for use on fruit or vegetable crops and is severely restricted in grain crop and other uses. Parathion has no approved residential use.

Exposure Routes and Pathways

Fatal poisonings have occurred after oral, dermal, and inhalation exposure to parathion. The vapor pressure of the pure compound is generally not sufficient to lead to respiratory exposure alone. However, fine dusts or aerosol preparations may result in severe poisoning through the respiratory tract.

Toxicokinetics

Parathion is efficiently absorbed through any route of exposure. Signs of toxicity due to parathion generally appear within several hours following dermal exposure. The rate of dermal absorption in rabbits was found to be $0.059\,\mu g\,min^{-1}\,cm^{-2}$. There is considerable individual variation in dermal absorption rates in animals and humans. About 0.1–2.8% of the applied compound was absorbed through skin in human volunteers. The kinetics of absorption studied in isolated perfused porcine skin flaps indicates a linear, three-compartment model of absorption.

Parathion is preferentially distributed in the liver, the kidneys, and ordinary adipose tissue. It is also concentrated to a fairly high degree in gastric and intestinal walls, thyroid, spleen, and lungs. It can cross the blood–brain barrier because of its nonpolar nature and it accumulates to a lesser extent in the central nervous system. Parathion is metabolized in the liver and other extrahepatic sites by the mixed function oxidase enzyme system to paraoxon, which is considerably more toxic than the parent compound. The conversion of parathion to paraoxon requires the presence of NADPH and oxygen. Parathion is also metabolized to *O*-ethyl phosphoric acid, phosphoric acid, and inorganic sulfate. Paraoxon is an extremely potent inhibitor of brain cholinesterase, with an IC_{50} of $18\,nmol\,l^{-1}$ in rat brain homogenates. Paraoxon is efficiently detoxified by binding to carboxylesterases and, to a much lesser extent by A-esterases.

Metabolites of parathion are exclusively eliminated through urine. However, some of the unmetabolized parent compound may also be excreted through sebum. The elimination half-life of parathion was found to be 2.1 days. It was reported that, following oral administration of parathion (1 or $2\,mg\,day^{-1}$) in humans, 60% of parathion was excreted within 4 h and 86% within 8 h in the form of *p*-nitrophenol. The rate of excretion of diethyl phosphate was found to be slower than that of *p*-nitrophenol. Following dermal exposure of 5 g of a 2% dust for 2 h, the *p*-nitrophenol concentration reached a peak level by 5 or 6 h after initial exposure. In case of dermal exposure, the rate of excretion of the metabolites of parathion increases with temperature.

Mechanism of Toxicity

The mechanism of toxicity for parathion is similar to that of chlorpyrifos. Following activation to the potent anticholinesterase paraoxon, acetylcholinesterase is inhibited within synapses and acetylcholine levels accumulate. This leads to overstimulation of cholinergic receptors of neurons, muscle cells, and end-organs culminating in cholinergic toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} for parathion is $2–30\,mg\,kg^{-1}$ in rats, $5–25\,mg\,kg^{-1}$ in mice, $8–32\,mg\,kg^{-1}$ in guinea pigs,

10 mg kg^{-1} in rabbits, 0.93 mg kg^{-1} in cats, and 3–5 mg kg^{-1} in dogs. The dermal LD_{50} is 6.8–50 mg kg^{-1} for rats, 19 mg kg^{-1} for mice, 45 mg kg^{-1} for guinea pigs, and 15 mg kg^{-1} for rabbits. The no-observed-adverse-effect level (NOAEL) based upon plasma and red-blood cell cholinesterase inhibition is 0.025 mg kg^{-1} day^{-1} in rats.

Human

The toxic effects of parathion in humans are similar to those of chlorpyrifos, although parathion is toxic at much lower levels. The lowest dose that results in toxic effects in humans has been estimated at 240 μg kg^{-1}. Parathion has the notorious distinction of being the synthetic pesticide that has killed more humans (both through accidental and intentional exposures) than any other.

Chronic Toxicity (or Exposure)

Animal

Parathion is neither mutagenic nor teratogenic. Parathion has been classified as a possible carcinogen. Parathion has been shown to produce adverse reproductive effects, although these are most likely secondary to the primary neurotoxic effects. Parathion has not been shown to cause delayed neuropathy. The lowest-observed-adverse-effect limit for parathion in rats has been determined to be 0.01 mg kg^{-1} day^{-1} in a 1 year feeding study in dogs, with no NOAEL established.

Human

Repeated or prolonged exposure to parathion can cause the same effects seen with acute exposures. In people working directly in the manufacture or application of parathion, impaired memory and concentration, disorientation, severe depressions, irritability, confusion, headache, speech difficulties, delayed reaction times, nightmares, and sleepwalking and drowsiness or insomnia have all been reported. Parathion cannot cause delayed neurotoxicity but has been reported to be associated with the intermediate syndrome.

In Vitro Toxicity Data

Paraoxon, the active metabolite of parathion, is a potent inhibitor of acetylcholinesterase, butyrylcholinesterase, and carboxylesterase, with IC_{50} values in the low- to mid-nanomolar range. Neither parathion nor paraoxon has been shown to be mutagenic when tested *in vitro*.

Clinical Management

Oral Exposure

Induction of emesis is contraindicated in the case of parathion poisoning due to the early onset of respiratory depression and seizures. Gastric lavage may be indicated if performed immediately after parathion ingestion. Activated charcoal/cathartic therapy may be adopted to retard the absorption from the gastrointestinal tract. Atropine should be administered intravenously until atropinization is achieved. In adults, 2–5 mg kg^{-1} should be administered every 10–15 min and in children 0.05 mg kg^{-1} must be given at the same frequency. Atropinization may require several hours to days depending on the severity of poisoning. 2-PAM (Pralidoxime) may be combined with atropine in case of severe poisoning (adult, 1 or 2 g intravenously at 0.5 g min^{-1}; children, 25–50 mg kg^{-1} over 5–30 min). Seizures may be treated with conventional anticonvulsants (e.g., diazepam, phenobarbital, and phenytoin).

Inhalation Exposure

The affected person should be moved immediately to fresh air and should be administered with 100% humidified supplemental oxygen with assisted ventilation.

Eye Exposure

Exposed eyes should be irrigated with copious amount of tepid water for 15 min. If irritation, photophobia, pain, or swelling persists, the patient should be admitted to a health care facility.

Dermal Exposure

The contaminated clothing should be removed and the contaminated area of the skin should be washed repeatedly with soapy water.

Environmental Fate

Parathion binds tightly to soil particles and has little or no potential for groundwater contamination. Residues of parathion may persist for days or weeks. Parathion readily undergoes photodegradation and sunlight can convert parathion into paraoxon. The breakdown of parathion in soil or water increases with alkalinity. Parathion residues on crops typically decay with a half-life of 1 day.

Ecotoxicology

Parathion is extremely toxic to birds, with LD_{50} values of 6, 3, and 2.1 mg kg^{-1} in bobwhite quail,

pigeons, and ducks, respectively. Parathion is moderately toxic to aquatic invertebrates and fish. The 96 h LC_{50}s for trout, catfish, and bluegill are 1.6, 2.7, and 0.02 mg l^{-1}. Parathion is extremely toxic to honeybees.

Exposure Standards and Guidelines

US Environmental Protection Agency has established an acute reference dose of 0.000 25 mg kg^{-1} day^{-1} and a chronic reference dose of 0.0000 33 mg kg^{-1} day^{-1} for parathion.

See also: A-Esterases; Carboxylesterases; Chlorpyrifos; Organophosphate Poisoning, Intermediate Syndrome; Organophosphates.

Further Reading

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Savolainen K (2001) Understanding the toxic actions of organophosphates. In: Krieger R (ed.) *Handbook of Pesticide Toxicology*, 2nd edn., pp. 1013–1041. San Diego, CA: Academic Press.

Relevant Website

http://www.inchem.org – International Programme on Chemical Safety.

Paregoric

Fermin Barrueto Jr.

- Chemical Abstracts Service Registry Number: CAS 8029-99-0
- Synonyms: Opium; Opium tincture; Hydrochloride of opium alkaloids, Camphorated tincture of opium
- Chemical/Pharmaceutical/Other Class: Paregoric is an opium preparation. It is composed of several alkaloids, including anhydrous morphine (9.5% or more) and smaller amounts of codeine and papaverine

Uses

Paregoric affects the gastrointestinal tract by inhibiting motility and propulsion and increasing smooth muscle tone. Thus, it has been used to treat diarrhea, opioid addiction, and neonatal abstinence syndrome.

Exposure Routes and Pathways

Paregoric is usually taken orally.

Toxicokinetics

Paregoric is well absorbed from the gastrointestinal tract. Peak serum levels are detectable ~1 h after ingestion. Opium preparations are metabolized in the liver by demethylation. Morphine undergoes conjugation with glucoronic acid at the 3-hydroxyl group. Secondary conjugation occurs at the 6-hydroxyl group to form 3,6-diglucuronide. Paregoric is 34–37% protein bound. The volume of distribution is 3 or 4 l kg^{-1}. From 8.5% to 12% is excreted unchanged in the urine, 7–10% excreted in feces as glucuronide conjugate, and 7–10% in the bile. The half-life is 1.9–2.6 h.

Mechanism of Toxicity

Opium and its derivatives cause depression of the central nervous system (CNS) and respiratory depression through binding of the opioid receptors (μ, δ, κ subtypes).

Acute and Short-Term Toxicity (or Exposure)

Animal

Cats and horses experience excitability and increased CNS effects. Dogs exhibit drowsiness, ataxia, seizures, coma, respiratory depression, hypotension, and vomiting. Treatment consists of decontaminating the gastrointestinal tract, maintaining the airway, administering naloxone, and controlling seizures with diazepam and/or phenobarbital. Hypotension is effectively treated with intravenous fluids and rarely needs pressor support. If ineffective, norepinephrine is the drug of choice. Dopamine and dobutamine can also be used. Monitoring needs to be

provided for at least 8 h after cessation of symptoms since relapse can occur.

Human

Paregoric produces CNS depression ranging from drowsiness to coma. These symptoms can be cyclic due to decreased gastric emptying. Respiratory depression occurs and progresses to Cheyne–Stokes respirations, cyanosis, and respiratory arrest. Pulmonary edema can also occur. Cardiac affects are characterized by bradycardia and hypotension. Other symptoms include hypothermia, flaccid skeletal muscles, cold and clammy skin.

Chronic Toxicity (or Exposure)

Human

Chronic use can produce psychological and physical dependence. Discontinuation of paregoric causes withdrawal symptoms.

Clinical Management

Life-support measures should be provided. Gastric decontamination with activated charcoal can be utilized in recent ingestions. Respiratory and CNS depression can be effectively reversed with naloxone, a pure opioid receptor antagonist. Naloxone administration will precipitate the opioid withdrawal syndrome and should be administered judiciously. Initial dose of 0.1 mg of naloxone can be administered intravenously and titrated until the respiratory depression is reversed. Until naloxone is administered, proper airway support is critical. If the patient has a second episode of respiratory depression after administration of naloxone, a drip utilizing two-thirds the dose needed to reverse the patient should be administered over 1 h.

See also: Codeine; Drugs of Abuse; Morphine; Opium.

Further Reading

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Wagner CL, Katikaneni LD, Cox TH, and Ryan RM (1998) The impact of prenatal drug exposure on the neonate. *Obstetrics and Gynecology Clinics of North America* 25(1): 169–194.

PBT (Persistent, Bioaccumulative, and Toxic) Chemicals

Thomas M Murray

- Representative Chemicals: Aldrin (CAS 309-00-2); Dieldrin (CAS 60-57-1); Alkyl-lead (CAS 7439-92-1); Benzo(*a*)pyrene (CAS 50-32-8); Chlordane (CAS 57-74-9); Dichlorodiphenyltrichloroethane (DDT) (CAS 50-29-3); Hexachlorobenzene (CAS 118-74-1); Mercury and compounds (CAS 7439-97-6); Mirex (CAS 2385-85-5); Octachlorostyrene (CAS 29082-74-4); Polychlorinated biphenyls (PCBs) (CAS 11097-69-1 for Arochlor 1254); 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) (CAS 1746-01-6) and chemically similar compounds collectively known as dioxins and furans; Camphechlor (toxaphene) (CAS 8001-35-2)

Persistent, bioaccumulative, and toxic (PBT) chemicals are a group of chemicals that meet the following criteria:

1. evidence of an environmental persistence value of more than 2 months in water and more than 6 months in soil or sediment or evidence of sufficient persistence to justify its consideration;
2. evidence of a bioconcentration factor (BCF) value or bioaccumulation factor (BAF) value of more than 5000 in aquatic species or, in lieu of such data, a log K_{OW} of more than 5 or evidence of high bioaccumulation in other species, evidence of high toxicity or ecotoxicity, or monitoring data in biota indicating a sufficient bioaccumulation potential to justify its consideration;
3. evidence of adverse effects to human health or the environment or toxicity or ecotoxicity data indicating the potential for damage to human health or to the environment; and
4. a long-range transport value of a half-life in air of more than 2 days, or other data/predictions of traveling long distances through air, water, or migratory species.

Uses

The uses of these PBT chemicals vary by chemical. The reader is referred to other sections of this encyclopedia for specific use information.

Exposure Routes and Pathways

PBT substances (and ones that could be classified as PBTs) enter the environment in various ways. Some, like dioxins, dioxin-like compounds, and numerous polycyclic aromatic hydrocarbons, are unintentional by-products of incomplete combustion or other high-temperature industrial processes involving organic materials. Some result from the environmental degradation of a released substance, such as hexachlorocyclohexane, which degrades to a γ-isomer with much higher PBT properties than the parent compound. Some substances, such as active ingredients of pesticide products, were designed to have a combination of molecular stability, biological uptake/retention, and a specific toxic endpoint – and only later were recognized to have broader environmental impacts. Finally, some substances were designed or discovered to have economically desirable properties like flame suppression, absorption of ultraviolet radiation, water and oil repellency, and even fragrance, and were later discovered to have unintended properties of toxicity, bioaccumulation, and persistence.

The food chain is the predominant source of human and wildlife exposure to most PBT chemicals, although drinking water (and dust and dirt for children) are also significant exposure pathways for lead. Within the food chain, the aquatic and marine food chains are significant sources of PBT exposure to humans and other terrestrial species. PBTs enter waterways in various amounts, both directly or by virtue of air deposition and runoff. Aquatic and marine organisms ingest PBT chemicals from the water column and sediments as they feed. Once ingested, the chemical properties of PBTs make them difficult for many organisms (depending on their physiological makeup) to excrete, thereby leading to bioaccumulation of PBTs in the organisms. As one organism feeds on another, this results in PBTs moving up the food chain. Many PBTs also biomagnify as they bioaccumulate, which means they increase in concentration within organisms as they move up the food chain. Mammals and birds high on the food chain can have levels of these PBTs that are at least 100 000–1 000 000 times greater than the concentrations found in ambient waters.

Bioconcentration in the marine food chain can lead to animals such as seals, beluga whales, seabirds, and polar bears having concentrations of toxaphene $\sim$10 million times higher than levels in the surrounding water. For polychlorinated biphenyls (PCBs), the amplification is $\sim$1000 million times.

Bioaccumulation also occurs directly within the terrestrial food chain for some PBT chemicals, without the involvement of aquatic organisms. For example, dioxins and furans are generated in combustion processes, are discharged to air, and then settle out on plant surfaces. These plants become food for farm animals and these PBTs become concentrated in animal tissue (many of the PBTs concentrate in fatty tissue). People are exposed via the terrestrial food chain when they consume meat, poultry, pork, and dairy products.

Mechanism of Toxicity

The toxic effects of PBT chemicals include neurotoxicity, reproductive toxicity, developmental toxicity, and cancer in humans and other species.

Animal

Birds and mammals high on the food chain are also at risk from exposure to PBTs, with similar concerns for their young exposed to PBTs in their eggs or through maternal milk. In both the North Temperate and Arctic zones, some marine mammal and bird populations are experiencing disease, reproductive problems, and population declines, probably in part or in whole due to contamination from PBT pollutants. Free-ranging Orca whales along the Pacific Northwest coast, whose numbers are falling appreciably, have PCB levels four to five times higher than highly-PCB-polluted St. Lawrence Beluga whales, who themselves have serious health problems. Populations of mink and otter continue to be depressed in certain regions where significant PCB concentrations have been reported. Canadian Arctic whales are providing the first statistical inference that PBT (specifically, PCB) levels in Arctic species may relate to subtle health effects.

Human

The developing human fetus and nursing infant are at particular risk for developmental problems. Studies show that mothers, who previously accumulated PBTs in their bodies, whether from the food chain or some other exposure pathway, transmit PBT contaminants through the fetal blood cord and breast milk to the fetus and nursing infant respectively. Most PBT chemical releases have occurred in the North Temperate zone, between the Arctic Circle and the Tropic of Cancer, where the majority of industrialized nations are located. In this region, the general population has detectable levels of 2,3,7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) – and chemically similar compounds collectively known as dioxins, furans, and coplanar PCBs – in their bodies as a result of eating meat, fish, eggs, and dairy products.

US Environmental Protection Agency's (EPA's) 2000 Draft Dioxin Reassessment estimated the cancer risk to the US population from this background exposure to be in the 1:10 000 to 1:1000 range, which is approaching levels associated with adverse noncancer effects.

For mercury, results from the 1999 US Natio nal Health and Nutrition Examination Surveys (NHANES), which measured mercury levels in hair and blood of US women, show that ~8% of US women of childbearing age have concentrations of mercury at blood levels higher than those associated with EPA's reference dose. About 75% of the 2618 US consumption advisories listed on US EPA's 2001 National Listing of Fish and Wildlife Advisories were issued at least partly due to mercury, and lake acres and river miles under mercury advisory continued to increase in 2001, a trend since 1993. Most US advisories involve mercury, PCBs, chlordane, dioxin, and DDT, with PCBs being the second highest cause of fish advisories.

Environmental Fate

Air deposition of PBTs can occur in three ways – as gases and particles trapped in rain, fog, or snow, as dry particles dropping onto surfaces, or as semivolatile organic chemicals (SVOCs) cycling between the gas phase in air and the particle phase in water. SVOCs and some trace metals like mercury can cycle between the atmosphere and the Earth's surface many times in the course of being transported long distances. This cycling slows or ceases in the colder Polar Regions and high-altitude regions, a phenomenon known as global distillation.

Atmospheric deposition of PBTs contributes significantly to the contamination of aquatic, marine, and terrestrial ecosystems and their food chains. Large water bodies, such as the Great Lakes, seas, and oceans, appear vulnerable to significant air-water exchange of SVOCs, and air deposition accounts for a significant percentage of toxics contained in water bodies such as the Great Lakes. Moreover, the circulation of PBTs in the atmosphere at regional and global scales can make it difficult to identify sources of contamination deposited via the atmosphere, since they may be far away. This point is perhaps best illustrated by the existence of PCBs in the Arctic snow pack and food chain, hundreds or thousands of miles from any possible source.

See also: Aldrin; Dieldrin; Dioxins; Polychlorinated Biphenyls (PCBs).

Further Reading

Arctic Monitoring and Assessment Programme (AMAP) (1997) *Arctic Pollution Issues: A State of the Arctic Environment Report*. ISBN 82-7655-060-6 (Oslo, Norway).

Arctic Monitoring and Assessment Programme (AMAP) (1998) *AMAP Assessment Report: Pollution Issues*. ISBN 82-7655-061-4 (Oslo, Norway).

Canada Department of Indian Affairs and Northern Development (1997) *Highlights of the Canadian Arctic Contaminants Assessment Report: A Community Reference Manual*.

Relevant Websites

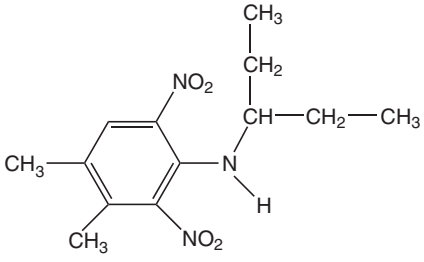
http://www.cdc.gov – Centers for Disease Control and Prevention, US Department of Health and Human Services, National Health and Nutrition Examination Surveys (NHANES), March 2, 2001: Mercury findings. Other NHANES results are available on the same website.

http://www.epa.gov – US Environmental Protection Agency (EPA), 2000 National Listing of Fish and Wildlife Advisories. Additional details and information on PBTs are also available on the same website.

Pendimethalin

K S Rao

- Chemical Abstracts Service Registry Number: CAS 40487-42-1
- Synonyms: (*N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine); Prowl; Squadron
- Chemical/Pharmaceutical/Other Class: Agricultural; Herbicide
- Chemical Formula: $C_{13}H_{19}N_3O_4$
- Chemical Structure:



Uses

Pendimethalin is used as a selective herbicide to control broadleaf weeds and grassy weed species in cereals, onions, garlic, corn, sorghum, rice, soy beans, peanuts, brassicas, carrots, celery, peas, potatoes, cotton, pome fruits, stone fruits, citrus, lettuce, tobacco, and tomatoes. It is also used on noncrop areas and on residential lawns and ornamentals.

Exposure Routes and Pathways

Primary exposure is through the dermal route to mixers, loaders, and applicators, during and after normal use. However, significant exposure to residues of pendimethalin in food crops can occur in consumers.

Toxicokinetics

Pendimethalin is absorbed rapidly and effectively by the oral route but less effectively by the dermal route. Following absorption, ~70% of pendimethalin is excreted in the feces and 20% in the urine in 24 h. The maximum tissue concentration of pendimethalin was seen at 6 h in liver, kidney, muscle, and fat. The major portion that is excreted in the feces is the parent compound. Pendimethalin is metabolized in rats mainly through oxidation of the 4-methyl group attached to the benzene ring as well as oxidation of the alkyl side chain of the N-substituted dinitroaniline compound.

Mechanism of Toxicity

The toxicity of pendimethalin is related to its effects on the thyroid. Treatment with pendimethalin (500 ppm for 90 days) produces a decreased total T_4, T_3, total free T_4 and increased percent T_3, increased follicular cell height and decreased area occupied by colloid. In addition, it produces an increased absolute (15%) and relative (23%) thyroid weight and ultrastructural thyroid changes. The ultrastructural thyroid changes are consistent with mild to moderate testicular stimulating hormone (TSH) stimulation except for the accumulation of dense bodies in the cytoplasm, which may be reaction products of pendimethalin. At higher doses (5000 ppm), increased liver weight, bile flow and cumulative biliary excretion of [^{125}I]-T_4 with a slight increase in T_4-glucuronytransferase activity was detected by generation of [^{125}I]-T glucuronide from [^{125}I]-T.

Acute and Short-Term Toxicity (or Exposure)

Animal

In studies using laboratory animals, pendimethalin generally has been shown to be of low acute toxicity with an oral LD_{50} of 1050 mg kg^{-1} in rats. It is slightly toxic to practically nontoxic by skin exposure, with reported dermal LD_{50} values of greater than 2000 mg kg^{-1}. It is not a skin sensitizer, but it causes mild eye irritation. The inhalation 4 h LC_{50} for pendimethalin in rats is 320 mg l^{-1}, indicating practically no toxicity via this route. Inhalation of dust or fumes of pendimethalin is irritating to the linings of the mouth, nose, throat, and lungs.

Human

A search in the Office of Pesticide Programs' Incident Data System identified 12 pendimethalin reports with three of these involving five humans (the remainder concern fish, wildlife, or domestic animals). The symptoms included signs of systemic illness: vomiting, diarrhea, chills, and shakiness. Three people were hospitalized when they were exposed to a mixture of pesticides including pendimethalin and nitrogen. The database does not indicate the associated use patterns or activities in which the poisoned individuals were involved. The California Pesticide Illness Surveillance Program for 1982–1992 contained six reports involving pendimethalin. In three of these reports, the effects were systemic (vomiting, diarrhea, etc.), two involved skin effects, and one involved eye effects. Pendimethalin was ranked 41st on a list of the top 200 active ingredients for which the National Pesticide Telecommunications Network (NPTN) received calls during 1982–1991. There were 682 calls, with 91 of them concerning human poisoning due to pendimethalin.

Chronic Toxicity (or Exposure)

Animal

In subchronic studies in rats at a low dose level of 100 ppm (5.0 mg kg^{-1} day^{-1}) there was decreased total T_4, rT_3, total free T_4 and increased percent T_3, increased follicular cell height and decreased area occupied by colloid. At 5000 ppm (245 mg kg^{-1} day^{-1}) exposed animals exhibited decreased body weight and food consumption compared to controls, increased thyroid weight, decreased total T_4, total T_3, rT_3, total free T_4 and [^{125}I]-T_4 to transthyretin bonding, increased percent free T_4, percent free T_3 and [^{125}I]-T_4 to albumin binding, increased follicular cell height and decreased area occupied by colloid and ultrastructural thyroid changes. Chronic exposure to pendimethalin has resulted in increased liver weights in test animals.

Pendimethalin induces a statistically significant increase in thyroid follicular cell adenomas in male and

female rats. Pendimethalin does not induce mutations, birth defects or reproductive effects.

Human

The Environmental Protection Agency has concluded that pendimethalin should be classified as a group C (possible human) carcinogen.

Clinical Management

In case of contact, the eyes and skin should be flushed immediately with water for at least 15 min to reduce exposure. Following accidental oral exposure, immediate dilution with 4–8 ounces (i.e., 118–237 ml) of milk or water is recommended. Following a known significant exposure, the patient should be subjected to a complete thyroid profile, to make sure thyroid function is intact. If thyroid function is affected, a specialist in endocrinology should be consulted.

Environmental Fate

Pendimethalin is moderately persistent, with a field half-life of 40 days. It does not undergo rapid microbial degradation. Pendimethalin is strongly adsorbed by most soils. Increasing soil organic matter and clay is associated with increased soil binding capacity. Under agricultural use conditions, pendimethalin is absorbed by plant roots and shoots and inhibits cell division.

Ecotoxicology

Pendimethalin is highly toxic to fish and aquatic invertebrates. The 96 h LC_{50} value for pendimethalin in rainbow trout is 138 $\mu g\,l^{-1}$. The bioconcentration factor for this compound in whole fish is 5100, indicating a moderate potential to accumulate in aquatic organisms.

Pendimethalin is slightly toxic to birds, with an acute oral LD_{50} of 1421 $mg\,kg^{-1}$ in mallard ducks.

See also: Pesticides; Thyroid Extract.

Relevant Websites

http://europa.eu.int – European Commission (2003) Review Report for the Active Substance Pendimethalin. Brussels: Health and Consumer Protection Directorate General.

http://www.epa.gov – US Environmental Protection Agency (1997) Reregistration Eligibility Decision (RED) Pendimethalin. Washington, DC: Office of Prevention, Pesticides and Toxic Substances.

Penicillin

Brenda Swanson-Biearman

- SYNONYMS: Aminopenicillins; Amoxicillin; Ampicillin; Carbenicillin; Bacampacillin. Extended spectrum: Carbenicillin; Mezlocillin, Piperacillins, Ticarcillins. Natural penicillins: Penicillin G; Penicillin V. Pencillinase-resistant: Cloxacillin; Dicloxacillin; Methicillin; Nafcillin; Oxacillin
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Antibiotic

Uses

Penicillin is used to treat infections caused by Gram-positive cocci, Gram-negative aerobic cocci, and anaerobic bacteria. The penicillins may be bactericidal or bacteriostatic in action, dependent upon the concentration of drug attained at the sire of infection and susceptibility of the organism.

Exposure Routes and Pathways

Penicillin is available in tablet and suspension form for oral use and in injectable form for intravenous and intramuscular use. Ingestion of tablets and the suspension forms are the most common poisoning exposures.

Toxicokinetics

Following oral administration, absorption of penicillin occurs mainly in the duodenum and upper jejunum, with a small percentage absorbed in the stomach and insignificant amounts in the large intestine. The extent of absorption is variable and depends on several factors including the penicillin derivative, the dosage form administered, gastric and intestinal pH, and the presence of food in the gastrointestinal tract. Peak concentrations are generally seen within 1–2 h. Protein binding and the volume of distribution vary with each derivative. Following absorption from either the gastrointestinal tract or from

injection sites, penicillins are widely distributed into most body tissues. Most penicillins and their microbiologically active metabolites are excreted primarily unchanged in the urine by renal tubular secretion. Nonrenal elimination includes hepatic inactivation and biliary excretion. Renal clearance of penicillin is delayed in the neonate due to an immature tubular secretion mechanism. Children older than 3 months generally excrete drugs similarly to adults. Excretion is also delayed in geriatric patients due to diminished tubular secretion ability. Patients with renal impairment may also have altered tubular secretion ability and, therefore, have higher and more prolonged serum concentrations of penicillins. The rate of absorption from parenteral administration depends upon the dose, concentration, and solubility of the particular salt being administered. The elimination half-life depends on the derivative, but ranges from 0.5 to 2.5 h in the parent compound.

Mechanism of Toxicity

The primary toxic manifestations of penicillin overdose are due to inability of renal excretion due to age, kidney disease, or anaphylaxis.

Acute and Short-Term Toxicity (or Exposure)

Animal

Toxic amounts of penicillin in animals are not established. Allergic reactions in animals have not been commonly reported in animals. In very large doses, penicillin VK has resulted in neurologic effects in mice and rats.

Human

Hypersensitivity reactions may follow exposure to any amount of penicillins and could result in anaphylaxis. Acute ingestion of large amounts of penicillins ($>250\,mg\,kg^{-1}$) in children less than 6 years of age may result in nausea, vomiting, diarrhea, and abdominal pain. Acute oliguric renal failure, hematuria, and crystalluria have been reported rarely. Intravenous use of penicillin in doses exceeding 10 million units may cause seizures and coma. Cardiac conduction defects have occurred after rapid infusion of potassium penicillin C and procaine penicillin C. Electrolyte abnormalities have been associated with large doses of potassium and sodium salts. Toxicity associated with chronic ingestions is expected to be similar.

Chronic Toxicity (or Exposure)

Animal

No evidence of carcinogenicity was documented in rats and mice receiving 500 or $1000\,mg\,kg^{-1}$ penicillin VK 5 days a week for 2 years.

Human

Most penicillins have significant renal clearance. Patients who have reduced renal function may accumulate large amounts of penicillin over time. Patients with very high serum levels are more likely to develop more serious toxicity (e.g., neurologic effects) than those with lower levels.

In Vitro Toxicity Data

Penicillin V was inconclusive in the *Bacillus subtilis* assay and negative in *Escherichia coli* and male mouse sperm assays.

Clinical Management

Gastric decontamination with activated charcoal may be warranted with large recent ingestions. Urinalysis, renal function tests, and evaluation of electrolytes may be indicated in large exposures. In all cases, regardless of the route of exposure, the mainstay of therapy is supportive care and discontinuation of the drug. For very large overdoses that result in renal impairment, dialysis may be considered for correction of acidosis and electrolytes, rather than for removal of penicillins. Ocular exposures necessitate thorough eye irrigation with water for 15 min. Anaphylaxis is treated with supportive care and the administration of epinephrine and diphenhydramine as symptoms dictate.

See also: Gastrointestinal System.

Further Reading

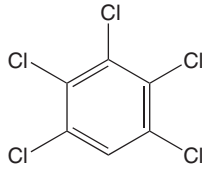
Swanson-Biearman B, Dean BS, and Lopez G (1988) The effects of penicillin and cephalosporin ingestions in children less than six years of age. *Veterinary & Human Toxicology* 30: 66–67.

Thethi AK and Van Dellen RG (2004) Dilemmas and controversies in penicillin allergy. *Immunology and Allergy Clinics of North America* 24(3): 445–461.

Pentachlorobenzene

Jing Liu

- Chemical Abstracts Service Registry Number: CAS 608935
- Synonyms: 1,2,3,4,5-Pentachlorobenzene; Benzene, pentachloro-; Quintochlorobenzene (QCB)
- Chemical/Pharmaceutical/Other Class: Chlorinated benzene
- Chemical Formula: C_6HCl_5
- Chemical Structure:



Uses

Pentachlorobenzene is a chemical intermediate in the production of the fungicide pentachloronitrobenzene. It can be found as a technical impurity in pentachloronitrobenzene formulations. It can also be used as a fire retardant.

Exposure Routes and Pathways

Inhalation and ingestion through contaminated food and water are the primary routes of human exposures.

Toxicokinetics

Pentachlorobenzene can be absorbed readily through gastrointestinal and respiratory tracts in humans and experimental animals. Chlorobenzenes accumulate primarily in fatty tissues and have been shown to cross the placenta. Pentachlorobenzene is metabolized via cytochrome P450-dependent processes to its major metabolites pentachlorophenol and 2,3,4,6-tetrachlorophenol. Food restriction was reported to increase its metabolism. Metabolites are excreted in the urine as mercapturic acids, glucuronic acid, or sulfate conjugates. Some proportion of the chemical is eliminated unchanged in the feces.

Mechanism of Toxicity

There is little information available that describes the exact mechanism of toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} values for pentachlorobenzene in rats were 940, 1080, and 1125 mg kg^{-1} for weanling females, adult females, and adult males, respectively. The oral LD_{50} values in mice were 1175 and 1370 mg kg^{-1} for males and females, respectively. With high dosages, animals exhibited signs of overt toxicity including tremors and narcosis. Acute toxicity of pentachlorobenzene has been reported to involve the liver, kidney, and thyroid. In one report, changes in plasma alanine aminotransferase (ALT) and liver histopathological profiles, presence of protein droplets in the tubular epithelial cells, and reduction of plasma thyroxine levels were observed in rats treated with relatively low intraperitoneal pentachlorobenzene exposures (1, 2, or 4 mmol kg^{-1}). In rats and mice exposed to pentachlorobenzene in the diet (33–2000 ppm) for 13 weeks, liver weights were increased and there was centrilobular hepatocellular hypertrophy and possible accumulation of porphyrins in the hepatocytes at higher concentrations. Male rats exhibited renal lesions that were characteristic of hyaline droplet nephropathy. Exacerbation of spontaneous nephropathy characterized by renal tubular cell regeneration and homogeneous intratubular protein casts was seen in both male and female rats. Urinary protein levels were increased at 1000 and 2000 ppm dietary pentachlorobenzene.

Human

The limited information regarding effects of chlorobenzenes on human health is restricted to case reports and to mono- and di-chloro congeners. Clinical signs and symptoms of excessive exposure include central nervous system effects, irritation of the eyes and upper respiratory tract, hardening of the skin, and hematological disorders. No report is available specifically regarding pentachlorobenzene in humans.

Chronic Toxicity (or Exposure)

As a family of chemicals, the long-term toxicity of chlorobenzenes increases with ring chloridation. The liver and kidney are the major target organs. Thyroid and hematologic toxicity including

hypothyroxinemia and decreased hematocrit, hemoglobin, and erythrocytes were also reported in animals exposed to higher doses. There is no available evidence that chlorobenzenes are teratogenic in rats or rabbits. Carcinogenicity of pentachlorobenzene has not been fully determined.

In Vitro Toxicity Data

The limited data from both *in vitro* and *in vivo* assays for chlorobenzene isomers other than 1,4-dichlorobenzene indicates that chlorobenzenes are not mutagenic.

Clinical Management

First aid should be provided based on the route of exposure. Clinical treatment is symptomatic.

Environmental Fate

Pentachlorobenzene is persistent and immobile in soil and sediment. Volatilization, adsorption, photooxidation, and aerobic biodegradation primarily control the fate of pentachlorobenzene in the environment. Bioaccumulation in the food chain may occur.

Ecotoxicology

Pentachlorobenzene is toxic to aquatic organisms. The 96 h LC_{50} value for the guppy (*Poecilia reticulate*) is $135\,\mu g\,l^{-1}$. For the water flea (*Daphnia magna*), a 48 h EC_{50} of $122\,\mu g\,l^{-1}$ was reported based on acute immobilization. No toxicity data are available on birds.

Exposure Standards and Guidelines

No threshold limit value is currently available. The reference dose is $0.0008\,mg\,kg^{-1}\,day^{-1}$ based on liver and kidney toxicities.

See also: Pentachloronitrobenzene; Pesticides.

Further Reading

den Besten C, Peters MM, and van Bladeren PJ (1989) The metabolism of pentachlorobenzene by rat liver microsomes: The nature of the reactive intermediates formed. *Biochemical Biophysical Research Communication* 163(3): 1275–1281.

den Besten C, Vet JJ, Besselink HT, *et al.* (1991) The liver, kidney, and thyroid toxicity of chlorinated benzenes. *Toxicology and Applied Pharmacology* 111(1): 69–81.

Linder R, Scotti T, Goldstein J, McElroy K, and Walsh D (1980) Acute and subchronic toxicity of pentachlorobenzene. *Journal of Environmental Pathology and Toxicology* 4(5–6): 183–196.

Umegaki K, Ikegami S, and Ichikawa T (1993) Effects of restricted feeding on the absorption, metabolism, and accumulation of pentachlorobenzene in rats. *Journal of Nutritional Science and Vitaminology* 39(1): 11–22.

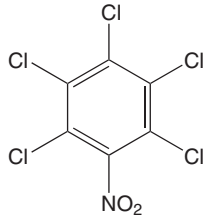
Relevant Websites

http://www.cdc.gov – National Institute of Occupational Safety and Health.
http://www.epa.gov – US Environmental Protection Agency.
http://www.hc-sc.gc.ca – Health Canada.
http://www.inchem.org – International Programme on Chemical Safety.
http://www.ntp-server.niehs.nih.gov – National Institute for Environmental Health Sciences.
http://www.speclab.com – Spectrum Laboratories.

Pentachloronitrobenzene

Jing Liu

- Chemical Abstracts Service Registry Number: CAS 82-68-8
- Synonyms: Quintozene (BSI and ISO preferred name); Avicol; Brassicol; Botrilex; Folosan; Fungiclor; Terraclor; Tilcarex; Tritisan; Tri-PCNB; Turfcide
- Chemical/Pharmaceutical/Other Class: Chlorinated nitrobenzenoid fungicide
- Chemical Formula: $C_6Cl_5NO_2$
- Chemical Structure:



Uses

Quintozene is used as a fungicide on seeds and soil. Most products containing quintozene have been banned in the United States.

Exposure Routes and Pathways

Dermal, oral, and inhalation routes of exposure are all possible. For the general population, exposure is mainly via residues in food.

Toxicokinetics

While very little information is available on absorption of pentachloronitrobenzene via inhalation or dermal contact, absorption from the gastrointestinal tract exhibited large differences among species. For example, quintozene is poorly absorbed from the gastrointestinal tract in rats. In contrast, it is well absorbed from the gastrointestinal tract in monkeys. In animals, quintozene is rapidly metabolized with main metabolites being pentachloroaniline (PCA) and mercapturic acids. A number of minor metabolites including pentachlorothioanisole (PCTA) and pentachlorophenol have also been identified. In soil and on plants, quintozene is metabolized to PCA and PCTA. Unabsorbed quintozene following oral exposure is excreted in the feces and the metabolites of quintozene are primarily excreted in the urine. There is virtually no bioaccumulation of quintozene in tissues. In dogs and rats fed at levels up to 1080 and 500 mg kg^{-1} of quintozene, respectively, for 24 and 33 weeks, no residues were detected in kidney, brain, fat, skeletal muscle, or liver.

Mechanism of Toxicity

There is no information on the exact mechanism of toxicity of quintozene. The aromatic nitro structure of quintozene and the aromatic amine structure of its main metabolite PCA, however, are the common parent structures of known methemoglobinemic agents. Hexachlorobenzene is a major contaminant in the technical material, which may be accountable for some aspects of quintozene's toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

The reported oral LD_{50} values of quintozene in male and female rats were 1710 and 1650 mg kg^{-1}, respectively. When given as an aqueous suspension, the oral LD_{50} was greater than 30 g kg^{-1}. Dogs can tolerate quintozene orally up to 2500 mg kg^{-1} without lethality. No signs of toxicity or skin irritation were observed in rabbits exposed dermally once to 4 g kg^{-1} quintozene as a 30% solution. Oral administration of quintozene to cats caused methemoglobinemia and Heinz body formation in erythrocytes.

Human

Among 50 human volunteers, quintozene did not cause skin irritation after a single 48 h dermal exposure. Four of the 50 subjects, immediately after a second exposure 2 weeks later, showed erythema, edema, formation of small vesicles, and itching. Another nine people developed a delayed reaction. A case of keratoconjunctivitis was reported following ocular exposure.

Chronic Toxicity (or Exposure)

Animal

Growth and survival of both sexes of rats were affected by dietary quintozene (5000 mg kg^{-1} for 3 months), with male rats exhibiting higher sensitivity. Liver hypertrophy and fine vacuolization of liver cell cytoplasm were observed in these rats. In dogs consuming 500, 1000, or 5000 mg kg^{-1} quintozene in the diet, liver changes including fibrosis, narrowing of hepatic cell cords, increased periportal areas, and leukocyte infiltration occurred in a dose-related manner. The highest dose of quintozene also caused reduced hematopoiesis and atrophy of the bone marrow. No effects on reproduction and no teratogenic effect were observed in rats that received quintozene orally. There is little information on quintozene's carcinogenic potential. It may cause liver tumors in mice.

Human

There is little information available on chronic effects of quintozene in humans.

In Vitro Toxicity Data

There is no indication for mutagenic activity. Quintozene elicited negative results in the Ames assay and reverse mutation assay.

Clinical Management

Treatment is symptomatic.

Environmental Fate

Pentachloronitrobenzene has an estimated half-life of 1.8 days in water. It is more stable in acidic and neutral conditions. Volatilization, adsorption, and sedimentation as detritus are the major processes responsible for the rapid decrease in quintozene in water. Biodegradation and photolysis are not relevant pathways. In soils, volatilization and biodegradation are important pathways, with anaerobic conditions being more favorable for the degradation.

Ecotoxicology

Quintozene appears to be moderately bioaccumulated in aquatic animals and plants. The toxicity of quintozene for aquatic organisms depends on the species tested. The LC_{50} values in rainbow trout and bluegill sunfish were reported to be 0.55 and 0.1 mg l^{-1}, respectively. On the other hand, a 48 h LC_{50} value of 10 mg l^{-1} for carp and a 3 h LC_{50} value of 40 mg l^{-1} for *Daphnia* have been reported. Quintozene is practically nontoxic to birds and no information is available for bees. Quintozene has a significant effect on earthworm reproduction and survival.

Exposure Standards and Guidelines

The oral reference dose is 0.003 mg kg^{-1} day^{-1}, the threshold limit value is 0.5 mg m^{-3} (8 h), and the acceptable daily intake is 0.007 mg kg^{-1} day^{-1}.

See also: Hexachlorobenzene; Pentachlorobenzene; Pesticides.

Further Reading

Borzelleca JF, Larson PS, Crawford EM, *et al.* (1971) Toxicologic and metabolic studies on pentachloronitrobenzene. *Toxicology and Applied Pharmacology* 18(3): 522–534.

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Edwards R, Ferry DG, and Temple WA (1991) Fungicides and related compounds: Quintozene. In: Hayes WJ Jr. and Laws ER Jr. (eds.) *Handbook of Pesticide Toxicology*, pp. 1420–1422. San Diego, CA: Academic Press.

Schumann AM and Borzelleca JF (1978) An assessment of the methemoglobin and Heinz-body-inducing capacity of pentachloronitrobenzene in the cat. *Toxicology and Applied Pharmacology* 44(3): 523–529.

Relevant Websites

http://extoxnet.orst.edu – Extension Toxicology Network. Oregon State University.

http://www.inchem.org – International Programme on Chemical Safety (IPCS).

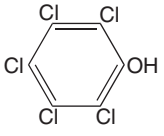
http://www.epa.gov – US Environmental Protection Agency.

http://www.speclab.com – Laboratory Inc.

Pentachlorophenol

Kevin N Baer

- Chemical Abstracts Service Registry Number: CAS 87-86-5
- Synonyms: PCP; Penchlorol; Penta; Pentacon; Penwar; Dowicide EC-7
- Chemical/Pharmaceutical/Other Class: Halogenated phenol
- Chemical Structure:



Uses

Pentachlorophenol was used extensively as an insecticide, herbicide, fungicide, and wood preservative. Since 1984, use of pentachlorophenol in the United States has been restricted to certified pesticide applicators. It is still used industrially as a wood preservative for utility poles, railroad ties, and wharf pilings.

Exposure Routes and Pathway

Dermal contact is the most frequent route of exposure and the majority of poisonings are occupational in origin.

Toxicokinetics

Pentachlorophenol is readily and almost completely absorbed through the skin and gastrointestinal tract. The majority of the compound is excreted primarily unchanged in the urine, although glucuronide conjugates have been detected. In humans, there is conflicting evidence as to whether pentachlorophenol is metabolized in the liver to any significant extent. One metabolite, tetrachlorohydroquinone, was produced in human liver homogenates and was detected in the urine of exposed workers, while in other studies no metabolites were found. Pentachlorophenol is well distributed throughout the tissues, with high concentrations found in the urine, liver, and kidneys. Greater than 90% in blood is bound to serum proteins. The kidney is the primary route of elimination with ~80% excreted in the urine and a smaller amount in the feces. In humans, discrepancies exist concerning the elimination half-life with values ranging from 10 h to 20 days. Although the exact reasons are not

known for the long half-lives, high protein binding with tubular reabsorption and possible enterohepatic circulation may be contributing factors.

Mechanism of Toxicity

Pentachlorophenol increases metabolic rate and elevates body temperature by uncoupling oxidative phosphorylation in tissues. The circulatory system and heart are particularly affected. Pentachlorophenol can be contaminated by dibenzodioxins and dibenzofurans.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute oral LD_{50} value for pentachlorophenol in rats, mice, and rabbits is $\sim$30–200 mg kg^{-1}. Dermal LD_{50} values in rats are somewhat higher ($\sim$100–300 mg kg^{-1}).

Human

Characteristic symptoms of poisoning are extremely high body temperature and profuse sweating. In fatal cases, rapid pulse, coma, heart failure, and death can occur within 3–30 h after initial symptoms appear. Symptoms in nonfatal poisonings include weakness, gastrointestinal upset, headache, dizziness, and seizures. Pentachlorophenol is a potent skin, eye, and upper respiratory tract irritant.

Chronic Toxicity (or Exposure)

Animal

Pentachlorophenol has been demonstrated to be fetotoxic and teratogenic with exposure during early gestation.

Human

Chloracne has been reported in chronic occupational exposure to pentachlorophenol. However, commercial preparations are commonly contaminated with dioxins and furans, and chloracne may be linked to these compounds. In addition, hemolytic and aplastic anemia and weight loss have been reported in humans. Pentachlorophenol is classified as a probable human carcinogen (group 2B).

Clinical Management

Rapid decontamination is important, especially with skin exposure. The primary treatment is supportive and symptomatic and consists of promoting heat loss, reducing anxiety, and replacing fluids and electrolytes lost during sweating. Following oral exposure, emesis, activated charcoal, and cathartics are recommended. Administration of salicylates to reduce the high body temperature is contrain dicated. Single-exchange transfusions have been successfully performed in infants poisoned by pentachlorophenol.

Environmental Fate

Pentachlorophenol is moderately persistent in the soil (half-life of 45 days). It degrades more rapidly under anaerobic conditions, higher temperatures, and in the presence of organic matter. Under alkaline conditions, it is less adsorbed and more mobile. It has been found in groundwater in a number of western states. In water, pentachlorophenol is primarily bound to sediments and suspended particles. The half-life in water ranges from hours to days. It is relatively nonvolatile and does not evaporate to a significant degree. Pentachlorophenol can be taken up by plants and is highly toxic in plants.

Exposure Standards and Guidelines

The time-weighted average (8 h) for pentachlorophenol is 0.5 mg m^{-3}. The reference dose is 0.03 mg kg^{-1} day^{-1}.

See also: Chlorophenols; Pesticides.

Further Reading

Proudfoot AT (2003) Pentachlorophenol poisoning. *Toxicological Reviews* 22(1): 3–11.

Relevant Website

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.

Pentane

Stephen R Clough

- Chemical Abstracts Service Registry Number: CAS 109-66-0
- Synonyms: Pentane (ACGIH, NIOSH, OSHA, DOT); Pentan (Polish); Pentanen (Dutch); Pentani (Italian); UN1265 (DOT)
- Chemical/Pharmaceutical/Other Class: Aliphatic hydrocarbon (C5)
- Chemical Formula: C_5H_{12}

Uses

Pentane is present in volatile petroleum fractions and is used: (1) as a fuel; (2) in the production of ammonia, olefin, and hydrogen; (3) in the manufacture of artificial ice; (4) in low-temperature thermometers; (5) as a blowing agent for plastics and foams; and (6) in solvent extraction processes. Neopentane is important in the manufacture of rubber.

Background Information

Pentane is a colorless, flammable liquid (the first liquid member of the alkanes) that is lighter than water. It has a pleasant odor that can be detected at 900 ppm, and a moderate odor intensity is observed at 5000 ppm. It occurs as two other isomers, including isopentane [$(CH_3)_2CHCH_2CH_3$] and neopentane [$C(CH_3)_4$]. Isopentane (2-methylbutane) apparently has physical and physiological characteristics similar to straight-chain pentane. Neopentane (2,2-dimethylpropane) is similar to butane in physical and physiological characteristics. In air, one part per million of C5 pentane is equivalent to 3 mg m^{-3}.

Exposure Routes and Pathways

As pentane may exist as a vapor or liquid at normal temperature and pressure, exposure would be expected to occur either by inhalation or dermal contact. Oral ingestion would be expected to be incidental or accidental. Typical background concentrations that have been detected in major cities within the United States range from 0.05 to 0.35 ppm.

Mechanism of Toxicity

As seen with other short-chain alkanes, upon inhalation, pentane is moderately toxic and may cause irritation of the respiratory tract and narcosis. The narcotic action of pentane (observed in 1 h at 90 000–120 000 ppm) is, however, much less pronounced than effects seen following exposure to the C1–C4 alkanes. Although the actual biochemical mechanism of toxicity has not been discerned, the narcotic effects seen are most likely related to its physical solvent properties. The effect is similar to the 'high' experienced upon exposure to other aliphatic hydrocarbon solvents.

Acute and Short-Term Toxicity (or Exposure)

Animal

Mice that are acutely exposed to a concentration of 200 000–300 000 mg m^{-3} show 'incoordination and inhibition of the righting reflex' and a pronounced anesthetic effect is seen after 10 min at 7% or 1.3 min at 9% (death will ensue after 37 min of 12.8%). Pentane exposure in dogs will sensitize the heart to epinephrine. In rats, air concentrations of 10.4, 50.9, and 94.7 mg m^{-3} resulted in brain damage in the offspring. As with other aliphatic hydrocarbons, studies have shown that pentane can be utilized by certain microorganisms as a nutrient.

Human

According to a 1981–83 National Occupational Exposure Survey (National Institute for Occupational Safety and Health, NIOSH), the majority of persons exposed to pentane in the workplace are operators of molding and casting machines, lathes and turning machines, separating/filtering/clarifying machines, hand molders and shapers, computer operators, chemists, and biological/chemical technicians. It is generally known that human exposure for 10 min at 5000 ppm does not cause any adverse effects or irritation of the mucous membranes. Lethal effects have been observed, however, in estimated air concentrations of 130 000 ppm; the lowest concentration known to cause a toxic effect is 90 000 ppm. Some studies have implicated pentane as a neurotoxicant, but these study results are confounded by the presence of other compounds in the mixture.

Chronic Toxicity (or Exposure)

Human

Prolonged skin contact may cause drying, cracking, and dermatitis.

Clinical Management

Persons exposed to high concentrations should vacate or be removed from the source of the liquid or

vapor and seek fresh air. Extreme care must be taken to keep areas of high concentration free from ignition sources, such as sparks from static electricity and use of explosion-proof apparatus.

Environmental Fate

When released into soil, pentane may biodegrade to a moderate extent, is expected to quickly evaporate, and is not expected to leach into groundwater. Pentane has a half-life of less than 1 day in water with an estimated bioconcentration factor of less than 100 and a log octanol–water partition coefficient of greater than 3.0. Thus, it is not expected to significantly bioaccumulate. When released into the air, pentane is expected to be readily degraded by reaction with photochemically produced hydroxyl radicals.

Ecotoxicology

Young Coho salmon exposed to pentane in the laboratory (salt water) show no effect on lethality below $100\,mg\,l^{-1}$. A freshwater EC_{50} of $9.74\,mg\,l^{-1}$ has been reported for *Daphnia magna* (or the water concentration required to immobilize approximately one-half of the test organisms).

Other Hazards

Pentane is highly flammable and readily forms explosive mixtures with air.

Exposure Standards and Guidelines

NIOSH recommends a workplace standard of $350\,mg\,m^{-3}$ (120 ppm) for the C5–C8 alkanes, and a short-term exposure limit (STEL) of $1800\,mg\,m^{-3}$ (610 ppm). The American Conference of Governmental Industrial Hygienists suggests a workplace environmental standard of $1770\,mg\,m^{-3}$ (600 ppm) and a STEL of $750\,mg\,m^{-3}$ (2210 ppm). Chemical exposure kits are available for individual monitoring (e.g., wearing chemical detection badges) of pentane in the workplace. Pentane is also highly flammable and is therefore an explosion and/or fire hazard. The upper and lower explosive limits are 1.5% and 7.8% by volume, respectively. No threshold limit values are available for other isomers of pentane, so time-weighted average and ceiling values established for pentane are therefore recommended.

See also: Petroleum Hydrocarbons.

Further Reading

Stadler JC, O'Neill AJ, Elliott GS, and Kennedy GL Jr. (2001) Repeated exposure inhalation study of pentane in rats. *Drug and Chemical Toxicology* 24(2): 75–86.

Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Pentane.

Pentazocine

Christopher P Holstege

- Chemical Abstracts Service Registry Number: CAS 359-83-1
- Synonyms: Fortral; Talacen; Talwin NX; Talwin
- Chemical/Pharmaceutical/Other Class: Synthetic opiate agonist and antagonist

Uses

Pentazocine is used as an analgesic. It may also be diverted as a drug of abuse.

Exposure Routes and Pathways

Exposures occur with ingestion or injection of solubilized tablets.

Toxicokinetics

Pentazocine is well absorbed from the gastrointestinal tract. The onset of action following oral administration occurs within 15–30 min, with peak blood levels occurring at 0.5–4.0 h. Following parenteral administration, the onset of effects are observed within 3 min with peak serum levels at 30 min. Protein binding is ~60%. The volume of distribution is $4–8\,l\,kg^{-1}$. Pentazocine is largely metabolized in the liver to *cis*-hydroxypentazocine and *trans*-carboxypentazocine. These metabolites are excreted in the urine with 5–8% of pentazocine excreted

unchanged in the urine. The elimination half-life ranges from 1.5 to 10 h.

Mechanism of Toxicity

Pentazocine is an opioid agonist and a partial opioid antagonist. It produces an analgesic effect by stimulating kappa and sigma receptors. It increases serotonin activity through sigma receptor agonism and produces partial opioid antagonism by inhibiting mu receptors. Pentazocine also potentially inhibits dopamine receptors and increases norepinephrine turnover.

Acute and Short-Term Toxicity (or Exposure)

Animal

In cases of acute exposure, dogs have experienced mild to moderate salivation, slight transient ataxia, fine tremors, and (infrequently) tonic convulsions. Horses, following intravenous administration, have experienced slight to moderate ataxia, localized muscular twitching, slight perspiration, unsteady gait, excitability, and nervousness.

Human

Acute toxicity induced by pentazocine is primarily associated with central nervous system (CNS) effects that include dizziness, anxiety, hallucinations, mood alterations, and seizures. Respiratory depression, increased P_aCO_2 levels, pulmonary edema, and apnea may occur. Tachycardia, increased systolic and diastolic blood pressure, pinpoint pupils, nausea, vomiting, and abdominal pain have also been reported. In a recently published case series, 40% of acute pentazocine overdose patients did not have the classic opioid toxidrome of CNS and respiratory depression with miosis.

Chronic Toxicity (or Exposure)

Human

Psychological and physical dependence may occur. Tolerance may develop, resulting in the need for higher and more frequent dosing. Some oral pentazocine preparations also contain naloxone (an opioid antagonist) to reduce parenteral abuse. Naloxone does not affect the efficacy of pentazocine administered by the oral route; naloxone does inhibit pentazocine's opioid effect if tablets are solubilized and injected. Pentazocine may be abused as a heroin alternative or in combination with other drugs. The most publicized combination was 'T's and Blues' (Talwin mixed with blue-colored pyribenzamine tablets). Since pentazocine can produce dependency, abrupt cessation may precipitate the opioid withdrawal syndrome. Chronic intramuscular pentazocine injections have been associated with fibrous myopathy and localized neuropathy.

In Vitro Toxicity Data

Pentazocine is used as a Sigma1 ligand in receptor binding studies. These ligands are of interest because of their potential role in the development of substance abuse syndromes as well as because of the changes in receptor quantity and binding affinity seen in humans and in animal brains during aging.

Clinical Management

Cardiac and respiratory stabilization are the first priorities following pentazocine poisoning. The patient's airway should be patent and adequate ventilation assured. If the patient has either inadequate ventilation or a poor gag reflex, then the patient may be at risk for subsequent CO_2 narcosis with worsening acidosis or aspiration. If necessary, endotracheal tube intubation should be performed. Close monitoring of the patient's pulmonary exam should be performed to assure that pulmonary edema does not develop. The health care providers should place the patient on continuous cardiac monitoring with pulse oximetry and make frequent neurological checks.

Gastrointestinal decontamination should be considered for patients who have ingested pentazocine only after initial supportive care has been provided and airway control has been assured. Activated charcoal ($1\,g\,kg^{-1}$) may be administered. Syrup of ipecac is contraindicated after overdose with pentazocine due to the potential for rapid clinical deterioration. Gastric lavage is not indicated. Naloxone can be infused in an attempt to reverse respiratory and CNS depression. Naloxone administration may precipitate opioid withdrawal and should be administered slowly. Recent case series have demonstrated that naloxone may not result in clinical improvement in the majority of patients who have overdosed on pentazocine.

See also: Drugs of Abuse; Heroin.

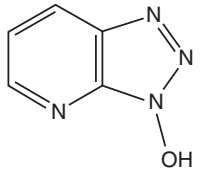
Further Reading

Challoner KR, McCarron MM, and Newton EJ (1990) Pentazocine (Talwin®) intoxication: Report of 57 cases. *Journal of Emergency Medicine* 8: 67–74.

Peptide Coupling Agents

Sang-Tae Kim

- Representative Chemicals: 1-Hydroxy-7-azabenzotriazole; *O*-(7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; 7-Azabenzotriazol-1-yl-*N*-oxy-tris(pyrrolidino)phosphonium hexafluorophosphate
- Chemical Abstracts Service Registry Numbers: CAS 39968-33-7; (1-Hydroxy-7-azabenzotriazole); CAS 148893-10-1 (*O*-(7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate); CAS 156311-83-0 (7-Azabenzotriazol-1-yl-*N*-oxy-tris(pyrrolidino)phosphonium hexafluorophosphate)
- Synonyms: HOAt: 1-Hydroxy-1*H*-v-triazolo[4,5-b]pyridine; 7-Aza-1-hydroxybenzotriazole; [1,2,3]Triazolo[4,5-*b*]pyridin-3-ol; 3-Hydroxy-3*H*-1,2,3-triazolo[4,5-*b*]pyridine HATU: 1*H*-1,2,3-Triazolo[4,5-*b*]pyridinium, 1-[bis(dimethylamino)methylene]-, hexafluorophosphate(1-), 3-oxide; HATU may crystallize as guanidinimum *N*-oxides (*N*-form), rather than the isomeric uranium structures (*O*-form) depending on storage conditions: *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridine-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate. PyAOP: Tri-1-pyrrolidinyl(3H-1,2,3-triazolo[4,5-b]pyridin-3-yloxy)-, hexafluorophosphate
- Chemical Structure:



HOAt

Uses

HOAt, as additives, and HOAt-based peptide coupling reagents, as activators, are used in peptide bond formation reactions both in solution and solid phase synthesis.

There are many different types of peptide coupling reagents (e.g., carbodiimides, aminium/uranium salts, and phosphonium salts). The choice of method(s) and reagent(s) depend on a variety of factors, including the specific sequence of peptide to be synthesized, the preferred method of deprotection, the preferred solvents, and the type of active intermediate desired.

Exposure Routes and Pathways

Exposure to HOAt- and HOAt-based peptide coupling reagents is most likely to occur in occupational settings. Inhalation and dermal exposure are the primary routes of exposure.

Acute and Short-Term Toxicity (or Exposure)

Animal

HOAt was nontoxic to rats when dosed at $2000\,mg\,kg^{-1}$ via the oral route. HOAt was not a skin irritant in rabbits. In rabbits, HOAt was found to be a slight eye irritant. Studies in guinea pigs revealed that HOAt did not induce contact allergy. HOAt did not induce micronuclei in bone marrow polychromatic erythrocytes in male mice when evaluated in the *in vivo* micronucleus assay.

HATU showed no mortality, but limited toxicity, to rats when dosed at $2000\,mg\,kg^{-1}$ via the oral route. HATU was not a skin irritant in rabbits. In rabbits, HATU was found to be a slight eye irritant. Studies in guinea pigs revealed that HATU induced contact allergy.

PyAOP was nontoxic to rats when dosed at $2000\,mg\,kg^{-1}$ via the oral route. PyAOP was not a skin irritant in rabbits. In rabbits, PyAOP was found to be a very severe eye irritant. Studies in guinea pigs revealed that PyAOP induced contact allergy.

Human

The principal hazard of concentrated HOAt-based peptide coupling reagents is the potential to cause contact allergy. Signs and symptoms of exposure to HATU may include skin rashes, 'red face', itching, local swelling, headaches, coughing, eye irritation and swelling, respiratory congestion, shortness of breath, and flue-like symptoms. Symptoms may progress to include severe dyspnea, cephalgia, rhinorrhea, flatulence, obstruction of bronchial airways, and permanent fine whistle of bronchial airways. The likelihood and severity of adverse health effects due to exposure to HATU depends on (1) the concentration in the air, (2) how long the individual is exposed, and (3) the individual's susceptibility to the effects of HATU.

Prolonged eye contact to the solid form of HOAt may cause eye irritation. Information on the toxic effects of HOAt by other routes of exposure in humans is not available.

In Vitro Toxicity Data

HOAt was mutagenic to *Salmonella typhimurium* and *Escherichia coli* strains.

HOAt was weakly mutagenic in mouse lymphoma L5178Y cells, in the absence of S9 mix. In the presence of S9 mix, the evidence was inconclusive. HATU and PyAOP were not mutagenic to *S. typhimurium* and *E. coli* strains.

Clinical Management

The victim should be removed from the exposure environment. Exposed skin and eye should be copiously flushed with water and thoroughly decontaminated to prevent further absorption. Contaminated clothing and shoes should be removed and isolated at the site.

Ecotoxicology

HOAt showed no significant inhibition in a study designed to evaluate the effects on the respiration rate of sewage sludge microorganisms contained in activated sludge. HOAt and HATU were not readily biodegradable when evaluated in 28 day modified Sturm tests.

See also: Skin.

Further Reading

Han S-Y and Kim Y-A (2004) Recent development of peptide coupling reagents in organic synthesis. *Tetrahedron* 60: 2447–2467.

Perchlorate

David R Mattie

Published by Elsevier Inc.

- Representative Chemicals: Ammonium perchlorate, Sodium perchlorate, Potassium perchlorate
- Chemical Abstracts Service Registry Numbers: CAS 14797-73-0 (Perchlorate); CAS 7790-98-9 (Ammonium perchlorate); CAS 7601-89-0 (Sodium perchlorate); CAS 7778-74-7 (Potassium perchlorate)
- Chemical/Pharmaceutical/Other Class: Ternary salts of alkali metals containing oxygen
- Chemical Formulas: ClO_4^- (Perchlorate); NH_4ClO_4 (Ammonium perchlorate); $NaClO_4$ (Sodium perchlorate); $KClO_4$ (Potassium perchlorate)
- Chemical Structures:

Uses

The primary source of perchlorate is the ammonium salt. Ammonium perchlorate is the oxidizer ingredient in solid propellant mixtures for rockets, missiles, and munitions. Other uses of perchlorate salts include medicine, matches, metal cation chemistry, and pyrotechnics (illuminating and signaling flares, colored and white smoke generators, tracers, incendiary delays, fuses, photo-flash compounds, and fireworks). Perchlorate is also found in lubricating oils, finished leather, fabric fixer, dyes, electroplating, aluminum refining, manufacture of rubber, paint and enamel production, as an additive in cattle feed, in magnesium batteries, and as a component of automobile air bag inflators.

Exposure Routes and Pathways

Perchlorate has been found in soil, surface and groundwater, at locations where perchlorate salts were manufactured, stored, or used. The salts are highly water soluble, fully ionize in water, and result in a perchlorate ion that is identical whether it comes from the ammonium, sodium, or potassium salt. The primary route of exposure for perchlorate is through ingestion of water from contaminated public drinking water supplies across the United States. Occupational exposure of workers during the commercial production or use of ammonium perchlorate is higher than potential exposures from drinking water sources. Exposure to perchlorate is primarily through inhalation of ammonium perchlorate dust with systemic absorption through mucous membranes in the respiratory and gastrointestinal tracts. Some ingestion through the oral route is possible, as is dermal contact, although significant absorption of perchlorate through intact skin is unlikely.

Toxicokinetics

Studies of absorption, distribution, metabolism, and elimination to measure perchlorate kinetics revealed

that there was no metabolism of perchlorate in either adult rats or humans. Perchlorate is rapidly excreted, with urinary half-lives on the order of 4 h in the rat and 6 h in humans. Kinetic studies were also conducted for fetal and lactational time points in rats. Kinetic studies were designed to aid quantitative interspecies extrapolation as well as form the basis for physiologically based pharmacokinetic (PBPK) models for adult rats and humans, as well as pregnant and lactating rats.

Mechanism of Toxicity

The perchlorate ion, because of its similarity to iodide in ionic size and charge, competes with iodide for uptake into the thyroid gland. At therapeutic dosage levels (100–1000 mg day^{-1}), this competitive inhibition results in reduced production of the thyroid hormones T_3 and T_4 and a consequent increase in thyroid stimulating hormone (TSH) via a negative feedback loop involving the thyroid, pituitary, and hypothalamus. The competitive inhibition of iodide uptake is the only direct perchlorate effect on the thyroid, leading to a reversible chemical-induced iodine deficiency. Inhibition of iodide uptake in the thyroid of adult male rats dosed intravenously was detected at a dose as low as 0.01 mg kg^{-1} perchlorate.

Acute and Short-Term Toxicity (or Exposure)

Animal

A 90 day subchronic bioassay determined that the thyroid was the only target organ in male and female rats exposed to perchlorate in drinking water (0, 0.01, 0.05, 0.2, 1.0, and 10 mg kg^{-1} day^{-1}) for 90 days. The no-observed-adverse-effect level (NOAEL) based on thyroid changes was 1 mg kg^{-1} day^{-1} but hormone changes, decreased T_4 and increased TSH, were still seen at lowest doses.

Developmental neurotoxicity studies exposed pregnant rats to perchlorate in drinking water (0, 0.1, 1.0, 3.0, and 10 mg kg^{-1} day^{-1}) during pregnancy through day 10 of lactation. No pup behavioral effects were seen except equivocal motor activity at one time point. An additional motor activity study with the same doses found no statistically significant effects in motor activity. However, Bayesian statistical analysis on the results of the two different motor activity studies combined resulted in a NOAEL at 1.0 mg kg^{-1} day^{-1}. Hormone changes, decreased T_4, increased TSH, were again seen at lower doses. Brain histology and morphometry observations in developmental studies are equivocal.

Genotoxicity assays showed that perchlorate is not genotoxic or mutagenic. Perchlorate is not a teratogen as no birth defects were found at doses as high as 100 mg kg^{-1} day^{-1} in the rabbit or as high as 30 mg kg^{-1} day^{-1} in the rat. Immunotoxicity studies were motivated by case reports of aplastic anemia and leukopenia in humans when perchlorate was used as an antithyroid drug. Studies using female mice did not demonstrate any adverse effects to the immune system. Evaluation of thyroid responses identified no alterations in T_3 and TSH, while T_4 was decreased after exposure to 1.0, 3.0, or 30 mg kg^{-1} day^{-1}. Thyroid changes detected histologically were not seen in all animals until the 30 mg kg^{-1} day^{-1} dose.

Human

Two 14 day studies were conducted in which 10 mg day^{-1} was provided in water to 10 male subjects and 3 mg day^{-1} was provided in drinking water to 8 male subjects. In both studies, each subject served as their own control by having measurements taken before and after perchlorate consumption. Iodide-123 was measured in the thyroid to obtain inhibition data, and iodide and perchlorate were determined in blood and urine. Perchlorate, at both 3 and 10 mg day^{-1}, caused inhibition of iodide uptake into the thyroid (38% and 10%, respectively). There were no changes seen in TSH or thyroid hormone levels in the blood. The extrapolated no-observed-effect level for iodide inhibition was 2 mg day^{-1} based on these two exposures.

Another 14 day study employed 10 subjects (5 male/5 female) for each dose (0.5, 0.1, 0.02, and 0.007 mg kg^{-1} day^{-1}) who also served as their own control. The parameters measured were iodide-123 uptake in the thyroid for inhibition data and iodide and perchlorate in blood and urine for kinetic data. There were no changes seen in TSH or thyroid hormone levels in the blood. The result of the iodide inhibition measurements was a NOAEL of 0.007 mg kg^{-1} day^{-1}, resulting in 4.8% iodide inhibition (equivalent to 0.5 mg day^{-1} perchlorate exposure). Data from these studies were used to develop the human PBPK model for perchlorate.

Chronic Toxicity (or Exposure)

Animal

A two-generation reproductive toxicity study was used to evaluate fertility in adult rats and viability/toxicity in their offspring. Reproductive parameters were tested over two generations of drinking water exposure to perchlorate. The reproductive NOAEL is

greater than the highest dose tested, 30 mg kg^{-1} day^{-1}. Thyroid histology changes were seen starting at 3 mg kg^{-1} day^{-1}. There were three rare benign thyroid tumors seen in two first generation (F1) pups at the 30 mg kg^{-1} day^{-1} dose.

Human

Employees were examined at an ammonium perchlorate production facility in Nevada and their findings compared to those of a large control population from the same chemical complex. The average working-lifetime cumulative dose in the higher exposure group was estimated to be 38 mg kg^{-1}. Based on both cumulative and single-shift perchlorate exposures there were no adverse effects on thyroid, kidney, liver, or bone marrow function. A cross-sectional health study of two similar worker populations, a group of ammonium perchlorate workers in three exposure groups and a comparison group of other workers from the same industrial complex, was conducted at a perchlorate manufacturing plant in Utah. More than 40% of the workers had been working with perchlorate for more than 5 years. There were no effects on blood and clinical chemical parameters at any level of exposure up to 34 mg day^{-1}.

School-age children were examined in three cities in Northern Chile where levels of perchlorate in the water supply were undetectable, 5–7, and 100–120 ppb. No changes were found in hormone levels, prevalence of goiter between cities, congenital hypothyroidism, and clinical differences between children from the three different cities.

Perchlorate has been found in one Southern Nevada county at levels averaging 14 μg l^{-1} and as high as 24 μg l^{-1}. The congenital hypothyroidism data from the neonatal screening program for 1996 and 1997 were examined, and no increase in congenital hypothyroidism was observed in the county. The monthly mean T_4 levels of neonates from Las Vegas (an area with perchlorate-contaminated drinking water at 9–15 ppb (μg l^{-1}) for eight of those months and nondetectable (i.e., <4 ppb) for 7 months) were compared with those of neonates from Reno (an area with no detectable perchlorate in its drinking water) for the 15 month period of April 1998 through June 1999. There were no differences in neonatal T_4 levels between Las Vegas and Reno. An analysis of the neonatal TSH levels of newborns from Las Vegas and Reno was conducted for those born between December 1998 and October 1999 with a birth weight of 2500–4500 g and sampled within the first month of life. The mean blood TSH levels were not different in Las Vegas versus Reno. Another Las Vegas versus Reno comparison examined prevalence rates among Medicaid-eligible residents for simple goiter, nodular goiter, thyrotoxicosis, congenital hypothyroidism, acquired hypothyroidism, thyroid cancer, or other thyroid diseases. Again there were no differences between Las Vegas and Reno.

Environmental Fate

Perchlorate is exceedingly mobile in aqueous systems and can persist for many decades under typical ground and surface water conditions. Plants and vegetables grown in perchlorate-containing soil or water may incorporate the perchlorate, posing a potential exposure if such vegetation is consumed.

Other Hazards

Safety concerns with ammonium perchlorate are greater than toxicity concerns because of its explosive potential.

Exposures Standards and Guideline

There is no occupational standard for perchlorate but the Occupational Safety and Health Administration regulates perchlorate as a nuisance dust, with an 8h time-weighted average permissible exposure limit of 15 mg m^{-3}. Studies have demonstrated that occupational exposures to perchlorate have not been hazardous to the health of workers at manufacturing plants.

There are no environmental standards for perchlorate; however, the Office of Research and Development of the US Environmental Protection Agency (ORD/EPA) released the 1999 Interim Guidance on June 18, 1999 because of significant concerns and uncertainties that needed to be addressed in order to finalize a human health oral risk benchmark for perchlorate. That guidance recommended that agency risk assessors and risk managers continue to use the standing provisional reference dose range of 0.0001–0.0005 mg kg^{-1} day^{-1} (4–18 ppb) for perchlorate-related assessment activities. In the absence of a finalized oral health risk benchmark for perchlorate, but in light of ongoing assessment activities by EPA, states, and other interested parties, the ORD/EPA reaffirmed this guidance on January 22, 2003 with an added suggestion to carefully consider the low end of the provisional 4–18 ppb range.

See also: Ammonium Perchlorate.

Further Reading

Motzer W (2001) Perchlorate: Problems, detection, and solutions. *Environmental Forensics* 2(4): 301–311.

Soldin O, Braverman L, and Lamm S (2001) Perchlorate clinical pharmacology and human health: A review. *Therapeutic Drug Monitoring* 23: 316–331.

Teitelbaum D (2000) The halogens: Section 12.0 – Ternary salts of alkali metals containing oxygen. In: Bingham E, Cohrssen B, and Powell CH (eds.) *Patty's Toxicology*, 5th edn., vol. 3, pp. 791–799. New York: Wiley.

Urbansky E (2002) Perchlorate as an environmental contaminant. *Environmental Science and Pollution Research* 9: 187–192.

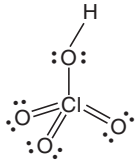
Von Burg R (1995) Perchlorates. *Journal of Applied Toxicology* 15: 237–241.

Wolff J (1998) Perchlorate and the thyroid gland. *Pharmacological Reviews* 50: 89–105.

Perchloric Acid

Samantha E Gad

- Chemical Abstracts Service Registry Number: CAS 7601-90-3
- Synonyms: Dioxonium perchlorate; Hydronium perchlorate
- Chemical/Pharmaceutical/Other Class: Halogenated acid
- Chemical Formula: $ClHO_4$
- Chemical Structure:



Uses

Perchloric acid is used to separate potassium from sodium, and in many laboratory tests and industrial processes. Uses for the salts of perchloric acid include explosives and plating metals. Perchloric acid may explode, and it decomposes on heating producing toxic and corrosive fumes. The substance is a strong oxidant and reacts violently with combustible and reducing materials, organic materials and strong bases, causing a fire and explosion hazard. It attacks many metals forming flammable/explosive gas. The acid is unstable if the concentration is over 72%; it may explode by shock or concussion when dry or drying. Mixtures with combustible material such as paper may ignite spontaneously at room temperature. Water should never be poured into perchloric acid; when dissolving or diluting always add perchloric acid slowly to the water. A mixture of perchloric acid and acetic anhydride exploded in a Los Angeles factory in 1947, killing 15, injuring 400, and causing $2 million in damage.

Exposure Routes and Pathways

Contact with skin and mucous membranes are the exposure pathways. Inhalation of mist formed from solutions is possible.

Toxicokinetics

Perchloric acid can be absorbed into the body by inhalation and by ingestion.

Mechanism of Toxicity

Perchloric acid's corrosive properties and ability to cause tissue oxidation are mechanisms of toxicity. Perchlorate (ClO_4^-) disrupts endocrine homeostasis by competitively inhibiting the transport of iodide (I^-) into the thyroid through the sodium iodide symporter. Potential human health risks exist from chronic exposure to perchlorate via drinking water. Such risks may include hypothyroidism, goiter, and mental retardation (if exposure occurs during critical periods in neurodevelopment).

Acute and Short-Term Toxicity (or Exposure)

Animal

Perchloric acid is a severe irritant and is corrosive to eyes, skin, and mucous membranes. The oral LD_{50} is $1100\,mg\,kg^{-1}$ in rats and $400\,mg\,kg^{-1}$ in dogs. The subcutaneous LD_{50} in mice is $250\,mg\,kg^{-1}$.

Human

Perchloric acid is corrosive to eyes, skin, and mucous membranes. Ingestion may produce mild to moderate oral and esophageal burns with more severe burns occurring in the stomach. Symptoms of lung edema might not become manifest until a few hours have passed, and are aggravated by physical effort.

Chronic Toxicity (or Exposure)

Human

Chronic exposure can cause bronchial irritation, gastrointestinal disturbances, rash, possible sensitization, dental erosion, and jaw necroses. Inhalation can cause upper respiratory tract effects and possible dyspenea and hemoptysis.

Clinical Management

Contaminated clothing should be removed and exposed body surfaces should be washed thoroughly. Respiratory assistance should be given if necessary. If ingested, a conscious patient should be given large amounts of water and 1 oz milk of magnesia if available. Vomiting should not be induced. Eyes should be flushed with large amounts of water if eye contact occurs. Treatment is the same as for exposure to any strong inorganic acid.

Ecotoxicology

Perchloric acid may be toxic to aquatic life. Degradation products are toxic.

See also: Acids; Ammonium Perchlorate; Corrosives.

Relevant Websites

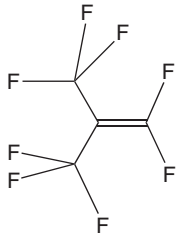
http://www.auburn.edu – Perchloric Acid (adapted from the *CRC Handbook of Laboratory Safety*).

http://www.inchem.org – International Programme on Chemical Safety (IPCS). Perchloric Acid (IPCS International Chemical Safety Card 1006).

Perfluoroisobutylene

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 3812-21-8
- Synonyms: Octafluoro-*s*-butene; Octafluoroisobutylene; PFIB; Isobutene; Octafluoroisobutene; Perfluoroisobutene
- Chemical/Pharmaceutical/Other Class: Perfluorocarbon
- Chemical Formula: C_4F_8
- Chemical Structure:



Uses

Perfluoroisobutylene or perfluoroisobutene (PFIB) is the monomer used in synthesis of Teflon®. It has possible use as chemical warfare agent, that is, it is a schedule 2A substance under the Chemical Weapons Convention (CWC).

Exposure Routes and Pathways

PFIB is produced by the pyrolysis, and as a by-product during the manufacture of 'Teflon' fluorocarbon resins, for example, polytetrafluorethylene (PTFE). Inhalation is the major exposure pathway.

Toxicokinetics

PFIB is readily absorbed. The retention in rats of inhaled PFIB in the upper airways and lungs was found to be ~25% of the amount inspired at the concentrations tested.

Mechanism of Toxicity

PFIB is a strong electrophile which reacts with nucleophiles. The toxicity of PFIB may be correlated with its susceptibility to nucleophilic attack and the generation of reactive intermediates.

Acute and Short-Term Toxicity (or Exposure)

Animal

Similar to phosgene but reported to be 10 times as acutely lethal. When PFIB is inhaled it produces a fulminating and sometimes fatal pulmonary edema similar to that of phosgene after a latent period of several hours. The rat LC_{50} is 500 ppb for a 6 h exposure. Lung injuries caused by the inhalation of PFIB have been examined in a study where rats were exposed to 50, 83, 90, 110, or 200 mg m^{-3} of PFIB for 10 min. At exposure to 90 mg m^{-3} or more, lung injuries began to be detected histologically within

hours after exposure, with the latency periods being inversely proportional to PFIB concentrations, so that at the high concentration of 200 mg m^{-3} no latency period was detectable. Significant accumulations of edema fluid were not apparent until 9 h after PFIB exposure. Significant amounts of fibrin were detected in alveolar spaces at 18–24 h after exposure, but no fibrin was evident at 48–72 h. Significant increases in alveolar macrophages (AMs) were observed at 10 h after exposure, with peak increases between 24 and 48 h postexposure. Even at 1 h after exposure, the alveolar epithelial cells and endothelial cells showed abnormal vacuolation and blebbing. Progressively, the alveolar surface was denuded, leading to edema and extravasation. The AM appeared relatively insensitive to the toxic effects of PFIB.

Human

The toxicity observed is called Polymer fume fever. The first symptom of poisoning is a cough and difficult breathing immediately after inhaling the fumes. The symptomology becomes progressively worse. Pathological changes in the lungs occur in the first 4–6 h postexposure and increase in severity in the first 2 days. Improvement occurs on the fifth to sixth days.

Chronic Toxicity (or Exposure)

Animal

Rats exposed to 0.1 ppm PFIB for 6 h day^{-1}, 5 day week^{-1} for 2 weeks showed no compound-related pathological changes and only mild respiratory impairment and restlessness during their exposure. A repeat study using the same experimental conditions (0.1 ppm) found no effects in rats.

Human

No information could be found on chronic toxicity of PFIB in humans.

Clinical Management

Exposure should be terminated and supportive management provided.

Ecotoxicology

PFIB decomposes rapidly when dissolved in water, forming various reactive intermediates and fluorophosgene, which then decomposes into carbon dioxide, a radical anion and hydrogen fluoride.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists, (US) Occupational Safety and Health Administration, and the (US) National Institute for Occupational Safety and Health have not set exposure standards for PFIB. Although there is no hygienic standard for the safe handling and use of 'Teflon' fluorocarbon resins, specifically PTFE and fluorinated-ethylene–propylene polymers manufactured by the DuPont Company, a publication from the American Industrial Hygiene Association many years ago noted that a maximum atmospheric concentration of 15 mg m^{-3} may be tolerated over an 8 h period on a nuisance basis without significant hazard, since the oral and inhalation toxicities of the undecomposed polymers are practically nil. Further, it noted that: (1) decomposition products appear only at temperatures above 200°C, (2) no practical way has yet been devised to express safe concentrations of the various possible mixtures of the decomposition products, which include PFIB, (3) above 250°C, toxicologically significant amounts of these products are evolved and polymer fume fever may result from exposure to them or from smoking Teflon-contaminated cigarettes, and (4) the decomposition products become flammable above 690°C.

See also: Combustion Toxicology; Phosgene.

Further Reading

Lehnert BE, Archuleta D, Behr MJ, and Stavert DM (1993) Lung injury after acute inhalation of perfluoroisobutylene: Exposure concentration–response relationships. *Inhalation Toxicology* 5: 1–32.

Maidment MP and Upshall DG (1992) Retention of inhaled perfluoroisobutene in the rat. *Journal of Applied Toxicology* 12: 393–400.

Patocka J and Bajgar J (1998) Toxicology of perfluoroisobutene. *The Applied Science and Analysis Newsletter.*

Smith LW, Gardner RJ, and Kennedy GL Jr. (1982) Short-term inhalation toxicity of perfluoroisobutylene. *Drug and Chemical Toxicology* 5: 295–303.

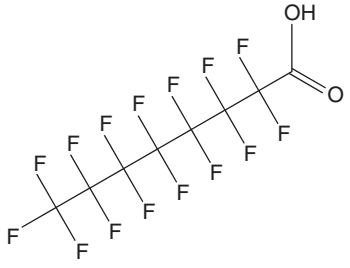
Van Helden HPM, van de Meent D, Oostdijk JP, *et al.* (2004) Protection of rats against perfluoroisobutene (PFIB)-induced pulmonary edema by curosurf and *N*-acetylcysteine. *Inhalation Toxicology* 16: 549–564.

Perfluorinated Chemicals *See* Perfluorooctanoic Acid (PFOA).

Perfluorooctanoic Acid (PFOA)

Cathy Villaroman and Ruth Custance

- Chemical Abstracts Service Registry Numbers:
  - Free acid ($X = OM^+$; $M = H$) (CAS 335-67-1)
  - Ammonium salt ($X = OM^+$; $M = NH_4$) (CAS 3825-26-1)
  - Sodium salt ($X = OM^+$; $M = Na$) (CAS 335-95-5)
  - Potassium salt ($X = OM^+$; $M = K$) (CAS 2395-00-8)
  - Silver salt ($X = OM^+$; $M = Ag$) (CAS 335-93-3)
  - Acid fluoride ($X = F$) (CAS 335-66-0)
  - Methyl ester ($X = CH_3$) (CAS 376-27-2)
  - Ethyl ester ($X = CH_2–CH_3$) (CAS 3108-24-5)
- Synonyms: 1-Octanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-PFOA; Pentadecafluorooctanoic acid; Pentadecafluoro-1-octanoic acid; Perfluorocaprylic acid; Perfluoroheptanecarboxylic acid; Perfluorooctanoic acid; Perfluoro-*n*-octanoic acid; Hexanoyl fluoride, 3,3,4,4,5,5,6,6,6-nonafluoro-2-oxo-; Pentadecafluoro-*n*-octanoic acid; Perfluoroctanoic acid; PFOA; Fluorad FC-26; 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-Pentadecafluorooctanoic acid
- Chemical Formula: $C_8HF_{15}O_2$
- Chemical Structure:



Uses

Perfluorooctanoic acid, also known as PFOA, is used as a processing aid in the manufacture of fluoropolymers, which are used in a wide variety of consumer and industrial applications, including nonstick surfaces on cookware. However, finished products are not expected to contain PFOA. It may also be formed during the degradation of related chemicals, such as small polymers called telomers, which are used in a range of commercial products including fire fighting foams, as well as soil-, stain-, and grease-resistant coatings on carpets, textiles, paper, and leather.

Exposure Routes and Pathways

PFOA has been detected in workers and it has also been measured in the general population. The highest levels reported to date in the general population are similar to some of the lowest levels in workers exposed to PFOA occupationally. Neither the environmental concentrations of PFOA nor the pathways of exposure to the general population are known. The limited geographic locations of fluorochemical plants making or using the chemical suggest that there may be additional sources of PFOA in the environment and exposures beyond those attributable to direct releases from industrial facilities. But whether human exposures are due to PFOA in air, water, on dusts or sediments, in dietary sources, or through some combination of routes is currently unknown.

Toxicokinetics

Animal studies have shown that the ammonium salt of PFOA (ammonium perfluorooctanoate; APFO) is well absorbed following oral and inhalation exposure, and to a lesser extent following dermal exposure. PFOA distributes primarily to the liver and plasma and may be detected in the blood stream after exposure. It does not partition to the lipid fraction or adipose tissue. PFOA is not metabolized and there is evidence of enterohepatic circulation of the compound.

Gender differences were observed in the elimination of PFOA in rats. The urine is the major route of excretion of PFOA in females, while the urine and feces are both main routes of excretion in males. Moreover, estimates of the serum half-life range from 1.9 to 24 h in female rats, while estimates of the serum half-life range from 4.4 to 9 days in male rats. Elimination of PFOA appears to be biphasic in female rats; a fast phase occurs with a half-life of ~2–4 h while a slow phase occurs with a half-life of ~24 h. The rapid excretion of PFOA by female rats is reportedly due to active renal tubular secretion (organic acid transport system), which is thought to be hormonally controlled. Hormonal changes during pregnancy do not appear to change the rate of elimination in rats.

Additionally, substantial differences have been observed in the half-life of PFOA in rats, monkeys, and humans. The gender and species differences are not completely understood; therefore, the extent of potential risks to humans is uncertain.

Mechanism of Toxicity

PFOA is thought to induce peroxisome proliferation and interfere with mitochondrial metabolic pathways. Direct measurements revealed that PFOA uncouple mitochondrial respiration by increasing

proton conductance. At sufficiently high concentrations, PFOA had the capacity to interfere with mitochondrial respiration by causing a slight increase in the intrinsic proton leak of the mitochondrial inner membrane, which resembled a surfactant-like change in membrane fluidity. The protonated nitrogen atom with a favorable pK_a is reportedly essential for the uncoupling action of perfluorooctane sulfonamides in mitochondria, which may be critical to the mechanism by which these compounds interfere with mitochondrial metabolism to induce peroxisome proliferation *in vivo*.

Acute and Short-Term Toxicity (or Exposure)

Animal

Most animal toxicity studies have been conducted with APFO, the most widely used salt of PFOA. Several animal toxicity studies have been conducted in rodents and monkeys and have shown that APFO exposure can result in a variety of toxic effects in animals including liver toxicity, developmental toxicity, and immunotoxicity.

Recent studies show organ weight changes among laboratory animals exposed to PFOA *in utero* and into early adulthood. The prenatal developmental toxicity studies in rats resulted in death and reduced body weight after exposure to oral doses of $100\,mg\,kg^{-1}\,day^{-1}$ or by inhalation to $25\,mg\,m^{-3}$ of APFO. There was no evidence of developmental toxicity after oral exposure to doses as high as $150\,mg\,kg^{-1}\,day^{-1}$, while inhalation exposure to $25\,mg\,m^{-3}$ resulted in reduced fetal body weights. In a rabbit oral developmental toxicity study, a significant increase in skeletal variations was observed after exposure to $50\,mg\,kg^{-1}\,day^{-1}$ APFO. No evidence of maternal toxicity at $50\,mg\,kg^{-1}\,day^{-1}$ was observed at the highest dose tested.

Human

APFO is a skin, eye, nose, and throat irritant. Evidence suggests that skin permeation can occur in amounts capable of producing the effects of systemic toxicity. Eye contact may cause eye irritation with discomfort, tearing, or blurring of vision. Inhalation may cause irritation of the upper respiratory passages, with coughing and discomfort. APFO ingestion could cause weight loss, gastrointestinal irritation and enlarged liver, or abnormal blood forming system function with anemia. Individuals with preexisting diseases of the liver or bone marrow may have increased susceptibility to the toxicity of excessive exposures.

A nonstatistically significant increase in estradiol levels in workers with the highest PFOA serum levels (>30 ppm) was reported; however, none of the other hormone levels analyzed indicated any adverse effects. At PFOA manufacturing plants where the serum PFOA levels were lower, cross-sectional and longitudinal studies found positive significant associations between PFOA and cholesterol and triglyceride levels. In addition, a positive, significant association was reported between PFOA and T3 hormone and a negative association with high-density lipoprotein in the cross-sectional study. These results must be interpreted carefully, as the studies conducted to date have many limitations.

Chronic Toxicity (or Exposure)

Animal

In a two-generation reproductive study, rats exposed to PFOA showed significant increases in absolute and relative liver and kidney weights, but reproductive indices were not affected in this F0 generation. In F1 animals exposed to PFOA *in utero* through early adulthood, a significant reduction in mean body weight was observed at the lowest doses tested. In F1 female rats, there was a significant increase in post-weaning mortality, a significant decrease in mean body weight, and a significant delay in sexual maturation at $30\,mg\,kg^{-1}\,day^{-1}$. Significant decreases in body weights and body weight gains, and significant changes in absolute liver and spleen weights and in the ratios of liver, kidney, and spleen weights-to-brain weights were observed in all treated F1 male groups. The lowest-observed-adverse-effect level (LOAEL) for the F1 females was $30\,mg\,kg^{-1}\,day^{-1}$, and the no-observed-adverse-effect level (NOAEL) was $10\,mg\,kg^{-1}\,day^{-1}$; the LOAEL for F1 males was $1\,mg\,kg^{-1}\,day^{-1}$ and an NOAEL was not determined. These gender differences in sensitivity are presumably related to gender differences in PFOA elimination. At the higher doses in the rat reproductive study (1 ppm), mortality was observed in 12% male and 10% female offspring. However, no excess mortality was found in F1 generation adult rats, which suggests that PFOA is less toxic in adults. The F2 generation pups were sacrificed at weaning; therefore, no treatment-related effects were observed.

Repeated exposures to APFO, the most widely used salt of PFOA, produced liver, kidney, pancreas and testes changes, anemia, and cyanosis. Tests in male rats demonstrated weak tumorigenic activity based on an increased incidence of benign testicular, pancreatic, and liver tumors. Tests in animals demonstrate no developmental toxicity.

Rodent bioassays have shown that chronic APFO (most widely used salt of PFOA) exposure is associated with a variety of tumor types. The mechanisms of APFO tumorigenesis are not clearly understood. The US Environmental Protection Agency (EPA) is currently evaluating the scientific evidence and has not reached any conclusions on the potential significance to humans of the rodent cancer data.

Human

Several epidemiological studies on the effects of PFOA in humans have been conducted on workers. These studies did not examine developmental outcomes. A retrospective cohort mortality study demonstrated a statistically significant association between prostate cancer mortality and employment duration in the chemical facility of a plant that manufactures PFOA. However, this result was not observed in an update to the study in which more specific exposure measures were used.

In Vitro Toxicity Data

In vitro mutagenicity assays with *Salmonella typhimurium* and *Saccharomyces cerevisiae* were conducted to evaluate the potential toxicity of APFO. All *in vitro* assays were negative for APFO mutagenicity.

Clinical Management

APFO is a skin, eye, nose, and throat irritant. If exposure to fumes from overheating or combustion occurs, the victim should be moved to fresh air and monitored for irritation. If eye or dermal contact occurs, affected areas should be flushed thoroughly with water for at least 15 min. The victim should be observed for resulting skin irritation. A physician should be consulted if symptoms persist. If ingestion occurs, two glasses of water should be immediately given and vomiting should be induced.

Environmental Fate

PFOA and related compounds are known to be highly persistent and widely distributed in the environment, and that they bioaccumulate. They do not further degrade and can remain in the body or environment for an extended period of time after exposure. PFOA also does not hydrolyze or photolyze under environmental conditions.

Although fluorinated telomers are not made using PFOA, some data indicate that certain telomers may break down or degrade to form PFOA in the environment, and may be metabolized to form PFOA if they manage to enter living organisms. Fluorinated telomers are small fluorine-containing polymers, synthetic chemicals produced by a specific process that utilizes the ability of certain chemicals to link together into chains of a defined length.

Ecotoxicology

Ammonium perfluorooctanoate can be very toxic to aquatic organisms and may cause long-term effects in the aquatic environment. It may produce a milky appearance if released into surface waters. The 96 h lethal concentration (LC_{50}) of APFO is 766 mg l^{-1} for fathead minnows and 569 mg l^{-1} for bluegill sunfish.

Other Hazards

Inhalation of fluoropolymer fumes from overheating or burning the resin may cause 'polymer fume fever'. High temperatures, such as in sintering operations, may release APFO vapors, which may condense as a solid or as a liquid solution in the oven, exhaust duct or stack, or on other cool surfaces.

Exposure Standards and Guidelines

The US EPA has entered into enforceable consent agreements with some parties under the Toxic Substance Control Act to control PFOA in the environment.

The occupational exposure standards and guidelines for APFO include the following:

American Conference of Governmental Industrial Hygienists threshold limit value of 0.01 mg m^{-3}.

The US Occupational Safety and Health Administration permissible exposure limit: not established.

See also: Blood; Carcinogenesis; Developmental Toxicology; Liver.

Further Reading

US EPA (2003) Environmental News: EPA Intensifies Scientific Investigation of a Chemical Processing Aid. Office of Pollution Prevention & Toxics (OPPT). April 14.

US EPA (2003) Fact Sheet. PFOA Q's & A's. Office of Pollution Prevention & Toxics (OPPT). April 14.

US EPA (2003) Perfluorooctanoic Acid (PFOA), Fluorinated Telomers. 18626 Federal Register, Vol. 68, No. 73, Wednesday, April 16.

US EPA (2003) Preliminary Risk Assessment of the Developmental Toxicity Associated with Exposure to Perfluorooctanoic Acid and Its Salts. Office of Pollution Prevention & Toxics (OPPT), Risk Assessment Division. April 10.

Relevant Websites

http://www.coating4ind.com – Material Safety Data Sheet. Manufacturer's Name: Coatings For Industry, Inc. Revised 9/00; reviewed 1/04.

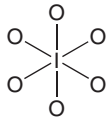
http://www.ewg.org – PFCs, A Family of Chemicals that Contaminate the Planet. Environmental Working Group.

Perfumes *See* Fragrances and Perfumes.

Periodic Acid

Samantha E Gad and Shayne C Gad

- Chemical Abstracts Service Registry Numbers: CAS 10450-60-9; CAS 13444-71-8
- Synonym: Paraperiodic acid
- Chemical/Pharmaceutical/Other Class: Inorganic acid; Periodate
- Chemical Formula: H_5IO_6
- Chemical Structure:



Uses

Periodic acid is used in organic synthesis. It is also used to fortify the wet strength of papers such as photographic paper, and in methods (e.g., oxidation in periodic acid followed by staining with Schiff's reagent) for staining tissues and cells for histopathology.

Exposure Routes and Pathways

Dermal contact, ingestion, and inhalation are possible routes of exposure.

Mechanism of Toxicity

Periodic acid is corrosive. It is also an oxidizing agent.

Acute and Short-Term Toxicity (or Exposure)

Human

The estimated lethal dose in humans is $1\,ml\,kg^{-1}$. Periodic acid is a strong corrosive to the skin, eyes, and mucous membranes. Symptoms of overexposure include respiratory distress, headache, nausea, and vomiting.

Clinical Management

The affected areas should be washed with copious amounts of water. If periodic acid has been ingested and the patient is conscious, the mouth should be washed with water and plenty of water given to drink.

Ecotoxicology

The US Environmental Protection Agency categorizes periodic acid as a corrosive hazardous waste. It is likely to reduce to iodides in the environment, and is possibly harmful to aquatic species.

See also: Corrosives.

Relevant Website

http://www.jtbaker.com – Periodic Acid (Material Safety Data Sheet from Mallinckrodt Baker, Inc.).

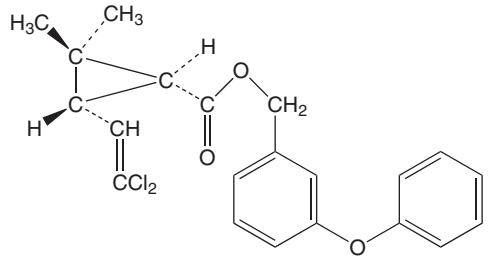
Permethrin

Paul R Harp

- Chemical Abstracts Service Registry Number: CAS 52645-53-1
- Synonyms: 3-Phenoxybenzyl-(1*R*,1*S*)-*cis*,*trans*-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropanecarboxylate; Adion; Ambush; Assithrin; Cliper; Coopex; Corsair; Dragnet; Dragon; Eksmin; Kafil; Pounce; FMC 33297; OMS 1821; NRDC 143;

SHA 109701. The *cis*-isomer is cispermethrin (NRDC 148) and the *trans*-isomer is biopermethrin (NRDC 147)

- Chemical/Pharmaceutical/Other Class: Type I pyrethroid insecticide
- Chemical Structure:



Uses

Permethrin is a broad-spectrum insecticide used in a variety of agricultural and commercial/residential applications. It is also used to control termites, ectoparasites on animals, and head lice and scabies (in combination with sulfur) in humans. Permethrin is available as a dust, smoke, wettable powder, emulsifiable concentrate, concentrate for ultra low volume application and as a lotion (for treatment of head lice).

Exposure Routes and Pathways

Human exposure to permethrin most commonly occurs through dermal contact.

Toxicokinetics

Pyrethroids are poorly absorbed through the skin and are only moderately absorbed in the gastrointestinal tract. One study estimated dermal absorption of permethrin to be $\leqslant 2$ mg per 12 h. Metabolism of permethrin occurs through hydrolysis, ester cleavage, and conjugation of the metabolites with glucuronic acid, glycine, or sulfuric acid. Isomeric configuration influences the rate of release from adipose tissues as well as the primary route of excretion (urinary versus fecal).

Mechanism of Toxicity

Several mechanisms of action have been identified for the pyrethroids with the primary mechanism related to a selective high affinity for membrane sodium channels. Closing of the channel, which ends the action potential, is slowed resulting in a prolonged 'tail' current and repetitive firing of presynaptic and accompanying postsynaptic cells following a single action potential. High enough doses can cause complete depolarization and blockade of nerve conduction. Permethrin also inhibits Ca^{2+},Mg^{2+}-ATPase.

Acute and Short-Term Toxicity (or Exposure)

Animal

In mammals, permethrin produces type I motor symptoms generally characterized by hyperexcitation, enhanced startle response, tremors, and prostration.

Human

Reports of human exposure have indicated only skin irritation.

Chronic Toxicity (or Exposure)

Chronic effects following permethrin exposure in humans have not been reported. US Environmental Protection Agency classifies permethrin as a possible human carcinogen based on findings of lung adenomas and combined adenomas/carcinomas and liver adenomas in mice.

Clinical Management

Exposed skin should be washed promptly with soap and water. Dermal application of vitamin E oil preparations may be used for both prophylaxis and treatment of paresthesia. For contact with eyes, eyes should be flushed immediately and for an extended period with generous amounts of clean water or saline. Gastric lavage is indicated if patient has ingested a large amount of pyrethroids and can be treated soon after exposure. For ingestion of smaller amounts or if treatment has been delayed, activated charcoal and catharsis are indicated. Seizures can be treated with intravenous benzodiazepines (diazepam or lorazepam); phenytoin or phenobarbital may be helpful for recurrent seizures. No specific antidotes for pyrethroid-induced neurotoxic effects have been approved for use in humans. Spontaneous recovery usually occurs with mild or moderate intoxication.

Environmental Fate

Permethrin is of low to moderate persistence in the soil (half-life of 30–38 days). Permethrin is readily degraded in most soils except those rich in organic matter, with microbial degradation predominant. Permethrin is tightly adsorbed to soil with little leaching and low mobility. Permethrin degrades rapidly

in water: the half-life in estuarine water was less than 3 days. Permethrin has little phytotoxic potential.

Ecotoxicology

Fish and crustaceans are extremely sensitive to pyrethroid compounds in laboratory settings. However, various factors (e.g., sediment binding) may reduce pyrethroid toxicity to these nontarget organisms in a natural environment.

Exposure Standards and Guidelines

The acceptable daily intake and reference dose for permethrin are both $0.05\,mg\,kg^{-1}\,day^{-1}$.

See also: Neurotoxicity; Pesticides; Pyrethrins/Pyrethroids.

Further Reading

Ray DE (2001) Pyrethroid insecticides: Mechanisms of toxicity, systemic poisoning syndromes, paresthesia, and therapy. In: Krieger R (ed.) *Handbook of Pesticide Toxicology*, 2nd edn., pp. 1289–1303. San Diego, CA: Academic Press.

Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Permethrin.

Permissible Exposure Limit *See* Occupational Exposure Limits.

Peroxisome Proliferators

Abraham Dalu and Harihara M Mehendale

Introduction

One of the most rapidly developing areas of organelle biology, which has a major involvement in biochemical pharmacology, is the research into peroxisomal function. The process of xenobiotic-induced proliferation of the cytoplasmic organelle, the peroxisome, in mammalian liver cells has received considerable attention because of the proposed relationship between the induction of hydrogen peroxide (H_2O_2)-producing peroxisomal enzymes and the development of hepatocellular carcinomas in mice and rats. In rodents, the number and the size of peroxisomes are dramatically increased in the liver and, to a lesser extent, the heart and kidney in response to treatment with a variety of different amphipathic acids, including hypolipidemic drugs and plasticizers, which are collectively referred as 'peroxisome proliferators' (PPs). They encompass a diverse group of compounds with dissimilar structures capable of producing pleiotropic responses in experimental animals. The pleiotropic responses are predominantly observed in hepatocytes and are characterized by enlargement of the liver, increases in a relative number of peroxisomes in a cell, marked induction of fatty acid β-oxidation, carnitine acetyltransferase, lauric acid ω-hydroxylation activities, and hypolipidemia. Long-term exposure to PPs is associated with the induction of hepatocellular carcinoma. Currently, there are over 100 known PPs in use including herbicides, industrial solvents, plasticizers, and hypolipidemic agents. Peroxisome proliferation can occur in many organs and tissues. The effects of PPs have been also reported in the kidneys, the heart, the prostate, the pancreas, and the thyroid. Immune reaction may also be observed. Although a significant advance has been made in this area over the past few years, there is much yet to be investigated to elucidate the molecular and cellular mechanisms underlying PPs associated with hepatocellular carcinoma.

Peroxisomes

Peroxisomes, cytoplasmic organells of $\sim 0.5\,\mu m$ in diameter, are ubiquitous in eukaryotes. Peroxisomes consist of a single membrane that separates them from the cytosol, and participate in several important metabolic functions, including simple respiration characterized by H_2O_2 production and H_2O_2 degradation, β-oxidative chain shortening of long chain and very long-chain fatty acids, metabolism of glyoxalate, degradation of uric acid; and synthesis of ether phospholipids, cholesterol, and bile acids. Peroxisomes were discovered by Christian de Duve in 1965. The term 'peroxisome' was introduced to

emphasize the biochemical property of H_2O_2 production by the peroxisomes as a result of respiration mediated by peroxisomal oxidases such as d-amino acid oxidase and fatty acyl-CoA. Unlike lysosomes, peroxisomes are not formed in the Golgi apparatus, but self-replicate by dividing peroxisomes, normally enlarge, then divide. Peroxisomes are 0.2–1 μm and are most abundant in the liver.

The biogenesis of the peroxisome requires the formation of a lipid bilayer, the import of membrane proteins into that bilayer, and the transport of soluble proteins across the membrane into the peroxisomal matrix. Among the different organisms in which this process has been studied, 24 genes have been identified that encode proteins involved in peroxisome biogenesis. These genes are called *PEX* genes (*PEX1*, *PEX2*, *PEX3*, etc.), and their protein products are termed peroxins. Peroxisomes are ubiquitous single-membrane organelle present in all organelles both in animal and plant cells which exhibit numerous oxidases involved in several catalytic and anabolic pathways such as β-oxidation of very long fatty acids. Peroxisomes are also involved in bile acid synthesis, cholesterol synthesis, plasmalogen synthesis, amino acid metabolism, and purine metabolism. The importance of the peroxisome and these processes is underscored by the existence of numerous genetic disorders associated with defects in the peroxisome, which can be divided into two categories.

One of the main functions of peroxisomes is to detoxify the cell by splitting hydrogen peroxide. They contain the enzyme catalase. Catalase converts H_2O_2 (hydrogen peroxide, a toxic by-product of cellular metabolism) to H_2O and O_2, with $4H_2O_2 \rightarrow 4H_2O + 2O_2$. Peroxisomes also degrade fatty acids and toxic compounds and catalyze the first two steps required in the synthesis of ether-linked phospholipids (which are later used to build membranes) and sterols. In addition, it plays a role in isoprenoid biosynthesis and amino acid metabolism.

Although the mitochondria are the primary site of oxidation for dietary and storage fats, the peroxisomal oxidation pathway is responsible for the oxidation of very long-chain fatty acids, β-methyl branched fatty acids, and bile acid precursors. The peroxisomal pathway also plays a role in the oxidation of dicarboxylic acids. In addition, it plays a role in isoprenoid biosynthesis and amino acid metabolism. Peroxisomes are also involved in bile acid biosynthesis, a part of plasmalogen synthesis and glyoxylate transamination. Furthermore, the literature indicates that peroxisomes participate in cholesterol biosynthesis, hydrogen peroxide-based cellular respiration, purine, fatty acid, long-chain dicarboxylic acid, prostaglandin, and xenobiotic metabolism. Currently, there are ~ 50 known enzymes associated with mammalian peroxisomes including catalase, oxidases (H_2O_2 generators), acetyltransferases (carnitine acetyl-CoA and carnitine octanoyl-CoA), dehydrogenases (NAD and NADP), and others (enoyl-CoA hydratase, thiolase, fatty acetyl-CoA synthetase). Thus, any chemical capable of disrupting these enzymes perturbs the normal functioning of peroxisomes, leading to long-term adverse health effects. Several techniques are available to identify these organelles in hepatocytes and other cells. One such technique is the recently developed immunohistochemical protocol using antibodies raised against peroxisomal enzymes, or the 'protein A-gold' method.

Peroxisome Proliferators

PP in mammalian cells, first described over 30 years ago, represents a fascinating field of research. Agents known as PPs exert peroxisome proliferation through binding to the steroid hormone receptors known as PP-activated receptors (PPARs). They are an important group of chemicals that include certain hypolipidemic drugs, plasticizers, and pollutants. The term 'PP' was introduced by Reddy and coworkers in 1975 to designate a drug or xenobiotic which induces the proliferation of peroxisomes in the liver cells. PPs are structurally diverse compounds, which induce peroxisome proliferation. Many of these agents are known rodent liver tumor promoters and debate exists as to whether humans are at increased cancer risk following exposure to PPs. They have been shown to regulate hepatic lipid metabolism via activation of the PP-activated receptor alpha (PPARα). Recent studies have revealed that PPs also exert considerable influence on certain extrahepatic tissues, including adipose tissue and lymphoid organs, in an indirect fashion. Inhibition of the proliferation of thymocytes and splenocytes and alteration of fatty acid uptake into and release from adipose tissue might be consequences of the hypolipidemic effect of PPs involving both PPARα-dependent and -independent pathways. Exposure to PPs reduces the cholesterol content of circulating low-density lipoprotein (LDL), which is the major supply of this steroid to most peripheral tissues. In addition, PPs increase serum levels of high-density lipoprotein (HDL), which extracts cholesterol from peripheral tissues and returns it to the liver, thereby further decreasing the cholesterol content of peripheral tissues.

Earlier studies with the hypolipidemic agent clofibrate revealed that it induced a marked

hepatomegaly in male rats. Fine structural changes seen primarily as a massive increase in the number of dense particles or 'microbodies' were first described by Paget. Catalase and three H_2O_2-producing oxidases (urate oxidase, D-amino acid oxidase, and 1-α-hydroxy acid oxidase) had been found to cosediment with liver cell fractions containing particles identical to microbodies, and the term 'peroxisome' was coined to describe this organelle as a site of compartmentalized peroxide metabolism.

Currently, over 100 compounds have been identified as PPs. The literature indicates that induction of peroxisome proliferation is not limited to exogenous chemicals. A number of endogenous substances, such as the steroid hormones, thyroid hormones, morphogenes, and fatty acids, are also involved in peroxisome proliferation. Peroxisome proliferation in hepatic parenchymal cells of rats and mice following the administration of clofibrate has been reported by numerous investigators. Compounds that are structurally unrelated to clofibrate, such as acetaminophen and Wy-14,643, can also cause peroxisome proliferation (**Table 1**). The industrial solvent trichloroethylene, the industrial plasticizers di(2-ethyl hexyl) phthalate (DEHP) and di(2-ethyl hexyl) adipate (DEHA), have also been found to be hepatic peroxisome proliferators.

Newer compounds of interest include the perfluoroalkanoic acids, the steroid hormones, and anticarcinogen dehydroepiandrosterone; some structurally related leukotriene D_4 antagonists; certain chlorinated hydrocarbons, primarily those metabolized to tri- or di-chloro acetic acid; as well as structurally unrelated herbicides such as tridiphane, lactofen, and several of the chlorophenoxy acids (2,4-dichlorophenoxy acetic acid and 4-chloro-2-methylphenoxy acetic acid) (**Table 1**). PPs comprise chemicals of wide structural dissimilarity (**Figure 1**) and share a common property of inducing characteristic effects in the liver of treated rats and mice. Within a few days of exposure they produce a striking dose-dependent hepatomegaly accompanied by characteristic proliferation of the peroxisomal and microsomal compartments as assessed morphologically and biochemically.

Peroxisomes are also responsive to dietary and hormonal changes such as high-fat diets, particularly those with long-chain fatty acids, high-cholesterol diets, and vitamin E deficiency. Thyroid hormones produce moderate increases in the content of hepatic peroxisomes and peroxisomal enzymes. While these diets and physiological influences rarely induce changes in peroxisomes to the same extent as many xenobiotics, they are useful in exploring the mechanisms of regulation of peroxisomal proliferation.

Table 1 Selected representative PPs

Fibric acid hypolipidemic agents
Beclobric acid
Ciprofibrate
Clofibrate
Gemfibrozil
Simfibrate
Other xenobiotics
Bleached kraft mill effluents
Citral
Dimethrin
Garlic, ether extracts
Trichloroethylene
Nonfibric acid hypolipidemic agents
Gemcadiol
Niadenate
Tiadenol
Tibric acid
Wy-14,643
Herbicides
2,4-D (2,4-dichlorophenoxyacetic acid)
MCPA (2-methyl-4-chlorophenoxyacetic acid)
2,4,5-T (2,4,5-trichlorophenoxyacetic acid)
Lactofen
Tridiphane
Other drugs
Acetylsalicyclic acid
Benzobromarone
Bifonazole
Flurbiprofen
Valprioic acid
Other chlorophenoxy acids
2-Phenyopropionic acid
4-Chlorophenoxypropionic acid
4-Chlorophenoxybutyric acid
Fatty acid analogs
2,2,4,4,6,8,8-Heptamethylnonane
Perfluorobutyric acid
Perfluorodecanoic acid
Sorbic acid
Tetradecylthioacetic acid

Biomedical Responses to Acute Toxicity of PPs

Peroxisome proliferation is consistently associated with hepatomegaly, which arises from a combination of cellular hypertrophy and hyperplasia. Studies on fine structure of hepatocytes revealed that the increase in hepatocyte size was associated with the predominant increase in peroxisomes and modest increase in smooth endoplasmic reticulum (SER). Rats exposed to peroxisome proliferators exhibit 7- to 10-fold increases in peroxisomal relative volume and surface area as evidenced by morphometric analysis of liver sections. The increase in peroxisomal relative volume is due to the increases in both volume and number of peroxisomes. In contrast, the increase in SER surface area and volume rarely exceeds two-fold. The magnitude of increase in cellular DNA and

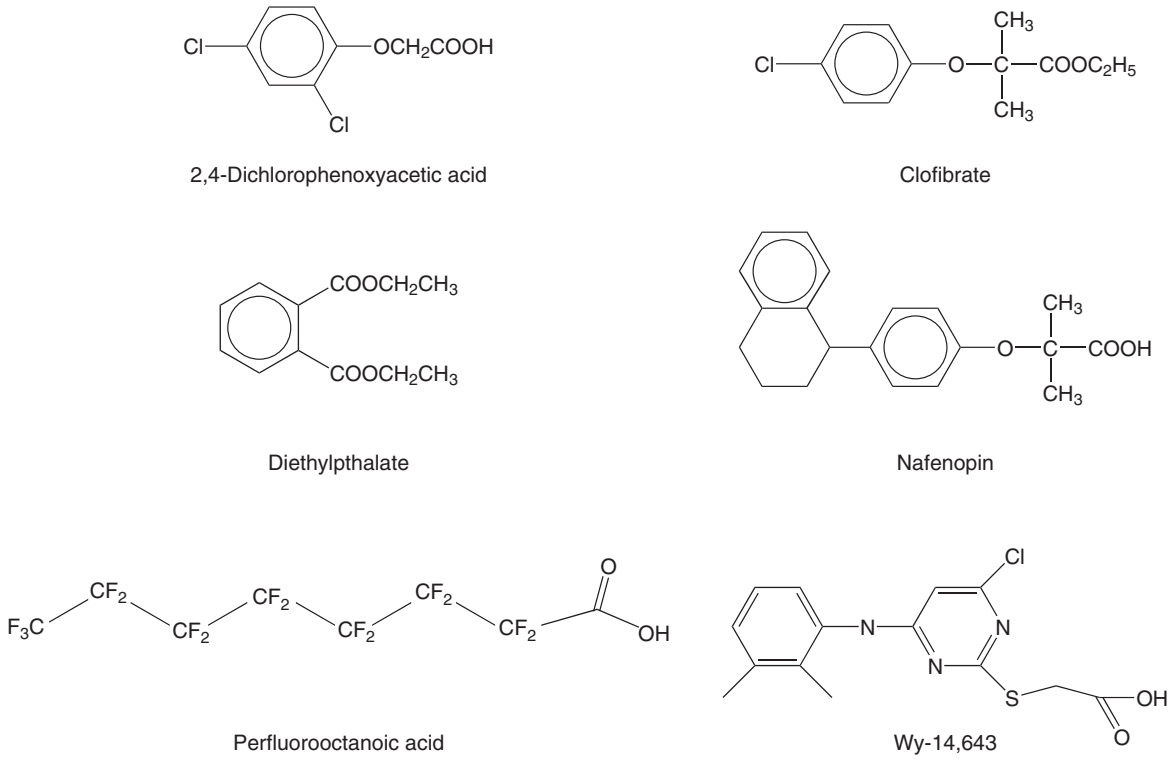


Figure 1 Structures of representative peroxisome proliferators.

peroxisomes is dose and compound dependent and correlates with the extent of hepatomegaly. Hyperplasia, however, does not correlate with the extent of peroxisomal proliferation. For example, clofibrate and DEHP produce little hyperplasia, while nefenopin and Wy-14,643 produce relatively extensive hyperplasia. Hypolipidemia is one of the characteristic responses to peroxisome proliferators. This can be evidenced by a remarkable decrease in triglyceride and cholesterol levels following exposure to nonhypolipidemic agents. Because of these properties, hypolipidemic drugs are primarily used to lower serum cholesterol and triglyceride. Earlier studies showed that clofibrate treatment increases liver carnitine acetyltransferase activity, but no correlation was established with peroxisome proliferation. It has also been reported by Moody and Reddy that an increase in carnitine acetyltransferase is a conforming response to a wide variety of peroxisome proliferators, with increase in specific activity ranging from 10- to 25-fold, the most predominant increase being in the peroxisomes. Increases in the medium- (5- to 10-fold) and long-chained (two- to fivefold) carnitine acetyltransferases also occur. Another obvious peroxisome response to peroxisome proliferators is increased β-oxidation of fatty acids in glyoxysome, a specific form of peroxisome, in germinated seeds which later was also reported to be present in rat liver peroxisome. Studies have shown that clofibrate treatment increases β-oxidation of fatty acids by $\sim$10-fold. Based on such studies, it has been concluded that β-oxidation of fatty acids is a generalized response to peroxisome proliferators.

Peroxisome proliferators are also involved in two other metabolic pathways of importance to lipid metabolism. Peroxisomes contain the most of dihydroxyacetone phosphate acetyltransferase and alkyldihydroxyacetone phosphate synthetase activities. Therefore, they are responsible for initiating most ether glycerolipid biosynthesis. These enzymes are also moderately induced by peroxisome proliferators. Induction of cytochrome P450s by peroxisome proliferators will be addressed separately.

Mechanism of Induction of Peroxisome Proliferation

Two widely accepted possible mechanisms for the induction of peroxisome proliferation are (1) activation of specific genes by the chemical or its metabolites, either directly or mediated by a specific receptor, and (2) substrate overload, either as a result of lipolysis occurring outside the liver and causing an influx of fatty acids into the liver or as a consequence of the peroxisome proliferators or their metabolites perturbing lipid metabolism.

The most likely mechanism of induction of peroxisome enzymes is interaction of peroxisome

proliferators with a cytoplasmic receptor of the hepatocytes. The peroxisome proliferator–receptor complex interacts with the chromatin to elicit selective increases in protein, translation of mRNA, and peroxisome-specific mRNAs. The second mechanism of peroxisome proliferation may be related to substrate overload in the hepatocytes of animals treated with various peroxisome proliferators. In rats, feeding of high-fat diet results in a very slight increase in peroxisome number. The administration of clofibrate or other peroxisome proliferators may lead to an influx of fatty acids into the liver as a result of lipolysis occurring outside the liver, or these compounds and their metabolites enhance the breakdown of triglycerides in the liver cell thereby causing an intrahepatic excess of fatty acids. The fatty acid overload may then trigger an increase in peroxisomal β-oxidation pathway.

In addition to the two previously mentioned mechanisms, some investigators have proposed another mechanism for induction of peroxisome proliferation. Peroxisomes contain a fatty acid β-oxidation system which preferentially oxidizes long-chain fatty acids (C_8–C_{20}). For example, the physicochemical properties of clofibric acid, the hydrolytic product of clofibrate, are very closely similar to those of naturally occurring C_{16}–C_{18} fatty acids. It is important, therefore, to examine whether the hypolipidemic drugs and/or their metabolites serve as substrates for the peroxisomal β-oxidation, thereby causing peroxisomal enzyme induction and possibly leading to increased production of H_2O_2 and other active oxygen species which ultimately lead to peroxisome proliferation.

Induction of Cytochrome P450s by PPs

Induction of the enzymes involved in xenobiotic biotransformation (phases I and II) is one of the characteristic responses to peroxisome proliferators. Attention has focused on these compounds initially because they were identified as epigenetic hepatocarcinogens in rodents. In addition, peroxisome proliferators received further attention as inducers of members of cytochrome P450 gene superfamily known to readily metabolize fatty acids. These metabolites have marked physiological activity, particularly those of arachidonic acid, which are vasoactive, regulate hormone release, and control renal ion flux. Therefore, regulation of cytochrome P450-dependent fatty acid hydroxylases by peroxisome proliferators is of particular interest in the field of physiology and pathophysiology. The cytochrome P450 gene superfamily consists of $\sim$250 different known genes which have substrate specificity in metabolizing a range of xenobiotics, including drugs, pesticides, food flavors and additives, and environmental chemicals. Furthermore, peroxisome proliferator-induced cytochrome P450s can also metabolize endogenous compounds other than fatty acids such as steroids, vitamins, and eicosanoids. It should be noted that only few of the cytochrome P450s induced by peroxisome proliferators have been isolated and studied. Cytochrome P450 4A1 in rat liver and P450 4A7 in rabbit lung are two such isozymes isolated and extensively characterized. Because a vast majority of endogenous and exogenous chemical substances are metabolized by cytochrome P450, their metabolism and effects can be modulated by exposure to peroxisome proliferators. The basic mechanism of action of peroxisome proliferators is shown in **Figure 2**.

Mechanisms of PP-Induced Cytochrome P450s and Other Enzymes

In general, the cytochrome P450 gene superfamily exhibits a range of induction mechanisms, including transcriptional gene activation, mRNA processing and mRNA stabilization, translational regulation, and protein stabilization. Current understanding indicates that xenobiotic-dependent transcriptional gene activation is the most common induction mechanism, and direct experimental evidence using nuclear run-on experiments has demonstrated that cytochrome P450 4A1 undergoes transcriptional gene activation by clofibrate. However, it is not clear whether or not the same induction mechanism is involved in the other cytochrome P450 4A gene superfamily. The question is then, do peroxisome proliferators directly activate the cytochrome P450 4A1 gene or do they require the intermediary of a protein factor/receptor to interact with the 5′ flanking regulatory element of the gene? Recent experimental findings suggest that peroxisome proliferator-induced cytochrome P450 and other enzymes are mediated through a receptor, better known as PPAR. The existence of multiple PPARs (PPAR-α, -β, and -γ) has been recently reported. These receptors are members of a superfamily that comprises at least 30 mammalian genes encoding receptors for the classical steroid hormones, thyroid hormones, vitamin D_3, and retinoic acid. These receptors have been implicated in the activation of some enzymes and have also been implicated in the activation of CYP4A6. Investigators have shown that the peroxisome proliferators' complex interacts with chromatin to result in selective increases in the transcription of peroxisomal fatty acid β-oxidation gene enzymes. The induction of P450 4A enzymes by PPs and fatty acids is now

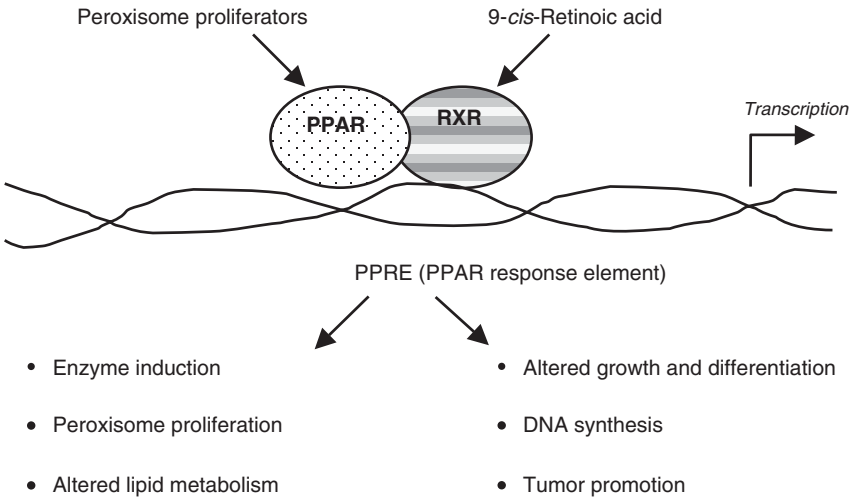


Figure 2 Basic mechanism of action of peroxisome proliferator-activated receptors. (Reproduced from Vanden Heuvel JP (1999) *Toxicological Sciences* 47: 1–8, with permission from The Society of Toxicology.)

known to be mediated by the PPARα that binds to response elements in target genes as a heterodimer with the RXR. The consensus sequence recognized by PPAR/RXR heterodimers contains an imperfect direct repeat of two nuclear receptor binding motifs separated by a single nucleotide. This repeat is preceded by a conserved A/T-rich sequence that is required for function. There is also evidence to indicate that the PPAR is inducible by the PP fenofibrate in rat liver as assessed by immunochemical methods and PPAR mRNA analysis by Northern blotting.

Peroxisome Proliferator-Activated Receptors

The past several years have seen an increasing interest in the PPARs, the subfamily of which consists of three isoforms by separate genes: PPAR alpha (α), PPAR delta (δ) (also referred to as PPAR beta (β)), and PPAR gamma (γ). PPARα was first cloned from mouse liver in 1990 by Issemann and Green; subsequently, Dreyer and associates cloned two other members of this subfamily (β (δ) and γ), which belong to the steroid receptor super-family. PPARs are now considered to be essential transcription factors regulating key cellular functions including lipid metabolism, xenobiotic metabolism, inflammation, cell differentiation, and cancer. These receptors are expressed in both embryonic and the adult organism. Each of the three PPAR subtypes is expressed in a distinct, tissue-specific pattern (**Table 2**) and differ considerably in their ligand-binding domains and specificities, attesting to the fact that they perform different functions in different cell types. PPARα is highly expressed in liver, skeletal muscle, kidney, heart and vascular wall, and brown adipose – tissues that are metabolically very active. PPARγ is expressed mainly in white and brown adipose tissue, large intestine, and spleen. In contrast to PPARα and PPARγ, which are abundantly expressed in just a few tissues, PPARδ is expressed in virtually all tissues at comparable levels and heterodimerized with retinoic acid X receptor (RXRα), another transcription factor activated by 9-*cis* retinoic acid. Like other members of the nuclear receptor superfamily, PPARα contains ~70 amino acid DNA-ligand-binding domain (LBD) of ~250 amino acids (**Figure 3a**). In addition to its ligand-binding capabilities, the LBD contains dimerization and transcriptional activation function 2 (AF-2), which is embedded in the extreme C-terminal portion of the receptor. The N-terminal domain of PPARα is less well characterized but appears to encode an additional transcriptional activation function.

Table 2 Tissue distribution of PPARs

PPAR isoform	*Liver*	*Intestine*	*Spleen*	*Fat*
α	++++	++++	+	–
β (δ)	++	+++	++	–
γ	–	++	+++	+++

PPARs contain a central cystein-rich zinc finger motif DNA-binding domain that recognizes DNA sequence elements, designated peroxisome proliferator response elements (PPREs), containing direct repeats of the hexanucleotide sequence AGGTCA separated by one nucleotide present in the 5′-flanking region of target genes. As shown in **Figures 2** and **3b**, PPARs bind to DNA as obligate

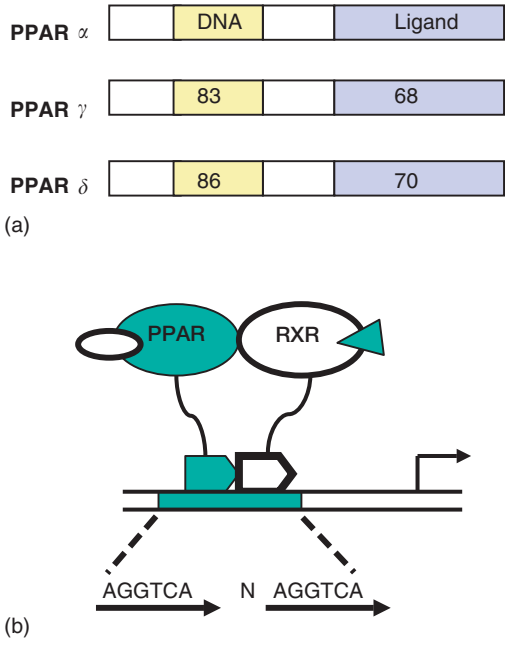


Figure 3 The PPAR family and its DNA properties. (a) The murine PPAR subfamily. The DNA and ligand-binding domains are indicated. Numbers represent percentage amino acid identity. (b) The PPARs bind to DR-1-type DNA response elements as heterodimers with RXR. The PPAR/RXR heterodimer can be activated by ligands for either PPAR or RXR. (Reproduced from Kliewer SA, Xu HE, Lambert MH, and Willson TM (2001) Peroxisome proliferator-activated receptors: From genes to physiology. *Recent Progress in Hormone Research* 56: 239–265, with permission from The Endocrine Society.)

heterodimers with the 9-*cis* retinoic acid receptors (RXRs). The PPAR/RXR heterodimers bind to two half sites of the consensus sequence AGGTCA. PPARs have been identified in the transcriptional regulatory regions of numerous genes involved in carbohydrate and lipid metabolism. There is emerging evidence that optimal binding sites differ slightly for each PPAR subtype. These subtle differences in binding site preference, together with differences with tissue expression patterns, undoubtedly contribute to the different biologies of the three PPAR subtypes.

PPARs (α and γ) are key regulators of lipid homeostasis and are activated by a structurally diverse group of compounds including fatty acids, eicosanoids, and hypolipidemic drugs such as fibrates and thiazolidinediones (see **Table 3** for representatives of the endogenous and exogenous ligands for PPARs). While thiazolidinediones and 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 have been shown to bind to PPARγ, although it is unclear whether other activators mediate their effects through direct interactions with the PPARs or via indirect mechanisms. The most conclusive evidence that PPARs are key elements in peroxisome proliferator-mediated events comes from

Table 3 PPAR ligands

PPARα	*PPARγ*
Exogenous PPAR ligands	
WY14,643	Indomethacin
Clorfibrate	Ibuprofen
Gemfibrozil	Piroxicam
Nafenopin	Pioglitazon
Bezafibrate	Ciglitazon
	Englitazon
	BRL-49653
Endogenous PARP ligands	
Palmitic acid	Arachidonic acid
Stearic acid	Eicosapentaenoic acid
Palmitoleic acid	PGJ2
Oleic acid	15 deoxy PGJ12
Linoleic acid	
Arachidonic acid	
Eicosapentaenoic acid	

studies with transgenic mice performed by Frank Gonzalez and colleagues at NCI. When PPARα expression was disrupted in transgenic mice, the response to peroxisome proliferators was greatly altered. In mice with abrogated PPARα expression the prototypical peroxisome proliferation, hepatomegaly, and induction of fatty acid metabolizing enzymes were not observed. In addition, PPARα-null mice were refractory to the carcinogenic effects of peroxisome proliferators. Therefore, PPAR is required for both the effects on gene expression as well as the tumor-promoting activity of peroxisome proliferators in rodents. PPARs also play an important role during rodent hepatocarcinogenesis, inflammation, atherosclerosis development, lipid metabolism, diabetes, and cancer.

Species Differences in Response to Peroxisome Proliferation

Studies with a number of peroxisome proliferators have shown a high degree of specificity in the responsiveness of species to peroxisome proliferators. The increase in growth and peroxisome proliferation seen in rat and mouse liver is not seen in guinea pigs, dogs, nonhuman primates, and more importantly, humans. In humans these findings have come from epidemiological studies with hypolipidemic drugs and from *in vitro* experiments with human hepatocytes. Fibrate acid derivatives such as clofibric acid and fenofibrate were among the first xenobiotics to be associated with increased numbers of peroxisomes and augmented enzymatic activity associated with this organelle. The peroxisome proliferator response is most notable in the liver and kidney, and also shows a strong species difference with rats

and mice being sensitive while humans are relatively refractory. Peroxisome proliferation, cytochrome P450 induction, and induction of peroxisomal enzymes by hypolipidemic agents and other chemicals such as DEHP is well-established in rats and mice. Hamster liver is also responsive to these compounds, although to a lesser extent. Examination of human liver biopsy material, obtained from patients receiving clofibrate, gemfibrozil, or fenofibrate, has demonstrated marginal or no increase in peroxisomal volume densities or numbers. These studies suggest that there is a marked species difference in sensitivity to chemicals that cause hepatomegaly and peroxisome proliferation. In general, rats and mice are most responsive; hamsters, chickens, and pigeons exhibit an intermediate responsiveness; and dogs, rabbits, marmosets, and rhesus monkeys are least responsive to peroxisome proliferators. Human peroxisomes are also insensitive to these compounds. In contrast, hypolipidemic effect is quite evident in all of the primates and most other species studied. One of the reasons for interspecies differences in response to peroxisome proliferators may be attributed to the existence of multiple PPARs, each having different ligand-binding specificity and being differentially regulated and expressed in a tissue- and species-specific manner. In mice, chronic exposure to PPs results in PPARα-mediated liver hypertrophy, hyperplasia, and carcinogenesis accompanied by a proliferation of peroxisomes. In contrast, humans exhibit a reduced sensitivity to PP pathogenesis. This could reflect >10-fold lower PPARα levels in humans relative to mice as well as differences in gene network regulated by PPARs. In addition, species differences in response to peroxisome proliferators are reproducible in cell cultures since hepatocytes from rats and mice respond *in vitro* to peroxisome proliferators, whereas monkey and human hepatocytes are far less sensitive. In sharp contrast, guinea pigs do not respond to peroxisome proliferators in both *in vivo* and *in vitro* systems. Therefore, this cell culture system may provide a good method to study the effects of peroxisome proliferators in humans.

Gender Differences in Peroxisome Proliferation

There appears to be a general misconception that female rats are not responsive to peroxisome proliferators such as clofibrate. This misconception was based on earlier studies in which male F-344 rats, but not females, maintained on 0.25% clofibrate diet for up to 4 weeks exhibited hepatic peroxisome proliferation. However, subsequent studies adequately demonstrated that clofibrate, when given at 0.5%, 1%, and 2% in a diet, resulted in a marked peroxisome proliferation in liver parenchymal cells of both male and female rats. Therefore, gender differences can be obviated by increasing the dose of the inducing agent. On the other hand, it would be interesting to investigate the nonresponsiveness of female rats to the low dose of clofibrate in terms of risk assessment. No gender differences in the induction of peroxisome were seen in adult, fetal, and neonatal rats treated with nafenopin or other potent PPs. In terms of gender-related differences in cytochrome P450 induction, recent studies have shown that P450 4A1 and 4A3 mRNAs are induced to a much greater extent in male compared to female rats following clofibrate treatment. Cytochrome P450 4A2 mRNA is altogether absent from the female rat liver. Male-specific expression of P450 4A2 mRNA was also observed in kidneys. These observations suggest that the lower responsiveness of female rats to clofibrate-induced PP may reflect the lower inducibility of the P450 4A fatty acid hydroxylase enzyme in female rats. There is no gender-related difference in the induction of P450 4A12 mRNA in mice treated with the potent PP methylclofenapate. Thus, gender-related differences in chemical-induced peroxisome proliferation are dependent by and large on species, strains, and doses of a given agent.

Recent studies indicate that there are gender-related differences in the induction of hepatic catalase activity by clofibrate. Catalase, which is located in peroxisomes, catalyzes the reduction of hydrogen peroxide to water either directly or using small molecules, such as ethanol, formate, or methanol, as electron donors. Basal and clofibrate-induced hepatic catalase activity in male Sprague–Dawley rats was reported to be higher as opposed to the corresponding levels in females. Induction of hepatic catalase activity by clofibrate was decreased by 50% in castrated males compared to intact male rats. Clofibrate-fed castrated male rats challenged with estradiol benzoate showed greatly diminished induction of catalase activity. Uninduced ovariectomized female rats had hepatic catalase activity levels comparable to those of induced intact females. A marginal increase in hepatic catalase activity was observed in induced ovariectomized females compared to ovariectomized control females. Furthermore, a substantial increase in hepatic catalase activity was seen in induced ovariectomized females challenged with testosterone propionate. These observations clearly demonstrate gender-related differences in the induction of hepatic catalase activity by clofibrate in rats depending on the exposure level. A basis for these different responses could be attributed to factors

such as gender-dependent metabolic pathways. Induction of acetyl-CoA oxidase activity by peroxisome proliferators has recently been shown to be receptor mediated. If induction of hepatic catalase activity by peroxisome proliferators is shown to also be receptor mediated, then hormonal status could potentially interact with signal transduction pathways, resulting in differences in induction of catalase and other biochemical endpoints between the genders. On the other hand, findings on the gender differences in the induction of catalase activity in other species and with other peroxisome proliferators are less compelling. Unlike clofibrate, other peroxisome proliferators show no consistent pattern of gender differences in induction of hepatic catalase activity in either mice or rats. Therefore, the observed differences may relate to effective dose of the inducer rather than responsiveness to peroxisome induction.

The mechanism of trichloroethylene-induced liver peroxisome proliferation and gender-related differences in response was investigated using a wild-type Sv/129 and PPARα-null mice. Trichloroethylene treatment ($0.75\,g\,kg^{-1}$ for 2 weeks by gavage) resulted in liver peroxisome proliferation in wild-type mice, but not in PPARα-null mice, suggesting that trichloroethylene-induced peroxisome proliferation is primarily mediated by PPARα. No remarkable sex difference was observed in induction of peroxisome proliferation, as measured morphologically, but a markedly higher induction of several enzymes and PPARα protein and mRNA was found in males. On the other hand, trichloroethylene induced liver cytochrome P450 2E1, the principal enzyme responsible for metabolizing trichloroethylene to chloral hydrate, only in males, which resulted in similar expression levels in both sexes after the treatment. Trichloroethylene influenced neither the level of catalase, an enzyme involved in the reduction of oxidative stress, nor aldehyde dehydrogenase, the main enzyme catalyzing the conversion to trichloroacetic acid. These results suggest that trichloroethylene treatment causes a male-specific PPARα-dependent increase in cellular oxidative stress.

Peroxisome Proliferators and Hepatocarcinogenesis

In recent years a growing concern has developed with regard to long-term exposure to hypolipidemic agents, certain herbicides and industrial plasticizers (DEHP and DEHA) and the possible effect on human health. These concerns have basically centered on the tumorigenic property of peroxisome proliferators. There is a large body of evidence to indicate an association between peroxisome proliferation and hepatocarcinogenesis in rats and mice. Hepatocellular carcinogenesis is a property of all peroxisome proliferators, with few exceptions after discounting any direct genotoxic action of these compounds. The increased production of H_2O_2, which may overwhelm protective enzymes within the hepatocyte and produce indirect genotoxic injury, and the propensity of peroxisome proliferators to induce hepatocyte replication have both been argued to contribute to the carcinogenic action of these compounds. Furthermore, there is no evidence for the covalent binding of peroxisome proliferators to DNA in experimental animals. The lack of covalent DNA binding and mutagenic activity suggests that peroxisome proliferators do not react directly with DNA to produce injury and that electrophilic species generated by peroxisome proliferators interact with non-DNA target. Therefore, since peroxisome proliferators do not directly interact with and impair DNA, their mechanism of action is considered to be nongenotoxic, and is classified as a novel class of epigenetic chemical carcinogen. The understanding of the carcinogenic process induced by peroxisome proliferators is a continuing challenge. It is generally believed that a major contributing factor to cancer formation by nongenotoxic carcinogens, including peroxisome proliferators, is altered gene expression. That is, these agents effect the expression of genes that regulate cellular growth and/or differentiation.

Furthermore, epigenetic carcinogens operate by mechanisms such as chronic tissue injury, immunosuppression, solid-state effects, hormonal imbalance, cocarcinogenesis, or promotional activity. In the presence of tumor-initiating agents, peroxisome proliferators accelerate tumor formation. The distinctive phenotypic markers (GGT-positive foci) of the early stages of hepatocarcinogenesis are not observed, suggesting that pathways specific to peroxisome proliferators underlie the transformation of rodent hepatocytes. Peroxisome proliferators such as Wy-14,643 and clofibrate promote tumors after cell initiation. Recent developments also indicate that commonly used phthalate ester plasticizers DEHA and DEHP are capable of inducing hepatocellular carcinoma in rats and mice. These observations are of a serious concern since ~400 million pounds of DEHP plasticizers are used every year in the United States and many more million pounds elsewhere in the world.

The molecular and cellular mechanism of peroxisome proliferator-mediated hepatocarcinogenecity is not well understood. However, several hypotheses have been proposed for the hepatocarcinogenecity of these compounds. Accumulated experimental

evidence does not favor any single triggering event to explain the hepatocarcinogenic process by peroxisome proliferators. Some of the hypotheses are calcium mobilization, a cascade of oncogene activation, sustained cell growth, increased turnover of specific hepatocyte population, the effect of the activated PPAR on the differentiation state, and the long-term consequences of the metabolic imbalance resulting from increased peroxisomal enzyme activities; oxidative stress may also be involved in the carcinogenic process. Oxidative stress in various forms can lead to activation of NFκB. Studies have shown that treatment of rats with ciprofibrate increased hepatic NFκB activity. Thus, it is possible that the oxidative stress induced by peroxisome proliferators is responsible for activation of NFκB. Overall, these data suggest that NFκB may play an important role in liver homeostasis. And, possibly regulation of hepatocyte proliferation following treatment with peroxisome proliferators.

Another hypothesis suggests the cell proliferation observed after peroxisome proliferators treatment is due to the expression and release of TNF-α from the Kupffer cells. Kupffer cells also contain NFκB and can become activated in response to oxidative stress. The hypothesis is that the oxidative stress produced in hepatocytes by peroxisome proliferator treatment may activate NFκB in Kupffer cells and lead to cytokine synthesis and release. It is this cytokine that stimulates neighboring hepatocytes to undergo mitogenesis. Ames and associates have proposed that chemical carcinogens or promoters that are not mutagenic in *Salmonella* mutagenicity tests interact with cellular membranes and may cause DNA damage through stimulation of arachidonic acid cascade or the induction of an oxidative burst and lipid peroxidation. It is likely that the carcinogenicity of halogenated compounds is owing to their ability to form radicals which cause lipid peroxidation. Because lipid peroxidation is a chain reaction, it causes the production of a considerable number of reactive oxygen species, such as the hydroxyl radicals ($OH^{\bullet}$), H_2O_2, and the superoxide radical (O_2^-), which can damage DNA.

Chronic exposure to peroxisome proliferators results in accumulation of autofluorescent lipofuscin pigments. The accumulation of lipofuscin pigments is indicative of increased lipid peroxidation and is generally related to increased production of biologically damaging free radicals such as $OH^{\bullet}$. Furthermore, peroxisome proliferators can alter the peroxisomal enzyme profile such that the output of the oxygen species produced can be enhanced in that the increase in the H_2O_2 destructive enzyme catalase is proportionally small compared to the peroxisomal volume and H_2O_2 generating fatty acid β-oxidation is increased by peroxisomal proliferators. Peroxisome proliferators also increase the activity of uricase, which results in decreased levels of uric acid, a powerful antioxidant which is a scavenger for oxygen radicals. While it is known that excessive H_2O_2 is formed in the liver as a result of sustained proliferation, it is not well known whether H_2O_2 or other reactive oxygen species are directly involved in hepatocarcinogenesis.

Relevance to Public Health

The effect of peroxisome proliferators on human health is a fundamental toxicological concern primarily due to the pervasive presence of these chemicals from clinical (hypolipidemic drugs), occupational and environmental sources (industrial plasticizers). Therefore, from the public health perspective the concern is the ultimate outcome from chronic occupational exposure and long-term therapeutic effects of peroxisome proliferators. The available evidence indicates that potent peroxisome proliferators are carcinogenic in rats and mice. However, neither the mechanism of proliferation nor the events leading to the development of hepatocellular carcinomas are sufficiently well understood. Since peroxisomal proliferation was not observed in nonhuman primates and certain other species in a preliminary screening study conducted several years ago with clofibrate, the peroxisome proliferator-induced carcinogenic effects have been readily dismissed by some as being of no importance to humans. The question, 'are rodents a good model for human risk?' cannot be answered directly in this instance due to large species differences in response to peroxisome proliferators. Species such as the mouse, rat, and hamster are responsive to peroxisome proliferation, whereas guinea pig and monkey are not. Importantly, the morphological effects of these chemicals in liver have not been seen in humans or in human hepatocytes in culture. Rat liver peroxisomal proliferation was characterized as a unique atypical phenomenon restricted to the biology of peroxisomes in these species. However, in light of recent evidence that peroxisomal proliferation can be induced in a wide range of species, including subhuman primates, it seems appropriate to consider the biological implications of peroxisomal proliferators and assess their risk to humans.

Hypolipidemic drugs are being developed with the assumption that reduction of elevated serum lipid is necessary in order to control mortality and morbidity associated with cardiovascular disease. Although the causal relationship between the level of certain serum lipids and the development of the atherosclerotic

lesion and its ischemic complications is supported by experimental and human studies, there is little proof that either prevention or amelioration of coronary heart and peripheral atherosclerotic disease is achieved by lipid-lowering therapy. Studies have shown that newer lipid-lowering agents reduce the short-term risk of nonfatal and fatal myocardial infarcts and other debilitating complications of hypolipidemias. However, this has to be balanced with any long-term delayed carcinogenic risk which usually develops in 10–20 years. Thus, patients should be informed of the risks/benefits related to hypolipidemic long-term therapy. In contrast to the hypolipidemic agents, there is limited evidence for carcinogenicity of industrial plasticizers such as DEHP and DEHA in experimental animals. However, because the two compounds are widely used in the formulation of plastics, they may present a wider danger to the general public. Additional studies are needed to establish the carcinogenicity of these industrial plasticizers.

Accumulated experimental evidence suggests that carcinogenic and proliferative effects of peroxisome proliferators may not be related. Thus, the assumption that lack of or a minimal peroxisome proliferative response observed in the liver of some animals or humans to therapeutic dose levels of hypolipidemic drugs poses no danger to humans could be misleading, if carcinogenesis by these drugs is not mediated by proliferated peroxisomes. On the other hand, if carcinogenesis is directly related to their ability to induce both hepatomegaly and peroxisome proliferation, carcinogenic risk to humans could be predicted with some assurance by quantitative morphometric analysis of the alterations in peroxisome volume, numerical densities, and by changes in the levels of H_2O_2-generating peroxisomal oxidases including the β-oxidation system.

In summary, substantial progress has been made over the past few years in understanding the cytoplasmic organelle peroxisome and factors that alter its normal functions. Peroxisome proliferator-induced increase in the liver peroxisomes is associated with an approximately two-fold increase in catalase activity and several-fold increases in the activity of the peroxisomal fatty acid β-oxidation system. It is also evident from the available literature that hepatic peroxisomal proliferation appears to be a carcinogenic event in rodents, and this may depend on the potency of the inducer. However, there is no single mechanism that is attributed to the peroxisome proliferation or carcinogenesis induced by these agents. The hypothesis that peroxisome proliferator-induced carcinogenesis is mediated by disturbances in subcellular organelle homeostasis requires continued investigational attention because of the importance of these hypolipidemic drugs and industrial plasticizers to our society. In conclusion, advances in molecular biology that led to the discovery of PPARs in 1990 significantly enhanced our understanding that peroxisome proliferators exert their effects through activation of PPARs. For example, humans express hepatic PPARα and this receptor functions nearly identical to its rodent counterpart. However, human cells do not respond identical to the effects of peroxisome proliferators. Whether or not humans are at risk to the tumor-promoting effects of peroxisome proliferators will not be realized until the sequence of events initiated by ligand activation of PPAR and ultimately resulting in altered parameters of growth and differentiation in sensitive species such as rat or mouse is delineated.

See also: Chlorophenoxy Herbicides; Liver.

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Personal Care Products *See* Cosmetics and Personal Care Products.

Pesticide Residues: Joint FAO/WHO Meeting *See* Joint FAO/WHO Expert Meetings (JECFA and JMPR).

Pesticides

Carey N Pope

The word pesticide literally means an agent used to kill an undesirable organism. In the amended US Federal Insecticide, Fungicide and Rodenticide Act, the definition of an 'economic poison' or pesticide was expanded to include

(1) Any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest (insect, rodent, nematode, fungus, weed, other forms of terrestrial or aquatic plant or animal life or viruses, bacteria, or other microorganisms, except viruses, bacteria, or other microorganisms on or in living man or other animals, which the Administrator declares to be a pest) and (2) any substance or mixture of substances intended for use a plant regulator, defoliant, or desiccant.

The major classes of pesticides in use today include herbicides, fungicides, rodenticides, insecticides, nematocides, acaricides, and molluscides. Prior to about 1940, pesticides were primarily inorganic chemicals (e.g., arsenic) and a few natural agents from plant origin (e.g., nicotine and pyrethrum). With the discovery of the insecticidal activity of DDT, however, a burgeoning increase in the development and utilization of synthetic organic chemicals occurred. From about 1940 to 1980, an exponential increase in the production and use of these synthetic pesticides was evident worldwide. The major chemical classes of pesticides in use today include inorganic and organic metals, chlorinated hydrocarbons, organophosphorus compounds, carbamates, pyrethroids, substituted phenols, substituted ureas, coumarins, organic acids, organic amides, triazines, and neonicotinoids. Currently, $\sim$1 billion pounds of $\sim$600 different pesticides (active ingredients) are produced each year in the United States alone, with total worldwide production estimated at $\sim$5 billion pounds.

Insects were the first major focus of pest control, whether to prevent the destruction of food or fiber crops or to limit the spread of insect vectors of disease. There is little doubt that the use of insecticides had a profound impact on the further development of civilization. The control of anopheline mosquitoes and malarial infection, as well as vectors for typhus, plague, and yellow fever, by DDT undoubtedly saved millions of lives. Over the past several decades, however, the use of herbicides has dramatically increased and such efforts have markedly altered the methods of modern agriculture. As a result, herbicides now represent the most extensively used class of pesticides in the United States. Some food and fiber crops reportedly increased yields by 300–600% after the introduction and widespread use of synthetic herbicides.

While the public health and economic benefits of synthetic pesticide use over the past 50 years are indisputable, these benefits have not been without costs. Widespread environmental contamination by DDT and other organochlorine pesticides, reaching global proportions, with concomitant deleterious effects on some members of the food web heralded the end of an era for their extensive use. DDT was banned from use in the United States in 1972 and most other organochlorines were subsequently banned, being replaced by the less environmentally persistent organophosphates and carbamates. While these agents had considerably lower abilities to accumulate in environmental and biological media, they tended to be much more acutely toxic and thus more hazardous to utilize. The pyrethroids are generally regarded as safer than the anticholinesterase organophosphates and carbamates, but still constitute a smaller proportion of total insecticidal use. In general, herbicides exhibit markedly lower acute mammalian toxicity than other classes of pesticides. The relative toxicities of these agents are generally scaled, however, on the basis of acute reactions. More recent findings suggest that many pesticides may have actions at lower levels of exposure that are more subtle in nature but with long-lasting consequences. For example, a number of studies suggest that the organophosphorus insecticide chlorpyrifos may alter neurodevelopmental processes in the mammalian brain and that those effects may not be elicited through the common mechanism of toxicity for this class of pesticides, that is, through acetylcholinesterase inhibition. While herbicides as a class typically elicit selective toxicity in plants with markedly less toxicity in mammalian species, the common herbicide and groundwater contaminant atrazine can disrupt luteinizing hormone and prolactin secretion

through direct action on the hypothalamic–pituitary axis, possibly altering reproductive success. Endocrine disruption by direct interaction with hormone receptors or alteration of hormone metabolism is a real concern for a number of pesticides in different chemical classes, with possible adverse health consequences for both wildlife and humans. Knowledge of the long-term health consequences of prolonged, low-level exposure to various pesticide classes is still limited. A major challenge for toxicologists in the future is the continued acquisition of data pertaining to the long-term effects of low-level pesticide exposures. In contrast, recent events worldwide heighten concern that easily accessible and common pesticides might be used in chemical terrorism, with either direct human health consequences or long-term environmental contamination.

See also: Carbamate Pesticides; Chlorophenoxy Herbicides; Federal Insecticide, Fungicide, and Rodenticide Act, US; Nematocides; Occupational Toxicology; Organochlorine Insecticides; Organophosphates; Pollution, Soil; Pollution, Water; Psychological Indices of Toxicity; Pyrethrins/Pyrethroids; Veterinary Toxicology.

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Relevant Website

http://www.epa.gov – US Environmental Protection Agency.

Petroleum Distillates

Stephen R Clough

- Representative Chemicals: Organic solvents
- Chemical Abstracts Service Registry Number: CAS 8052-41-3
- Synonyms: Petroleum distillates; Petroleum naphtha; Naphtha
- Chemical/Pharmaceutical/Other Class: Aromatic hydrocarbons

Uses

Petroleum distillates are used as general extractants and universal solvents for paints, varnishes, fats, furniture polishes, and waxes. They are also used as vehicles for medication and pesticide applications, as counter-irritants, and as degreasers, detergents, and fuel. Most can be purchased 'over the counter', particularly mineral spirits, which is a widely used solvent or diluent for nonlatex paints.

Background Information

The term 'petroleum distillates' generally refers to petroleum naphtha or petroleum ether, which contain the lower boiling fractions (boiling point range, 86–140°F) of petroleum, principally pentanes and hexanes, with minor amounts of paraffins ranging up to 13 carbons. Petroleum naphtha is also known by the following synonyms: Amsco H-J, Amsco H-SB, Benzin B-70, HI-Flash naphtha, hydrotreated naphtha, naphtha coal tar, naphtha, petroleum naphtha, solvent naphtha, petroleum benzin, petroleum-derived naphtha, petroleum oil, Super VMP, UN1255, UN1256, UN1270, UN2553.

A solvent obtained from higher boiling distillates (boiling point range, 203–320°F), generically known as ligroin or 'varnish-makers' and painters' naphtha' (VM&P naphtha), may also occasionally be referred to as 'petroleum distillates'. Other synonyms for this solvent include benzin, benzine (light petroleum distillate), benzoline, canadol, ligroin, painters naphtha, petroleum ether, petroleum spirit, refined solvent naphtha, Skellysolve F, Skellysolve G, UN1271, varnish makers' naphtha, VM and P naphtha, VM&P naphtha.

Mineral spirits, also known as petroleum spirits, is another commonly used solvent that distills at an even higher temperature than naphtha (boiling point range, 302–392°F). It is also known as Stoddard

solvent, refined petroleum spirits, white spirits, Amsco 140, Soltrol, Soltrol 50, Soltrol 100, or Soltrol 180.

Thus, the term petroleum distillates may be used generically and interchangeably for two or three different types of petroleum distillation fractions. It can be confusing in that many commercial products will list the term as an ingredient even though it does not contain petroleum naphtha (e.g., the label of a solvent may say 'contains petroleum distillates' simply because some of the components were derived from crude oil).

Exposure Routes and Pathways

The primary exposure pathway for these solvents is inhalation of volatile components or absorption through skin. Inhalation of fumes can be hazardous, especially in environments with poor ventilation. Residential exposures to vapors may be significant if volatile components are allowed to accumulate in enclosed areas (e.g., using paint containing petroleum distillates in a poorly ventilated, enclosed space such as closets).

Toxicokinetics

Petroleum distillates are well absorbed through the gastrointestinal track if ingested and through the skin if contact occurs. Vapors are well absorbed through the lung. Because distilled fractions are mixtures, the distribution, metabolism, and excretion would be different for the different types of naphthas or spirits.

Mechanism of Toxicity

Although the toxicity of Stoddard solvent is not attributable to any one type of constituent, the aromatic components are considered to be more toxic than the paraffin or naphtha/naphthene components. Solvents tend to cause a nonspecific narcosis. This is generally believed to be due to the disrupting (or solublizing) effect that the solvent has on cellular membranes, particularly those of the nervous system.

Acute and Short-Term Toxicity (or Exposure)

Acute toxicity is generally defined as a single (or short-term) exposure to a fairly high concentration of the chemical in question. Acute effects are generally reversible upon removal of the animal or person from the chemical source.

It can be assumed, based on the adverse effects following exposure to these mixtures, that the central nervous system (CNS) is a primary target organ. The most common symptom of overexposure is dizziness and headache. Effects are generally similar to those seen with the methane series.

Animal

Acute toxicity information on mineral spirits (as Stoddard solvent) is sparse. Short-term animal studies have shown depression of the CNS and irritation of the eyes, nose, and throat. Draize skin irritation tests on rabbits resulted in a final score of 'moderate'.

Toxicity tests on laboratory animals using different types of petroleum distillate formulations have shown oral LD_{50} values ranging from 4.5 to 25 ml kg^{-1}. Inhalation LC_{50} values have ranged from 1600 to 73 000 ppm. The majority of laboratory rats subjected to chemical aspiration experiments (up to 0.2 ml) do not survive.

The acute inhalation LC_{50} for the rat is greater than 5500 mg m^{-3} (4 h whole body exposure). The acute oral LD_{50} in the rat is greater than 5000 mg kg^{-1} and the acute dermal LD_{50} in the rabbit is greater than 3000 mg kg^{-1}. All three of these acute values indicate that the overall toxicity to laboratory animals is relatively low.

Human

Irritation of the skin and/or respiratory tract is a common symptom following acute exposures to petroleum ethers. Reactions of human skin include edema (swelling), erythema (reddening), and disruption of the horny layer. Acute inhalation of high concentrations of petroleum ether may cause cerebral edema. Accidental ingestion may cause aspiration pneumonia and pneumatoceles in children.

Chronic Toxicity (or Exposure)

Chronic toxicity is generally defined as the repeated exposure to relatively low concentrations of a chemical over a long period of time. Chronic effects are generally less reversible than acute affects and may have serious long-term consequences (such as emphysema or cancer).

Animal

In experiments with VM&P naphtha, temporary hematological effects have been observed. As in humans, CNS depression is commonly seen following exposure; at high concentrations, convulsions are sometimes seen.

Using Stoddard solvent, long-term (chronic inhalation) rat studies have shown no outward signs of distress and only slight effects on the lung (irritation) and the liver and kidney (190–330 ppm for 13 weeks). Chronic inhalation tests in other animals (dogs, monkeys, guinea pigs, rabbits) show similar findings: no significant outward signs of toxicity (80–200 ppm in air) with lung irritation (e.g., congestion, emphysema) as the primary adverse effect.

No adverse teratogenic effects were seen in rats exposed to air concentrations that were high enough to induce maternal toxicity (950 ppm).

Human

The naphtha mixtures that are distilled at a lower boiling temperature have a higher volatility and, generally speaking, a higher degree of toxicity than the higher boiling fractions. In some occupational settings, chronic exposure to petroleum distillate has resulted in damage to the CNS, sometimes irreversible. Adverse effects on blood-forming components have also been reported, although the frequency of this effect has decreased considerably since the removal of benzene from these mixtures.

Petroleum ether (ligroin) consists primarily of *n*-pentane and *n*-hexane. Therefore, the primary effects seen are on the CNS, including peripheral nerve damage and depression.

Effects related to *n*-hexane intoxication, including paresthesia, loss of appetite, muscle weakness, and impaired motor function have been seen in workers chronically exposed to petroleum ether in inadequately ventilated buildings.

VM&P naphtha, also known as 'light naphtha' and 'spotting naphtha', is used extensively in the thinning of lacquers, varnishes, and rapidly evaporating paint thinner. It is a mildly irritating to the nose and eyes. Workers exposed to this mixture have been known to experience symptoms typical of intoxication with aliphatic compounds, including lightheadedness, labored breathing, tremors, hyperactivity, and nausea. Petroleum-derived distillates have not been shown to be carcinogenic in humans.

In Vitro Toxicity Data

Stoddard solvent has not been shown to be mutagenic in rat or mouse bioassays.

Clinical Management

If overexposure occurs, medical attention should be sought immediately. Persons exposed to high vapor concentrations should vacate or be removed from the source of the vapor and put in fresh air. If there are breathing problems, respiratory support should be provided (artificial respiration or oxygen, as appropriate). If skin has been exposed, the exposed area should be washed promptly with soap and large amounts of tepid water. Contaminated clothing should be removed. If eyes have been exposed, they must be irrigated immediately with tepid water. If swallowed, vomiting should not be induced. Emergency treatments that could result in introduction of solvents in the lung should be avoided. Aspiration pneumonia can also occur in children who have ingested solvents and then accidentally inhaled the solvent during vomiting. Symptoms should be treated and medical attention should be sought.

Rescuers should take care in areas with high vapor concentration. Care should be taken to control any potential ignition sources, such as sparks from static electricity.

Environmental Fate

Petroleum distillates that are spilled onto the ground may migrate to, and contaminate, groundwater supplies. Because they are volatile chemicals, however, most environmental releases will ultimately end up migrating to the atmosphere.

Ecotoxicology

No environmental guidelines or criteria for petroleum distillates in water, sediment, or air were identified. Some state regulatory agencies, such as Massachusetts Department of Environmental Protection, do have health-based environmental criteria (principally for soil) for various 'fractions' of aliphatic or aromatic hydrocarbons.

Other Hazards

Petroleum distillates are highly flammable so care must be taken when using these solvents near ignition sources or devices that can induce sparks. Contact with strong oxidizers should be avoided. Persons using paint(s) containing petroleum distillates should exercise caution when painting in poorly ventilated, enclosed areas.

Exposure Standards and Guidelines

For Stoddard solvent, the Occupational Safety and Health Administration has established a time-weighted average (TWA) standard of 500 ppm (2900 mg m^{-3} of air) for an 8 h workday, 40 h workweek to prevent nervous system and skin damage.

The National Institute for Occupational Safety and Health (NIOSH) recommended exposure limit TWA for VM&P naphtha is 350 mg m^{-3} TWA for a 10 h workday and a ceiling level of 1800 mg m^{-3} (15 min sampling period). NIOSH recommends preventing contact with eyes and skin. The immediately dangerous to life or health value is 10 000 ppm.

The American Conference of Governmental Industrial Hygienists threshold limit value, TWA, is 1370 mg m^{-3} (300 ppm) for VM&P naphtha. The lower explosive limit is 1.2%.

See also: Heptane; Hexane; Neurotoxicity; Octane; Pentane; Petroleum Ether; Petroleum Hydrocarbons; Stoddard Solvent.

Relevant Websites

http://www.intox.org – Canadian Center for Occupational Health and Safety. Cheminfo. Chemical Profiles Created by CCOHS.

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Petroleum Distillates.

Petroleum Ether

Patricia J Beattie

- Chemical Abstracts Service Registry Number: CAS 8032-32-4
- Synonyms: Ligroin; Refined solvent naphtha; Varnish makers' and painters' naphtha (VM&P naphtha); Skellysolve
- Chemical/Pharmaceutical/Other Class: Petroleum ether is a petroleum distillate made up primarily of aliphatic and alicyclic hydrocarbons in the C_5–C_{10} range
- Chemical Formula: Petroleum ether is a complex mixture. The following is a typical composition; however, this will vary depending on petroleum feedstock and refining process:
  - 50–80% paraffins (C_5–C_{10}) (% by volume)
  - 20–40% monocycloparaffins
  - 2–10% aromatics
  - The boiling point range for petroleum ether is 38–150°C; the flash point range is −18–13°C. The most common production process used today results in petroleum ether with $\leqslant$0.002% benzene and $\leqslant$5.0% *n*-hexane

Uses

Petroleum solvents are typically grouped into three classes based on volatility and aromatic content. They are (1) special boiling range solvents, (2) white spirits, and (3) high boiling aromatics. Petroleum ether is in the special boiling range solvent class. It is used in the rubber industry and as a degreasing agent. Petroleum ether is a common constituent in adhesives, inks, paints, varnishes, and lacquers.

Exposure Routes and Pathways

Exposure occurs most commonly by either inhalation or through skin contact.

Toxicokinetics

Petroleum ether is absorbed by the lungs following inhalation exposure. It is metabolized by the liver with a biological half-life of 46–48 h.

Mechanism of Toxicity

The acute toxicity from overexposure to petroleum ether is manifested primarily in central nervous system (CNS) effects. The mechanism of toxicity is unknown; however, the general anoxia observed is most likely due to oxygen deprivation. The mechanism of toxicity from long-term overexposure to petroleum ether is dependent on the chemical makeup of the distillate. For example, if peripheral neuropathy is observed, it is most likely due to a high concentration of *n*-hexane in the petroleum ether. *n*-Hexane is known to cause axonal damage in peripheral nerves.

Acute and Short-Term Toxicity (or Exposure)

Animal

In general, the toxicity reported from exposure to petroleum ether is more pronounced with samples containing higher concentrations of aromatic compounds. The irritation potential of petroleum ether to skin and eyes as tested in rabbits in the Draize protocol ranged from minimally to moderately irritating. Inhalation toxicity of VM&P naphtha was studied in mice, rats, cats, and dogs at concentrations ranging from 280 to 15 000 ppm. Acute exposure to high concentrations resulted in loss of motor coordination and CNS depression. Eye irritation was reported in rats at 3400 ppm after 4 h and dogs at 3400 ppm after 2 h. Respiration rate was decreased

in mice after exposure for 1 min to $\geqslant$2600 ppm. The 4 h LC_{50} in rats was reported to be 3400 ppm.

Human

Skin contact with petroleum solvents can cause allergic contact dermatitis. Preexisting skin disease may increase the potential for adverse effects. Overexposure via inhalation of petroleum ether affects primarily the CNS. Short-term, high overexposure is associated with an excitatory phase followed by a depressive phase. Exposures of 100–400 ppm for 7 h have resulted in headaches, fatigue, and incoordination with dose-associated effects on equilibrium, reaction time, visuomotor coordination, and memory. Inhalation exposures of 445–1250 ppm resulted in blurred vision, a cold sensation in extremities, fatiguability, headache, fatty demylination of muscle fibers, and demylination and mild axonal degeneration. Exposure to 880 ppm produced eye and throat irritation with temporary olfactory fatigue.

Chronic Toxicity

Animal

Beagle dogs were exposed to 1200 ppm of petroleum ether for 6 h day^{-1}, 5 days $week^{-1}$ for 13 weeks. No significant toxicity was reported. In long-term mouse skin painting studies using petroleum distillate fractions similar to petroleum ether, local necrosis, ulceration, marked regenerative epidermal hyperplasia, and, in some cases, squamous cell carcinomas have been reported.

Human

Several cross-sectional epidemiology studies have investigated the CNS effects observed in industrial painters, house painters, car painters, shipyard painters, and floor layers. Subjective symptoms such as headache, fatigue, poor coordination, emotional instability, impaired memory and other intellectual functions, and impaired psychomotor performance have been reported. Because most of these workers were exposed to a multitude of chemicals, in addition to petroleum ether, it is difficult to evaluate the cause of the reported effects.

In Vitro Toxicity Data

The majority of data suggest that petroleum ether is not mutagenic, based on *in vitro* tests using cultured mammalian cells, yeast, or bacterial test systems. Genotoxic potential is correlated with polynuclear aromatic hydrocarbon concentration.

Clinical Management

Overexposure to vapors of petroleum ether is treated by removing the patient to fresh air. If skin or eye contact occurs, the affected areas should be flushed with water for at least 15 min to remove residual solvent. Good personal hygiene and regular washing of skin and clothes minimizes the potential for developing allergic contact dermatitis. If ingestion of petroleum ether occurs, vomiting should not be induced. This could result in aspiration of solvent into the lungs, leading to chemical pneumonitis, and pulmonary edema, which can be fatal. If ingestion is suspected and the patient is coughing, there is a good possibility that aspiration has occurred. The patient should be monitored closely; hospitalization may be indicated.

Environmental Fate

Petroleum ether degrades rapidly in soil and water. In air, it reacts with photochemically produced hydroxyl radicals with an estimated half-life of 4–8 days. Based on water solubility and estimated bioconcentration factors, petroleum ether is not expected to bioconcentrate in aquatic organisms.

Exposure Standards and Guidelines

- Occupational Safety and Health Administration: Permissible exposure limit, 8 h time-weighted average (TWA) is 100 ppm (400 mg m^{-3}).
- American Conference of Governmental Industrial Hygienists: Threshold limit value, 8 h TWA is 300 ppm (1370 mg m^{-3}). A3 – Confirmed animal carcinogen with unknown relevance to humans.
- National Institute for Occupational Safety and Health: Recommended exposure limit, 10 h TWA is 100 ppm (400 mg m^{-3}).
- Immediately dangerous to life or health limit is 1000 ppm.

See also: Neurotoxicity.

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Petroleum Hydrocarbons

Shayne C Gad

Petroleum is a naturally occurring, oily, flammable liquid composed principally of hydrocarbons; it is occasionally found in springs or pools but is usually obtained from beneath the Earth's surface by drilling wells. Formerly called rock oil, unrefined petroleum is now usually termed crude oil. Crude oil is a highly complex mixture of paraffin, cycloparaffinic (naphthenic), and aromatic hydrocarbons, containing a low percentage of sulfur and trace amounts of nitrogen and oxygen compounds.

The most important petroleum fractions, obtained by cracking or distillation, are various hydrocarbon gases (butane, ethane, and propane), naphtha of several grades, gasoline, kerosene, fuel oils, gas oil, lubricating oils, paraffin wax, and asphalt. From the hydrocarbon gases, ethylene, butylene, and propylene are obtained; these are important industrial intermediates, being the source of alcohols, ethylene glycols, and monomers for a wide range of plastics, elastomers, and pharmaceuticals. Benzene, phenol, toluene, and xylene can be made from petroleum. Hundreds of other products, including biosynthetically produced proteins, are petroleum derived.

Petroleum is separated by distillation into fractions designated as (1) straight-run gasoline, boiling at up to ~390°F (200°C); (2) middle distillate, boiling at ~365–653°F (185–345°C), from which are obtained kerosene, heating oils, and diesel, jet, rocket, and gas turbine fuels; (3) wide-cut gas oil, which boils at ~653–1000°F (345–540°C), and from which are obtained waxes, lubrication oils, and feedstock for catalytic cracking to gasoline; and (4) residual oil, which may be asphaltic.

The physical properties and chemical composition of petroleum vary markedly, depending on its source. As it comes from the earth, it ranges from an occasional nearly colorless liquid consisting chiefly of gasoline to a heavy black tarry material high in asphalt content. Most crudes are black; many are amber, red, or brown by transmitted light and show a greenish fluorescence by reflected light and have a specific gravity in the range ~0.82–0.95.

Hydrocarbons constitute 50–98% of petroleum, and the remainder is composed chiefly of organic compounds containing oxygen, nitrogen, or sulfur and trace amounts of organometallic compounds. The hydrocarbon types found in petroleum are paraffins (alkanes), cycloparaffins (naphthenes or cycloaldanes), and aromatics. Olefins (alkenes) and other unsaturated hydrocarbons are usually absent.

The number of carbon atoms in hydrocarbons of a given boiling range depends on the hydrocarbon type. In general, gasoline will include hydrocarbons having 4–12 carbon atoms; kerosene, 10–14; middle distillate, 12–20; and wide-cut gas oil, 20–36. Five main classes of compounds are present in the gasoline fraction: straight-chain paraffins, branched-chain paraffins, alkylcyclopentanes, alkylcyclohexanes, and alkylbenzenes. Several physiochemical properties (viscosity, surface tension, and volatility) significantly influence toxicity.

Asphalt is a dark-brown to black solid or semisolid consisting of carbon, hydrogen, oxygen, sulfur, and sometimes nitrogen. It is made up of three components: (1) asphaltene, a hard, friable, infusible powder, (2) resin, a semisolid to solid ductile and adhesive material, and (3) oil, which is structurally similar to the lubricating oil fraction from which it is derived.

Acute exposure to unleaded gasoline and a variety of light hydrocarbons present in gasoline produces a nephropathy in male rats characterized by (1) an excessive accumulation of protein (hyaline droplets) in epithelial cells of proximal tubule, (2) accumulation of casts at the corticomedullary junction, and (3) evidence of mild tubular regeneration. This nephropathy only occurs in male rats; female rats and mice do not show any renal pathology. A number of chemicals present in unleaded petrol when tested alone have been shown to produce nephropathy and, in particular, 2,2,4-trimethylpentane and decalin have been used as model compounds. Certain other industrial chemicals (1,4-dichlorobenzene and isophorone), natural products (D-limonene), and pharmaceuticals (levamisole) also produce this male-rat-specific nephropathy. Chronic exposure of male rats to unleaded petrol, 1,4-dichlorobenzene, isophorone, or D-limonene ultimately leads to the induction of a low incidence of renal adenomas and carcinomas.

Studies on the mechanism of pathogenesis have shown that the protein which accumulates in the proximal tubular cells is α_{2u}-globulin, a low-molecular weight protein of 18 700 Da that is synthesized in the liver of adult rats and is freely filtered at the glomerulus. Female rats excrete less than 1% of the α_{2u}-globulin that male rats excrete. The chemical itself or a metabolite has been shown to bind reversibly to α_{2u}-globulin and this chemical-protein complex is then thought to be taken up by the proximal tubular cells (primarily in the S2 segment) by

endocytosis. These complexes appear to be quite resistant to, or impair, lysosomal degradation, which leads to their accumulation of polyangular droplets. Lysosomal overload is thought to lead to individual cellular necrosis, which is followed by repair and regeneration. It has been suggested that a sustained increase in renal cell proliferation can promote initiated cells to form preneoplastic foci and lead to renal neoplasia. The development of the renal toxicity and increased cell proliferation is dependent on the presence of α_{2u}-globulin The NCI Black–Reiter strain of male rat cannot synthesize α_{2u}-globulin and, by inference, would not be expected to be at risk. However, it is not known whether these hydrocarbons or their metabolites can bind to other low-molecular-weight proteins and, if so, whether the same biochemical events as those observed with α_{2u}-globulin could occur.

Products with viscosity in the range of 30–35 or lower present an extreme aspiration risk and include agents such as mineral seal oil, which is found in furniture polishes. It is important to realize that even small amounts of a low-viscosity material, once aspirated, can involve a significant portion of the lung and produce a chemical pneumonitis. Oral ingestion of hydrocarbons often is associated with symptoms of mucous membrane irritation, vomiting, and central nervous system depression. Cyanosis, tachycardia, and tachypnea may appear as a result of aspiration, with subsequent development of chemical pneumonitis. Other clinical findings include albuminuria, hematuria, hepatic enzyme derangement, and cardiac arrhythmias. Doses as low as 10 ml orally have been reported to be potentially fatal, whereas some patients have survived the ingestion of 60 ml of petroleum distillates. A history of coughing or choking in association with vomiting strongly suggests aspiration and hydrocarbon pneumonia. Hydrocarbon pneumonia is an acute hemorrhagic necrotizing disease that can develop within 24 h after the ingestion. Pneumonia may require several weeks for complete resolution.

Activated charcoal and/or emesis may be indicated in some hydrocarbon ingestions in which absorption may produce systemic effects. Agents such as asphalt, tar, heavy lubricants, vaseline, and mineral oil are considered relatively nontoxic and do not require removal. Chlorinated hydrocarbon solvents or any hydrocarbon or petroleum distillate with a potentially dangerous additive (camphor, pesticide, and heavy metals) in some cases may be treated with activated charcoal or emesis. Petroleum naphtha derivatives, gasoline, kerosene, and mineral seal oil (or signal oil) as found in furniture polish and oil polishes produce severe and often prolonged chemical pneumonitis. These compounds are poorly absorbed from the stomach but are very damaging to the lung if inhaled. They should not be removed by emesis unless very large amounts are ingested ($\geqslant$12–18 ml kg^{-1}). Gastric lavage is not indicated for hydrocarbon ingestion because of the risk of aspiration if the patient vomits around the lavage tube. X-rays taken early in the course of ingestion may not demonstrate chemical pneumonia; even if it is demonstrated, the clinical severity does not correlate well with the degree of X-ray findings. However, X-rays should be repeated on follow-up to detect the development of pneumonitis or demonstrate pneumatoceles. Patients who arrive coughing probably already have aspirated and should be monitored closely for the development of pneumonitis. The decision for hospitalization should be based on clinical criteria (e.g., cyanosis and respiratory distress) rather than on X-ray findings alone. Steroid therapy may be harmful. Antibiotics, oxygen, and positive end expiratory pressure should be instituted as indicated.

The usual cutaneous response to oil-based materials is an oil folliculitis that arises as a result of chemical irritation and mechanical plugging of the follicular canals. Onset of the problem usually occurs soon after the first exposure and is marked by acute reactions starting on the dorsal surfaces of the hands and fingers, the extensor surfaces of the forearms and thighs, and the abdomen (i.e., those surfaces that are in contact with oil or oil-soaked clothing). Comedones and perifollicular papules and pustules ('oil boils') develop. Secondary infections may occur, but the bacteria in the oil are rarely primary skin pathogens and are rarely the single cause of the folliculitis. Melanosis may appear later. Clinical manifestations clear rapidly with the termination of exposure and do not resolve if the exposure is continued. Exposure is controlled through proper machine design to prevent spattering, clean clothing, protective garments, and careful attention to hand washing and other aspects of personal hygiene.

Since emulsion and synthetic fluids are potent defatting agents, the skin reaction to them may include maceration, dryness and 'chapping', reddening, and vesiculation. Bacterial growths in the fluid do not appear to be directly injurious to workers, but rancid fluids and products of bacterial action can lead to skin disorders. As in the case of insoluble oils, both treatment and prevention are based on the control of exposure. Corticosteroid creams may be used as an adjunct in the treatment. The value of 'barrier' creams and other protective gels is not universally accepted but they do offer modest usefulness in certain situations and have been shown to reduce ultrastructural and cytoarchitectural changes in human epidermis after applications of acetone and kerosene.

Individual additives in cutting fluids can be a cause of either primary irritative or hypersensitive dermatitis. Detergents, soaps, and wetting agents defat the skin, and alkaline materials damage the keratin of the upper, protective skin layers. Ulcerative and erythematous lesions on the genitals and buttocks have been reported for workers wearing coveralls that had been dry-cleaned with Stoddard solvent, a mixture of petroleum distillates. Formalin in germicides is a sensitizer. Additives containing sulfur and chlorine are direct irritants, although so-called chloracne is not associated with cutting fluids. Nickel or chromates derived from metals being cut can be a source of allergic dermatitis. Harsh abrasive soaps and solvents, such as gasoline and kerosene, may contribute to chemical and traumatic dermatitis since these cleaning materials are common in machine shops. While grime and grease can certainly be removed from the skin with these substances, it is safer to utilize less injurious cleansers available commercially.

Certain petroleum oils have carcinogenic constituents; this is especially the case with shale oils, which are currently extracted and used outside of the United States. Since American potential supplies of oil shale tars constitute 94% of the known world resources, these substances may present toxic problems in the United States in the future. There are no good data that would establish the prevalence of skin cancers among machinists in this country, but scrotal and other skin cancers have been reported among British cotton mule spinners prior to 1953 and more recently among tool setters and machine operators in the British Midlands. Knowledge of occupational malignancies of the skin has a long and important history that dates back to 1775 when Pott identified scrotal cancer in English chimney sweeps. A particular set of carcinogenicity bioassays, the mouse skin painting studies, were developed specifically to assess the carcinogenic potential of petroleum products.

Exposure to mist sprays or insoluble oils used as coolants, cutting fluids, and lubricants in machine operations are usually not harmful to the respiratory tract, although worker discomfort occurs at oil mist levels above $5\,\mathrm{mg\,m^{-3}}$. Mineral oil droplets $< 5\,\mu\mathrm{m}$ in diameter may be inhaled and result in fibrotic nodules, paraffinomas, or in lipoid pneumonitis. There was no evidence that machinists exposed to cutting-oil mists had any unusual mortality from respiratory tract cancer.

See also: Kerosene; Oil, Crude; Oil, Lubricating; Polycyclic Aromatic Hydrocarbons (PAHs).

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Relevant Website

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Peyote

Amanda Lofton

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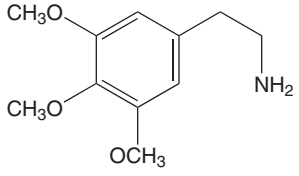
- Chemical Abstracts Service Registry Number: CAS 11006-96-5
- Synonyms: 3,4,5-Trimethoxyphenethylamine; Anhalonium; Bad seed; Big chief; Button; Cactus; Indian dope; *Lophophora williamsii*; Mesc; Mescal; Mescal button; Mescaline; Moon tops; Peyotl; Turnip cactus
- Chemical/Pharmaceutical/Other Class: Mescaline, the major active alkaloid found in peyote, is a narcotic hallucinogen
- Chemical Formula: $C_{11}H_{17}NO_3$
- Chemical Structure:



Uses

Peyote is used as a Native American religious intoxicant, a hallucinogenic agent, and a folk remedy.

Background Information

Peyote is the common name given to *Lophophora williamsii*, the North American dumpling cactus. The

peyote cactus is a flowering plant of the family Cactaceae, a group of fleshy spiny plants found primarily in dry climates. Spines are present only in very young seedlings. The cactus areole, the area on the stem that usually produces flowers and spines, is well pronounced in peyote and is identified by a tuft of hairs or trichomes. Flowers arise from within the center of the plant. Leaves are greatly reduced and only microscopic in size. The plant also produces a pink bitter-tasting berry that contains black seeds.

Peyote has been used in tribal ceremonies by indigenous cultures in North America since 1000 BC. In the year 1560, Spanish priest Bernardino de Sahagún wrote about the use of peyote and hallucinogenic mushrooms by the Aztecs. The first proper botanical description of peyote was made by Hernandez, the naturalist of Philip II of Spain, in 1638. Dried peyote buttons were processed and distributed by Parke Davis and Company in 1887. By 1930, over a dozen states in the United States had outlawed the possession of peyote and in 1967 peyote was banned nationwide by the federal government.

Exposure Routes and Pathways

Peyote is ingested in various forms, including dried buttons, tincture of peyote (70% alcohol), and pan-peyote (chloroform extract of ground peyote). Synthetic mescaline has been administered orally, intravenously, subcutaneously, and intramusculary.

Toxicokinetics

Mescaline is rapidly absorbed, with peak blood levels noted within 2 h of ingestion. Gastrointestinal effects appear ~30–60 min after exposure and sensory effects peak between 4 and 6 h postingestion. Symptoms usually resolve within 12–14 h. Mescaline is not bound to plasma proteins, but to liver proteins. The volume of distribution is large, with the agent widely distributed among a number of organs. Brain and blood levels are nearly equal; concentrations in the kidneys, liver, and spleen are 3 to 5 times greater. Mescaline is metabolized in the liver to a variety of inactive metabolites. Approximately 60% of a dose is excreted unchanged in urine. The elimination half-life of the compound is ~55 min.

Mechanism of Toxicity

Mescaline causes hallucinogenic effects by stimulating serotonin and dopamine receptors in the central nervous system. The sympathomimetic effects of mescaline are probably also centrally mediated. Changes in catecholamine metabolism and adrenal medullary function may be responsible for the agent's peripheral effects. In animals, mescaline decreases the synthesis of the cofactor nicotinamide adenine dinucleotide in the brain. Mescaline may also produce cerebral vasospasm.

Acute and Short-Term Toxicity (or Exposure)

Animal

Doses of 20 mg kg^{-1} in animal studies led to bradycardia, hypotension, and peripheral vasodilation. The lethal dose of mescaline in animals ranged from 150 to more than 500 mg kg^{-1}, depending on the species and the route of administration. The terminal events in animals given mescaline overdoses were seizures followed by respiratory arrest.

Human

A dose of 5–8 mg kg^{-1} by any route causes the desired psychedelic effects. One dried peyote button contains ~45 mg of mescaline. Nausea, chills, and vomiting, which are often accompanied by anxiety and terror, occur first in most users. Diaphoresis, tachycardia, and hypertension are common. Photophobia secondary to mydriasis, nystagmus, tremors, ataxia, and hyperreflexia may also present. These sympathomimetic effects are followed by vivid visual hallucinations and exaggerated sensitivity to sound and other sensory perceptions. Users describe increased clarity and intensity of thought. Death from mescaline has not been reported. Hallucinations may lead to psychotic or suicidal behavior resulting in trauma and death. The qualitative presence of mescaline in urine can confirm the diagnosis; blood levels do not correlate with toxicity.

Chronic Toxicity (or Exposure)

Human

Flashbacks, or reoccurrence of hallucinogenic effects, have been reported. Persistent psychosis, anxiety, and depression have been described following mescaline use. Tolerance, but not physical dependence, to mescaline's effects has been reported in humans. Additionally, chronic users may demonstrate cross-tolerance to the effects of LSD or psilocybin.

Clinical Management

Treatment consists mainly of supportive care. A nonthreatening environment should be maintained and calm reassurance provided to the patient. Because mescaline is rapidly absorbed and vomiting is common, gastric decontamination is usually not necessary. However, activated charcoal will adsorb

mescaline and can be used prior to the occurrence of symptoms. Benzodiazepines are recommended to sedate agitated patients. Haloperidol can be administered to patients who fail to respond to benzodiazepines, but should not be used in children. Phenothiazines may increase the risk of flashbacks in later years. Patients with massive mescaline ingestion may require ventilatory support.

See also: LSD (Lysergic Acid Diethylamide); Mescaline; Plants, Poisonous.

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Pharmacokinetic Models

Natalie Eddington

Pharmacokinetics/toxicokinetics is the area of toxicology that is concerned with the role of absorption, distribution, metabolism, and excretion of toxicants in the body. These events, some of which may be interdependent, often have a very significant impact on the toxicity of a chemical in a specific species. Quantitative characterization of the time profile of absorption, distribution, metabolism, and excretion of xenobiotic compounds is included in the area of pharmacokinetics. In this sense, pharmacokinetics is used synonymously with toxicokinetics.

One of the methods of examining the kinetics of absorption, distribution, metabolism, and excretion of a xenobiotic, particularly of a toxicant, is the use of physiologically based pharmacokinetic (PBPK) models. PBPK models are mathematical models that permit predictions about body burdens, clearance profiles following cessation of the exposure, and provide other information that may aid in assessing the hazards of chemicals to humans. In these models, body is described as a series of relevant compartments in contact with the venous and arterial supplies of blood wherein anatomy and physiology decides the structure of model. The physiological information, such as blood flow rates to each compartment, the partition coefficients between the blood and organ tissues, and the different volumes of the various compartments, can then be used to build differential mass-balance equations that describe the rate of change of concentrations of the chemical of interest in the compartments.

PBPK models are especially useful for characterizing tissue-level doses when external exposures (exposures contacting the biological barriers or membranes of the organism (e.g., skin)) are repeated or intermittent in nature. Such models utilize physiological parameters and biochemical transformation data to determine the temporal relationships of the distribution and disposition of an administered dose.

PBPK models require three different types of information: (1) partition coefficients that describe the relative solubility or affinity of the compound for blood versus other tissues; (2) physiological constants, such as tissue and organ volumes and the relevant blood flows; and (3) rate constants for the key elimination pathways.

Often, PBPK models for toxicokinetics application require special considerations (e.g., volatile toxicants may incur tissue–air partition coefficients and alveolar elimination rates). Partition coefficients are generally obtained by measurement in the laboratory, tissue volume/blood flow data are mostly available from the scientific literature (with allometric scaling from species to species), and biotransformation data are usually obtained from *in vivo* and *in vitro* kinetic studies. Biochemical constants for metabolic pathways are captured using the maximum rate of reaction, or V_{max}, and the binding affinity of the particular substrate for the metabolizing enzyme.

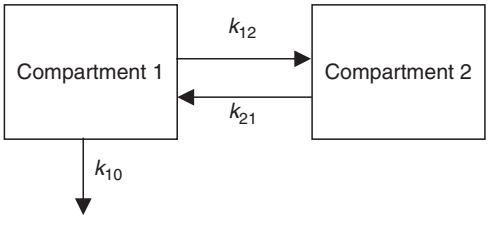
Two limiting cases relating to permeability of the cell membrane should be considered in developing a pharmacokinetic model for a given chemical. In the flow-limited model, transport of the chemical across the cell membrane is assumed to be so rapid that the rate of blood flow is taken to be the limiting process that determines the rate of uptake. In the membrane-limited model, cell permeability is assumed to be low, and thus the rate-limiting step, compared with the blood perfusion rate. The pharmacokinetics of volatile hydrocarbons and halogenated hydrocarbons, such as methylene chloride, are adequately described by a flow-limited model. Larger molecular weight compounds and many drugs, such as digoxin and

methotrexate, are most appropriately described by a membrane-limited model.

In constructing a pharmacokinetic model, it is not necessarily critical to place each tissue or organ into a separate compartment; rather, organs with similar blood flows, diffusion characteristics, and permeability properties can often be combined into single compartments. For example, the adrenals, kidneys, thyroid, brain, heart, and heptoportal system are sometimes pooled into one compartment because their perfusion-to-volume ratios are relatively high, facilitating their classification as a vessel-rich group. The liver, lungs, and gastrointestinal tract are usually represented separately. For toxicokinetics applications, however, the decision of inclusion/exclusion or lumping of compartments is greatly influenced by the properties of toxicant and nature of its toxicity (e.g., a localized toxicity to a specific organ tissue may require it to be included as an individual compartment).

PBPK models are particularly useful for interspecies extrapolations of dose–response data. In using a PBPK model of uptake, distribution, and elimination, an exponential power (e.g., 0.75) of the body weight is used to scale the cardiac output and ventilation rate between the laboratory species (typically rat) and humans. A PBPK model will therefore contain adequate logic to account for routes of administration, storage tissues and residence time therein, elimination rates, and sufficient mathematical detail to mimic the integration of these processes. It is important that the model parameters (e.g., elimination rates) be validated as much as possible by separate kinetic studies in the relevant species. The ultimate test of the model is how the model predictions are for parameters such as blood levels, rate of metabolism, and tissue concentrations relative to real-life animal data for the chemical.

The simplest model is a two-compartment model in which there is a plasma compartment and tissue compartment which have reversible flows of compound or metabolites or both between them. The compartment model for a drug which follows bi-exponential pharmacokinetics is shown below. In addition, the general forms of the equations that describe the rate of change of drug in the two compartments are presented below for both the central (compartment 1, eqn (1)) and the peripheral (compartment 2, eqn (2)) model, respectviely:



$$dX_1 = -(k_{12} + k_{10}) \cdot X_1 + k_{12} \cdot X_1 + k_{21} \cdot X_2 \quad (1)$$

$$dX_2 = k_{12} \cdot X_1 - k_{21} \cdot X_2 \quad (2)$$

X_1 and X_2 represent the doses of drug in the central compartment (1) and the peripheral compartments, respectively, k_{12} represents the intercompartment rate constant from the central to the peripheral compartment, k_{21} represents the intercompartment rate constant from the peripheral back to the central compartment, and k_{10} is the elimination rate constant.

Simultaneous integration of these two equations gives the explicit solution as a multiexponential equation, the exponents being expressed as a function of the distribution (α) and elimination rate constants (β), and factoring in the volumes of the compartment (V_c). The following equation (eqn(3)) represents the concentration versus time for a drug which follows a two-compartment model:

$$C_p = \frac{X_0(\alpha - k_{21})}{V_c(\alpha - \beta)} e^{-\alpha t} + \frac{X_0(k_{21} - \beta)}{V_c(\alpha - \beta)} e^{-\beta t} \quad (3)$$

PBPK have been developed and parameters defined for a number of chemicals, including methylene chloride, perchloroethylene, and pharmaceuticals (including anticancer drugs). The US Environmental Protection Agency commonly uses the Integrated Exposure Uptake Bio-kinetic Model for Lead to estimate the blood lead levels in children (up to 6 years) associated with multipathway environmental exposures. Acceptability of the concentration of lead in various environmental media is linked to a blood lead level in children believed to be protective of health. For methylene chloride, the mass-balance equations that form the model adequately account for the removal of the compound by the liver as well as the significant excretion through the lungs and incomplete retention of an inhaled dose, and they are useful for comparing organ-specific doses between different routes of exposure (e.g., oral and inhalation). Because of these features, the PBPK model prevents overestimation of dose that would be obtained, for example, if total absorption of an inhaled dose were assumed and if removal by the liver were ignored. Thus, such a model provides an effective tool for exposure assessment by quantifying the internal doses that are ultimately the most appropriate dose metrics to use in route-to-route comparisons.

See also: Absorption; Distribution; Excretion; Pharmacokinetics/Toxicokinetics.

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Pharmacokinetics/Toxicokinetics

Robert Tardif and Jules Brodeur

It is generally accepted that the intensity of toxic effects exerted by foreign chemicals (xenobiotics) is related to the concentration of the toxic moiety at the site of action in a target tissue or organ, whereas in many cases, the duration of the toxic effects depends on the time during which the toxic moiety remains at the site of action. However, there are numerous examples showing that the administration of two xenobiotics, at the same dose and with similar toxic potential, does not yield the same concentration of the toxic moiety for the same duration of time at a given site of action. The most plausible reason for such behavior is that the respective disposition of the two chemicals in the body can differ. Disposition may be regarded as the result of the absorption, distribution, and elimination processes acting on xenobiotics. In other words, disposition is what governs the fate of chemicals in the various compartments of the body and as such plays a key role in determining the concentration and toxicity of these chemicals at the site of action.

Pharmacokinetics/toxicokinetics may be defined as the study of the dynamic movements of xenobiotics during their passage through the body and as such encompass the concept of disposition described previously (**Figure 1**). In simpler words, it tells us what the body does to foreign chemicals. To that end, pharmacokinetic/toxicokinetic analysis uses mathematical terms, or equations, to describe the time course of the absorption and disposition of xenobiotics in the body and proposes simplified representations (models) of the relationship between time and movements of xenobiotics. Once the information on the concentration of a chemical in biologically relevant parts of the body is provided by pharmacokinetic/toxicokinetic studies, it then usually becomes possible to better understand, interpret, and even predict the nature and the extent of the biological effects of xenobiotics.

Etymologically, the term pharmacokinetics relates to the study of the movements of medicines or therapeutic agents within the body. From an historical point of view, principles and methods dealing with the study of the movements of chemicals within the body have evolved from data pertaining precisely

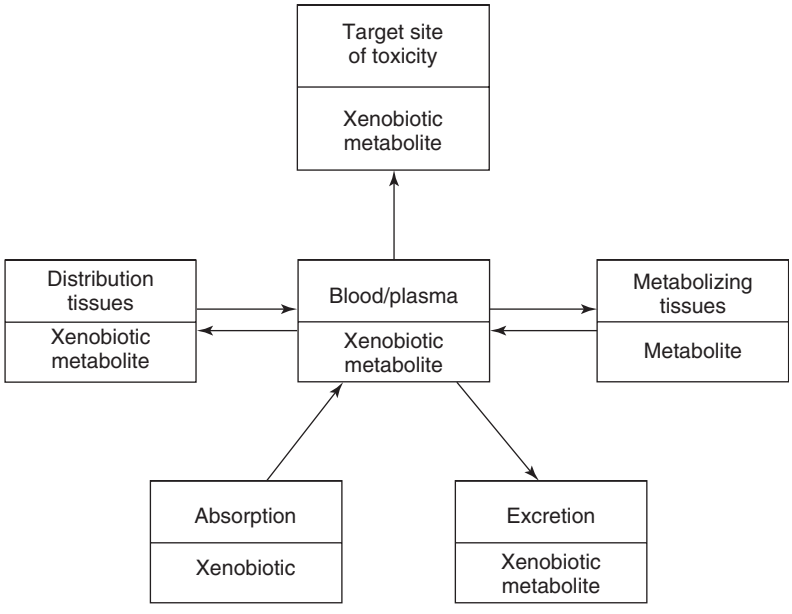

Figure 1 Schematic representation of the main biological processes involved in the disposition of xenobiotics in the body.

to medication. For example, pharmacokinetic data are used for optimal adjustment of the dosage regimen to obtain the best therapeutic effect without eliciting adverse side effects. The term toxicokinetics has a broader meaning in that encompasses the application of pharmacokinetic principles and methods to the prediction of occurrence and time course of toxic events related to foreign chemicals (medication, food additives, workplace products, environmental contaminants, etc.) encountered at levels of exposure likely to induce toxicity.

To facilitate the description of the biological fate of xenobiotics, toxicologists represent the body as compartments that correspond to the various tissues, organs, or fluids of the body. Such functional representation is known as pharmacokinetic modeling. Two types of models are currently available to toxicologists for that purpose: compartmental models and physiologically based pharmacokinetic (PBPK) models. Compartmental models have been extensively studied and used in pharmacokinetic modeling, but recently PBPK modeling has received increasing attention.

The purpose of this article is to introduce the reader to simple basic concepts and principles of pharmacokinetic/toxicokinetic analysis using both types of models – compartmental and physiologically based.

Pharmacokinetic Models

Compartmental Models

Compartmental models, also known as data-based models, are essentially used to fit curves to experimental data on blood, plasma, or urine concentrations of a chemical or its metabolite(s). In this approach, the body is represented as a single or a series of compartments that do not necessarily correspond to any physiological or anatomical reality. As mentioned previously, toxicologists are mainly concerned with avoiding toxicity in target organs/tissues that are presented with time-dependent concentrations of a chemical. It is not feasible, at least in humans, to determine the time course of the concentration of a xenobiotic at a target site (e.g., brain, liver, and kidneys). To overcome this problem, it is assumed, when using the compartmental modeling approach, that the biological effects which depend on the concentration at target site are also related to the concentration of a chemical in blood or plasma. This is the reason why almost all pharmacokinetic analyses are based on blood concentration.

Compartmental modeling consists of finding the proper mathematical equation of the curve that provides the best fit to the kinetic behavior of a xenobiotic (e.g., blood levels). In the simplest case, the body is represented as a single compartment (e.g., one-compartment model). When necessary, however, additional and usually limited numbers of compartments can be added to achieve a better description of the kinetic behavior of a particular xenobiotic (e.g., two- and three-compartment models).

When using such models, it is assumed that the disposition of a chemical is governed by first-order processes. This means that the rate of disappearance of a xenobiotic from the body, as a result of excretion and/or biotransformation, is proportional to the amount of the xenobiotic in the body at that time. In other words, the quantity of a xenobiotic that leaves the body is large when the amount of xenobiotic in the body is large (e.g., immediately after exposure), whereas this quantity is small when the amount in the body is small (e.g., several hours after exposure). Most xenobiotics exhibit this type of behavior, provided that the several biological mechanisms responsible for disposition are not saturated, i.e., not overwhelmed by large concentrations of xenobiotics (see section Dose-Dependent Kinetics).

One-Compartment Open Model In this simple model, the body is treated as a homogenous unit with an entry and an exit (i.e., open model) (**Figure 2a**). It is assumed that changes occurring in blood concentrations reflect similar changes in tissue levels as the xenobiotic rapidly equilibrates between blood and all the various tissues of the body.

Figure 2b illustrates the time dependency of the concentration of a xenobiotic in blood, following rapid intravenous administration. It is seen that the blood concentration decreases rapidly at the beginning and then falls more slowly thereafter. This is typical of first-order elimination as described previously. This curve can be described by using the following exponential term:

$$C_p = C_p^0 \times e^{-k \times t}$$

where C_p represents the blood (plasma) concentration of a xenobiotic at time t, C_p^0 is the blood initial concentration (i.e., extrapolated at time 0), and k is the first-order elimination rate constant. A more practical form of this equation is obtained by substituting the base 10 logarithm (**Figure 2c**):

$$\log_{10} C_p = \log_{10} C_p^0 - \frac{k \times t}{2.303}$$

This simple mathematical description is very useful for determining various kinetic parameters

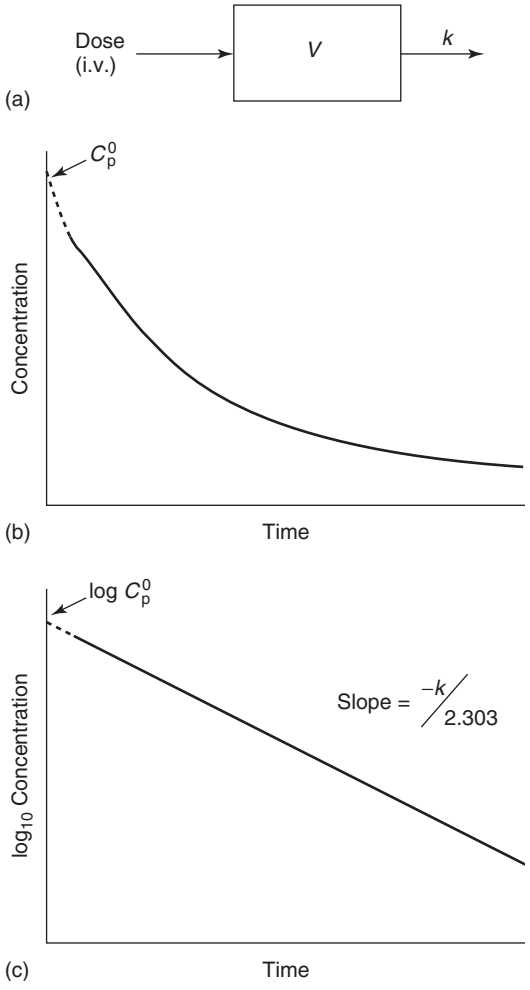

Figure 2 (a) Schematic representation of a one-compartment open model: i.v., intravenous administration; *V*, volume of the compartment; *k*, elimination rate constant (see text). (b) and (c) represent the time course of blood concentration of a xenobiotic following intravenous administration (b, linear scale; c, logarithmic scale); C_p^0, blood or plasma concentration at time zero.

that characterize the kinetic behavior of a given xenobiotic. These kinetics parameters are the volume of distribution, the elimination rate constant, the half-life of elimination, the clearance, the area-under-the-blood-concentration curve, and the bioavailability.

Volume of distribution (V_D) The volume of distribution is defined as the apparent volume (e.g., liter and milliliter) into which a chemical appears to have been dissolved, once it has penetrated in the body, to give an initial blood concentration equal to C_p:

$$V_D = \text{DOSE}/C_p$$

Usually, V_D does not correspond to any real biological volume and as such has no direct physiological meaning. In some cases, V_D may show values even larger than the volume of a standard human body. Indeed, since the value of V_D is inversely proportional to the blood concentration, chemicals showing an especially high affinity for fatty tissues (e.g., insecticides of the DDT family and several industrial solvents) and therefore having a strong tendency to leave the blood pool may exhibit large V_D values. On the other hand, a chemical that is firmly bound to blood components, such as the red cells and the proteins (e.g., warfarin, a blood anticoagulant), will remain in the blood pool and exhibit V_D values close to blood volume only.

Elimination rate constant (k) The elimination rate constant (i.e., usually a first-order rate constant) is a very useful value that represents the fraction of an agent that is eliminated from the body during a given period of time. For instance, when the value of the elimination rate constant of a xenobiotic is 0.25 per hour, this means that ~25% of the amount remaining in the body is excreted each hour. The rate constant is calculated from the slope ($-k/2.303$) of the curve relating blood concentration with time as shown in **Figure 2c**. Its value is affected by all processes (e.g., distribution, biotransformation, and excretion) that contribute to clear the substance from the blood.

Half-life of elimination ($t_{1/2}$) This is the time period (e.g., minutes, hours, and days) during which the blood concentration of a xenobiotic falls to one-half of its original value as a result of all processes of distribution, biotransformation, and excretion. The determination of $t_{1/2}$ is based on the calculation of the elimination rate constant described above (k):

$$t_{1/2} = 0.693/k$$

Xenobiotics that show small $t_{1/2}$ values (i.e., short half-lives) are those that are cleared rapidly from the body, whereas those with high values (i.e., long half-lives) are cleared more slowly and in some cases may accumulate in the body. Insecticides of the DDT family and heavy metals like lead, cadmium, and mercury all display long half-lives, whereas aspirin is a drug that exhibits a short half-life.

Clearance (CL) Clearance represents the volume of blood (e.g., milliliter and liter) that is completely cleared of a xenobiotic during a given period of time, usually 1 min or 1 h (e.g., ml min^{-1}, l h^{-1}). As such, the clearance is a quantitative measure of the rate of removal of a compound from the body. All routes of elimination (e.g., hepatic biotransformation, urinary, biliary, and pulmonary excretion) contribute to the clearance of a chemical from the body, and each one

exhibits a specific clearance value. Specific clearance values provide an indication of the ability of a particular organ to dispose of a substance. When the value for clearance is high, it suggests that the compound is removed rapidly from the body, whereas a low clearance value indicates slower removal. The value of the clearance is the product of the elimination rate constant (k) and the apparent volume of distribution (V_D) as described by the following equation:

$$CL = k \times V_D$$

Therefore, CL may be regarded as the apparent volume of blood from which the compound is removed during a given period of time.

Bioavailability (F) Bioavailability is a term used to describe the percentage (or the fraction F) of an administered dose of a xenobiotic that reaches the systemic circulation. Bioavailability is practically 100% ($F = 1$) following an intravenous administration. Bioavailability could be lower ($F \leqslant 1$) and in some cases almost negligible for other routes (e.g., oral, dermal, and pulmonary), depending on how efficiently a xenobiotic crosses various biological membranes (e.g., lungs, skin, and stomach) or whether or not tissues or organs (e.g., lungs, skin, and liver) through which xenobiotics pass before reaching the systemic circulation are capable of metabolizing the substance; the latter phenomenon is known as a first-pass effect. Bioavailability may vary considerably between compounds or even between batches of a given compound. For example, drugs commonly used as therapeutic agents must undergo bioavailability testing to ensure reliable dosing throughout treatment. The blood concentration of the administered drug is used as an index of bioavailability.

Area under the curve (AUC) The area-under-the-blood-concentration-time curve reflects the amount of a xenobiotic that has effectively reached the systemic circulation and as such is influenced both by the degree of bioavailability and by the rate at which a chemical is removed from the body. AUC is a good indicator of the internal exposure dose in the body since it takes into consideration not only the blood concentration of a xenobiotic but also the time a xenobiotic is present in the blood compartment and thus in the body.

In summary, the kinetic parameters described previously are used to describe the behavior of xenobiotics in the body following exposure via several routes: the extent of distribution within the body, the amount available for action and elimination, the contribution of specific organs in elimination, and the rate of elimination. Such information can be used to establish therapeutic drug regimens or to predict the extent and duration of contamination of exposed organisms.

Two-Compartment Open Model In certain circumstances, following the completion of the absorption phase, the curve that describes the time course of the blood concentration of a xenobiotic does not exhibit a single straight line but rather two segments (**Figure 3b**). Such biexponential decline can best be described by a two-compartment model (**Figure 3a**): a central compartment that usually refers to the blood pool and a peripheral compartment that represents various fluids and tissues of the body for which a xenobiotic may have a particular affinity. This system can be described mathematically by a differential equation comprising two exponential terms, one for each segment of the curve. Taken individually, each one of these terms is essentially similar to the one used to describe the curve corresponding to the one-compartment model:

$$\log_{10} C_p = \log_{10} A - \frac{\alpha \times t}{2.303} + \log_{10} B - \frac{\beta \times t}{2.303}$$

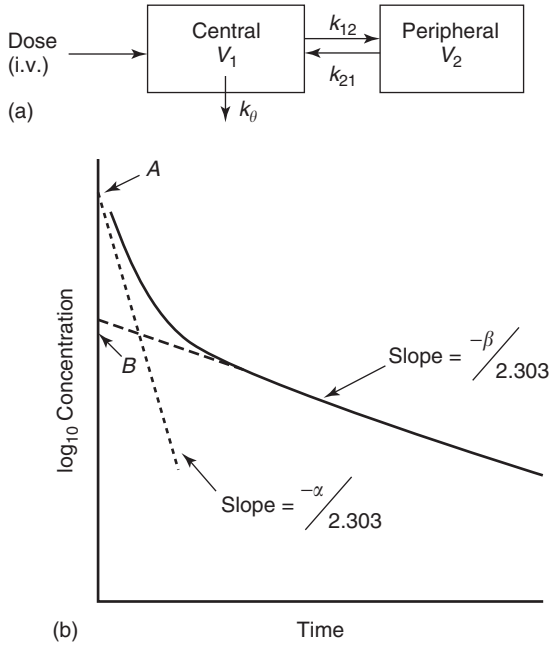

Figure 3 (a) Schematic representation of a two-compartment open model: i.v., intravenous administration, V_1 and V_2, respective volumes of compartments 1 and 2; k_{21} and k_{12}, transfer rate constants between compartments 1 and 2; k_θ, elimination rate constant. (b) Time course of blood concentration following intravenous administration: A and B, proportionality constants; α and β, elimination rate constants corresponding to each segment of the curve.

where A and B are proportionality constants for each compartment ($A + B = C_p$), and α and β are composite rate constants that can be regarded as the elimination rate constant of each segment of the curve (i.e., each compartment). The first segment is known as the α-phase, during which a chemical leaves the blood circulation to be distributed among the various organs and tissues, whereas the second segment corresponds to the β-phase, which mainly characterizes the processes leading to the elimination of a chemical. Accordingly, the $t_{1/2}$ of a xenobiotic displaying such kinetic behavior is calculated from the β-phase using an equation similar to the one previously described for a one-compartment model:

$$t_{1/2} = 0.693/\beta$$

How values A, B, α, and β are calculated falls beyond the scope of this text. Suffice it to say that these parameters represent values that contribute additively to the equation describing the two-compartment open model.

To facilitate the understanding of the pharmacokinetic concepts, the examples given previously are for the simplest and the most effective route of administration, that is, intravenous administration. When exposure is to toxic compounds (e.g., occupational or environmental exposure), however, other routes are frequently involved. These routes include respiratory, cutaneous, mucous, or oral uptake. In such cases, pharmacokinetic analyses are more complex since they should take into account the various processes responsible for the uptake of a xenobiotic. Usually, this consists of introducing into equations an additional term that contains a rate constant describing the uptake, operating in a direction opposite to, yet not conceptually different from the elimination rate constant.

Physiologically Based Models

Whereas compartmental models are abstract mathematical representations of an animal or a human body, in the form of a certain number of boxes, PBPK models describe the behavior of xenobiotics on the basis of the actual anatomy, physiology, and biochemistry of human beings and animals. Being realistically modeled on how the body functions, PBPK models take into consideration the complex relationships that exist between critical biological and physicochemical determinants such as blood flow, ventilation rates, metabolic rate constants, tissue solubilities, and binding to proteins (e.g., albumin and glycoproteins) or other macromolecules (e.g., DNA and hemoglobin).

Contrary to compartmental models, PBPK models allow one to describe the time course of xenobiotic concentration in any organ or tissue represented in the model. Since these models include anatomical, physiological, and biochemical determinants, they can account for any quantitative alterations of such determinants – for example, ventilation rates, organ pathology, or metabolic enzyme activity. Not only can they describe and model what is actually occurring under a given set of exposure conditions but also they can build on such a description and expand to any other condition likely to happen within the range of variation of the anatomical, physiological, and biochemical parameters.

A PBPK model comprises a series of anatomically well-defined compartments that represent organs or tissues in which a xenobiotic distributes or exerts its toxic effects (**Figure 4**). These anatomical compartments are interconnected by the blood circulation (i.e., arterial blood to and venous blood from the tissues). The physiological and anatomical determinants for different species, including humans (e.g., alveolar ventilation rate, blood flow rates, and tissue volumes), are usually abundantly documented in the literature. Physicochemical parameters – namely partition coefficients that describe the relative solubility

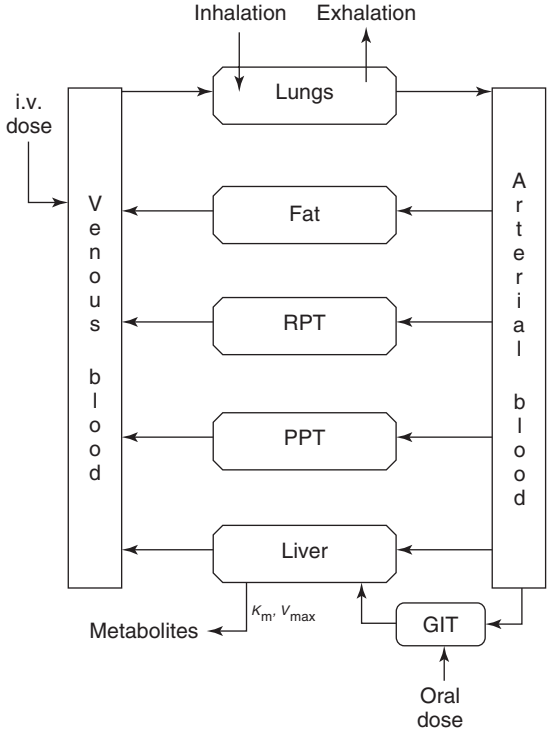

Figure 4 Schematic representation of a PBPK model with different routes of entry. RPT, richly perfused tissues (e.g., brain, kidneys, and spleen); PPT, poorly perfused tissues (e.g., muscles, skin, and bone; GIT, gastrointestinal tract. K_m and V_{max} are constants that characterize metabolizing tissues like the liver.

of a xenobiotic between air present in the lungs and blood, on the one hand, and between blood and tissues, on the other hand – may be obtained in some cases from the literature or otherwise determined experimentally in the laboratory. Usually, biochemical parameters, namely metabolic rate constants that describe the metabolic capacity of a tissue toward a given xenobiotic, are determined experimentally in the laboratory.

To each compartment corresponds a mass-balance differential rate equation that describes the rate of change in the amount (Amt) of a xenobiotic in this h tissue compartment, as the xenobiotic enters in, distributes within, and exits the tissue:

$$\frac{\mathrm{d}\ \mathrm{Amt}_i}{\mathrm{d}t} = Q_i(C_\mathrm{a} - C_\mathrm{vi})$$

where Q_i represents the volume of blood circulating throughout the tissue i per unit of time, C_a is the concentration of the xenobiotic in arterial blood entering the tissue, and C_vi is the concentration of the xenobiotic in venous blood leaving the tissue. For metabolizing tissues (e.g., liver), an additional term that takes into account the capacity of such tissues to operate the metabolic transformation of the xenobiotic is added to the basic differential equation described previously. Since the capacity of the liver and other metabolizing tissues is limited when large amounts of a xenobiotic are presented to the tissues, the basic equation contains terms (K_m and V_max) that account for such limitations:

$$\frac{\mathrm{d}\ \mathrm{Amt}_i}{\mathrm{d}t} = Q_i(C_\mathrm{a} - C_\mathrm{vi}) - \frac{V_\mathrm{max} \times C_\mathrm{vi}}{K_\mathrm{m} + C_\mathrm{vi}}$$

where the new terms K_m and V_max describe, respectively, the affinity of a xenobiotic for metabolizing enzymes and the maximum velocity of the enzymatic reactions.

The previously described equations are characteristic of blood flow rate-limited models; it is assumed that xenobiotics cross the cell membrane by simple diffusion and that equilibrium takes place instantaneously between blood and tissue compartments. This assumption is valid for a great number of chemicals. For certain xenobiotics, however, the kinetics of tissue uptake are not consistent with blood flow rate-limited processes since their distribution in a given tissue is limited by the resistance of the cell membrane to the passage of a xenobiotic. In these cases, the basic equation should account for such phenomena to describe adequately the time course of the xenobiotic disposition in the tissue.

Of course, various exposure routes (e.g., inhalation, intravenous, oral, and dermal) can be accounted for in PBPK models by incorporating the proper equation describing these uptake processes.

Once formulated, a PBPK model can be used to simulate the kinetic behavior of a xenobiotic (e.g., amount metabolized, blood or tissue concentrations, and percentage of dose excreted) in animals or humans. An important step in the development of PBPK model is its validation. Validation is usually based on the visual or statistical comparison of model predictions with experimental observations in humans or animals.

Once validated, PBPK models can be used by toxicologists for many purposes. For example, PBPK models can (1) provide an estimate of the time course distribution of xenobiotics and their metabolites in various parts of the body, including target organs/tissues; (2) allow various types of metabolic extrapolations between various species, from high doses of exposure to low doses, or from one route of exposure to another; (3) allow the examination of pharmacokinetic differences between species; (4) facilitate the setting and adjustment of exposure standards since it becomes possible to better estimate the concentration of a xenobiotic and its metabolite(s) in various body fluids or tissues, resulting from various exposure scenarios; and (5) predict changes in the disposition kinetics of xenobiotics resulting from physiological and pathological alterations in body function.

For all these reasons, PBPK models are and will continue to be increasingly used in toxicology. This is especially true in risk assessment studies since better definition of the internal tissue dose, may contribute to reduce the uncertainty associated with extrapolation to human beings of responses observed in animal toxicity studies in which animals usually receive high doses of xenobiotics by routes often different from the one(s) anticipated in human exposures.

Repetitive Exposures

Frequently, individuals are exposed repetitively to xenobiotics, be they medication, food additives, or environmental contaminants.

In general, chemicals exhibiting a short half-life (i.e., smaller than the period of time between each new exposure) are almost completely eliminated between exposures. Inversely, chemicals with a long half-life (i.e., longer than the period of time between exposures) tend to accumulate in the body leading eventually to increased risk of toxicity. In the latter case, if exposure continues at a relatively constant level, the accumulated chemical will reach a plateau, also called a steady-state level, when the amount of a xenobiotic that enters the body equals the amount eliminated during a given period of time.

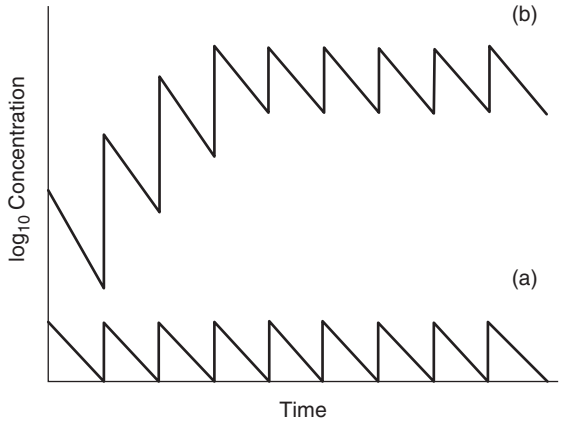

Figure 5 Time-course blood concentration of a xenobiotic following repeated intravenous administration: (a) xenobiotic half-life shorter than the period of time between exposures; (b) xenobiotic half-life longer than the period of time between exposures.

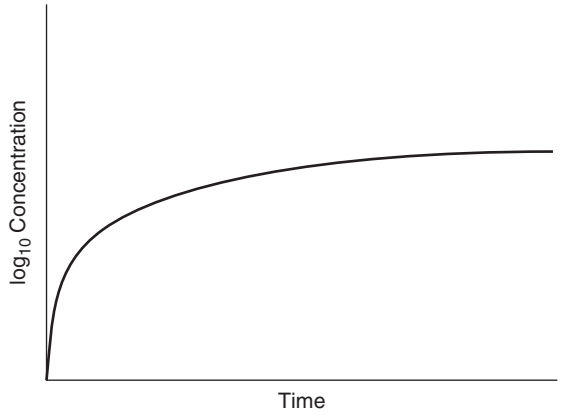

Figure 6 Time-course blood concentration of xenobiotic following continuous intravenous infusion or pulmonary inhalation until steady state.

Figure 5a and **b** illustrates the kinetics of a xenobiotic in blood resulting from repeated intravenous exposures. The time that is necessary to reach the steady state depends on the half-life of the xenobiotic and corresponds to about five times the half-life value, whereas the blood concentration is a function of the absorbed dose.

In contrast to the sawtooth pattern of blood concentrations during repeated, noncontinuous exposure to a xenobiotic (**Figure 5a** and **b**), the pattern resulting from continuous exposure is characterized by a single stable line (**Figure 6**). However, for both situations, the time to reach a plateau concentration and the amount present in blood obey the same rules of kinetics.

Thus, for a compound administered intravenously and described by a one-compartment model, the average steady-state blood concentration (C_{ss}) is

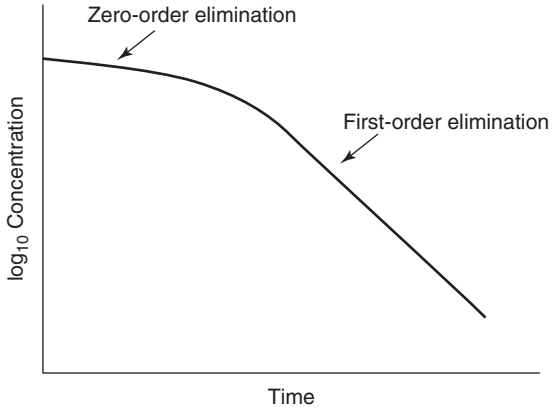

Figure 7 Time-course blood concentration of a xenobiotic exhibiting nonlinear (zero-order) kinetics following intravenous administration; when the biological processes responsible for the disposition of the xenobiotic are no longer saturated, first-order kinetics resume.

determined by the following equation:

$$C_{ss} = \frac{\text{Dosing rate}_{\text{i.v.}}}{V_D \times k \times \tau}$$

where V_D is the apparent volume of distribution, k is the elimination rate constant, and τ is the time interval between administered doses. As can be seen, the blood concentration is related proportionally to the administered dose but bears an inverse relationship to all other parameters.

Dose-Dependent Kinetics

As seen earlier, exposure conditions amenable to pharmacokinetic/toxicokinetic analysis are such that the rate of the biological processes (e.g., diffusion across membranes, biotransformation, excretion by glomerular filtration, etc.) is proportional to the concentration or amount of a xenobiotic in a given compartment such as blood. The rate is then said to be governed by first-order kinetics (see **Figures 2** and **3**).

There are biological processes, however, that involve saturable carrier or enzymatic systems, with a finite capacity for transport or catalysis. For instance, processes like active uptake at absorption sites, renal tubular secretion, or hepatic biotransformation of xenobiotics may become saturated at high exposure levels, yielding rates of disposition that are constant and independent of the concentration in blood. This is characteristic of zero-order kinetics. Biotransformations of ethanol in the liver and active tubular renal secretion of penicillin in urine are examples of biological processes that obey zero-order kinetics. **Figure 7** illustrates the blood concentration

of a chemical eliminated by zero-order kinetics. Since at high concentrations, the amount of a chemical that is biotransformated or excreted is limited by saturable processes, blood concentration falls less rapidly than when first-order kinetics prevails. This may result in more or less accumulation of that chemical in several tissues, including those that are especially sensitive to its toxic action.

Conclusion

Pharmacokinetic/toxicokinetic analysis is a very important tool that can help toxicologists understand how the body handles foreign chemicals. With a good knowledge of the time course relationship between exposure to chemicals and their concentration in various tissues and organs, toxicologists are in a position to better interpret and predict the nature and extent of toxicity.

More specifically, data pertaining to toxicokinetics are, and will increasingly continue to be, essential to properly:

- Predict the body burden of toxic chemicals in a critical organ or tissue.
- Understand the dose–response relationship of toxic chemicals.
- Assist in the selection of animal species that can act as a surrogate of human toxicity.
- Make rational extrapolations from high doses, as used in animal toxicity studies, to low doses, as encountered in the human environment.
- Set exposure limits to toxic chemicals for all kinds of living organisms, including humans.
- Identify potentially at-risk subgroups of exposed living organisms.

See also: Absorption; Distribution; Excretion; Pharmacokinetic Models.

Further Reading

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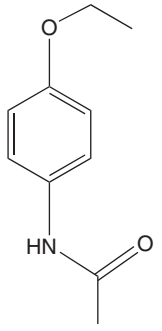
Pharmacology and Safety *See* Safety Pharmacology.

Phenacetin

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 62-44-2
- Synonyms: *p*-Acetophenetidide; 1-Acetamido-4-ethoxybenzene; Acetophenetin; 4-Ethoxy acetanilide; *p*-Acetylphenetidin
- Chemical/Pharmaceutical/Other Class: An NSAID (nonsteroidal antiinflammatory drug) with analgesic, antipyretic, and antirheumatic properties
- Chemical Formula: $C_4H_{13}O_2N$
- Chemical Structure:



Background Information

Phenacetin was introduced into the pharmaceutical market in 1887, and was withdrawn in 1983 in the United States due to unacceptable levels of interstitial nephritis in patients and potential risks of tumorgenicity. Like in the United States, most Western countries did not ban phenacetin from marketing until 1983.

Exposure Routes and Pathways

Possible exposure routes are oral and inhalation.

Toxicokinetics

Phenacetin is metabolized to acetaminophen and sulfhemaglobin-forming metabolite and other toxic metabolites. In the absence of adequate glutathione or a glutathione substitute, acetaminophen is further metabolized to cytotoxic and hepatotoxic molecules.

Acute and Short-Term Toxicity (or Exposure)

Animal

Oral rat LD_{50}: 3600 mg kg^{-1}; mouse 866 mg kg^{-1}; rabbit $>$500 mg kg^{-1}.

Human

Phenacetin is harmful if swallowed or inhaled, and may cause kidney, liver, and blood disorders. It may cause methemoglobinemia and hemolytic anemia due to acute toxicities, but more commonly as a result of chronic overdosage. A therapeutic plasma level was less than 20 μg ml^{-1}, with 50–150 μg ml^{-1} being toxic plasma levels in humans.

Chronic Toxicity (or Exposure)

Animal

Phenacetin is nephrotoxic, with positive results in mutagenicity and tumorgenicity studies.

An International Agency for Research on Cancer (IARC) Working Group reported that there is limited evidence of carcinogenicity of analgesic mixtures containing phenacetin in experimental animals.

Human

Phenacetin is group 2A (reasonably anticipated to be a human carcinogen) based on sufficient evidence of carcinogenicity in experimental animals according to the IARC Working Group noted above. The same group found that analgesic mixtures containing phenacetin are known to be human carcinogens based on sufficient evidence of carcinogenicity in humans. The Human Health Assessment Group in (US) Environmental Protection Agency's Office of Health and Environmental Assessment has evaluated phenacetin for carcinogenicity. According to their analysis, the weight of evidence for phenacetin is group B2 (considered probably carcinogenic to humans), which is based on inadequate evidence in humans and sufficient evidence in animals.

Phenacetin is linked to hypertension, cardiovascular disease, and cancer, but was removed from the market primarily due to induction of chronic renal disease.

In Vitro Toxicity Data

Phenacetin was mutagenic to *Salmonella typhimurium* bacteria when tested in the presence of a metabolic system derived from hamster but not mouse or rat liver. The urine from phenacetin-treated Chinese hamsters, but not that from rats, was mutagenic to bacteria. It is activated to direct-acting mutagens by deacetylation, occurring more frequently in hamsters than rats. Phenacetin induced chromosomal aberrations in Chinese hamster cells *in vitro*, but not DNA strand breaks in rat hepatocytes. It did not induce sex-linked recessive lethal mutations in *Drosophila*.

Clinical Management

Methylene blue therapy (unlike with methemoglobinemia) does not help with the hematological effects.

Environmental Fate

Phenacetin is expected to leach into groundwater when released into the soil. When released into the water, this material is expected to have a half-life of more than 30 days. This material has an estimated bioconcentration factor of less than 100, and is not expected to significantly bioaccumulate. This material is expected to be readily degraded by reaction with photochemically produced hydroxyl radicals when released into the air. This material is expected to have a half-life of less than 1 day when released into the air.

Exposure Standards and Guidelines

The US Occupational Safety and Health Administration regulates phenacetin under the Hazard Communication Standard, and as a chemical hazard in laboratories. A reportable quantity (RQ) of 100 lb has been proposed for phenacetin under the EPA's

Comprehensive Environmental Response, Compensation, and Liability Act.

See also: Carcinogen Classification Schemes.

Further Reading

Bakhe OM, Wardell WM, and Lasagna L (1984) Drug discontinuations in the United Kingdom and the United States, 1964–1984: Issues of safety. *Clinical Pharmacology and Therapy* 35: 559–567.

Fishbein L (1981) Phenacetin: an overview of its toxicity, metabolism and analysis. *IARC Science Publications* 40: 287–310.

Ronco PM and Flahault A (1994) Drug-induced end-stage renal disease. *New England Journal of Medicine* 331: 1711–1712.

Schnuelle P and van der Woude FJ (2003) Analgesics and renal disease in the postphenacetin era. *American Journal of Kidney Diseases* 42: 385–387.

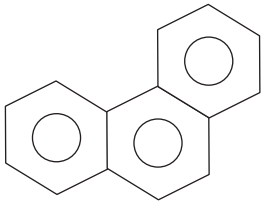
Relevant Website

http://ehp.niehs.nih.gov – US National Institute of Environmental Heath Sciences (NIEHS) National Toxicology Program (NTP) (2002) Phenacetin and Mixtures Containing Phenacetin (from the 10th Report on Carcinogens of the National Toxicology Program, 2002).

Phenanthrene

Samantha E Gad

- Chemical Abstracts Service Registry Number: CAS 85-01-8
- Synonyms: Coal tar pitch volatiles; Phenanthrin
- Chemical/Pharmaceutical/Other Class: Polycyclic aromatic hydrocarbon
- Chemical Formula: $C_{14}H_{10}$
- Chemical Structure:



Uses

Phenanthrene is a polycyclic aromatic hydrocarbon (PAH) that can be derived from coal tar. Phenanthrene is used in the production of dyes, pharmaceuticals, and explosives, and in biochemical research. A derivative, cyclopentenophenanthrene, has been used as a starting material for synthesizing bile acids, cholesterol, and other steroids.

Exposure Routes and Pathways

Phenanthrene occurs in fossil fuels and is present in products of incomplete combustion. Some of the known sources of phenanthrene in the atmosphere are vehicular emissions, coal and oil burning, wood combustion, coke plants, aluminum plants, iron and steel works, foundries, municipal incinerators, oil shale plants, and tobacco smoke. It is widely distributed in the aquatic environment and has been identified in surface water, tap water, wastewater, and dried lake sediments. It has also been identified in seafood collected from contaminated waters and in smoked and charcoal-broiled foods. Phenanthrene has been identified in foods.

Human exposure occurs primarily through inhalation of tobacco smoke and other polluted air, and via ingestion of food or water contaminated by combustion effluents.

Toxicokinetics

Since it is the smallest aromatic hydrocarbon to have a 'bay-region' and a 'K-region', phenanthrene is often used as a model substrate for studies on metabolism of carcinogenic PAHs. Phenanthrene is absorbed following oral and dermal exposure. Data from structurally related PAHs suggest that phenanthrene would be absorbed from the lungs. Metabolites of phenanthrene identified in *in vivo* and *in vitro* studies indicate that metabolism proceeds by epoxidation at the 1-2, 3-4, and 9-10 carbons, with dihydrodiols as the primary metabolites.

Mechanism of Toxicity

Phenanthrene absorbs ultraviolet light and causes production of singlet oxygen, which in turn leads to free radical production. Although a large body of literature exists on the toxicity and carcinogenicity of other PAHs, primarily benzo[*a*]pyrene, toxicity data for phenanthrene are limited.

Acute and Short-Term Toxicity (or Exposure)

Animal

The LD_{Lo} in mice is 71 mg kg^{-1}. In mice, the oral LD_{50} is 700 mg kg^{-1}, the intraperitoneal LD_{50} is 700 mg kg^{-1}, and the intravenous LD_{50} is 56 mg kg^{-1}. Single doses of 100 mg kg^{-1} day^{-1} of phenanthrene administered by gavage for 4 days suppressed carboxylestrase activity in the intestinal mucosa of rats, but did not produce other signs of gastrointestinal toxicity. Phenanthrene had no effect on hepatic or extrahepatic carboxylesterase activities. Single intraperitoneal injections of phenanthrene produced slight hepatotoxicity in rats.

Human

Phenanthrene can cause phototoxicity and photosensitization of the skin. No other human data were available that addressed the acute toxicity profile of phenanthrene.

Chronic Toxicity (or Exposure)

Animal

Phenanthrene may cause skin allergy, and is considered phototoxic. It has induced sister chromatid exchanges in Chinese hamster cells. The available data are inadequate to permit an evaluation of the carcinogenicity of phenanthrene to experimental animals; however, a number of other PAHs have caused tumors in laboratory animals via oral, inhalation, and dermal exposures. A single oral dose of phenanthrene did not induce mammary tumors in rats, and a single subcutaneous injection did not result in treatment-related increases in tumor incidence in mice. Neonate mice administered intraperitoneal or subcutaneous injections of phenanthrene also did not develop tumors. No skin tumors were reported in two skin painting assays with mice. Phenanthrene was also tested in several mouse skin initiation-promotion assays. It was active as an initiator in one study, inactive as an initiator in four others, and inactive as a promoter in one study.

Human

Phenanthrene is classified as category D for human carcinogenicity by the US Environmental Protection Agency, that is, it is not classifiable as to human carcinogenicity.

In Vitro Toxicity Data

Phenanthrene induced mutations in a human cell in culture and in the Ames *Salmonella*. Phenanthrene was shown to inhibit colony formation of HeLa cells.

Environmental Fate

Release of phenanthrene most likely results from the incomplete combustion of a variety of organic compounds including wood and fossil fuels. Release to the soil will likely result in biodegradation. Volatilization from soil is not expected to be significant. Phenanthrene is expected to bind strongly to soil and not leach extensively to groundwater. When released to water, adsorption of phenanthrene to suspended sediments is expected to remove most of the compound from solution. Photolysis is expected to occur near the water surface and biodegradation in the water column is expected. Bioconcentration is not expected to be significant. Phenanthrene released to the atmosphere is expected to rapidly adsorb to particulate matter. It will react with hydroxyl radicals with an estimated half-life of less than 2 days. Uptake, accumulation, and translocation of phenanthrene and pyrene by 12 plant species grown in various treated soils was investigated, and the plant uptake and accumulation of both compounds was correlated with their soil concentrations and plant compositions.

Ecotoxicology

Phenanthrene has been shown to be toxic to marine diatoms, gastropods, mussels, crustaceans, and fish. The toxic effects of several aromatic hydrocarbons (benzene, toluene, naphthalene, 1-methylnaphthalene, anthracene, 9-methylanthracene, and phenanthrene) on the productivity growth rate of various marine planktonic algae (*Dunaliela biocula*, *Phaeodactylum tricornutum*, and *Isochysis galbaya*) increased with an increasing number of aromatic rings. The methylated compounds were most toxic. The TL_m (median lethal dose) for exposure of *Neanthes arenaceodentata*, a member of the polychaete family, to phenanthrene is 0.6 ppm for a 96 h exposure in seawater at 22°C in a static bioassay.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value, 8 h time-weighted average (TWA) is 0.2 mg m^{-3} for coal tar pitch volatiles, as is the (US) Occupational Safety and Health Administration permissible exposure limit, 8 h TWA. The (US) National Institute for Occupational Safety and Health (NIOSH) recommended exposure level, averaged over a 10 h workday, is 0.1 mg m^{-3} for coal tar pitch volatiles. Further, NIOSH considers coal tar

products carcinogenic and conditions should be made to keep exposures as low as possible. Current NIOSH research indicates that asphalt products are carcinogenic to laboratory animals and, therefore may be more toxic to humans than previously believed. Phenanthrene is a toxic pollutant designated pursuant to Section 307(a) (1) of the US Clean Water Act, and is subject to effluent limitations.

See also: Coal Tar; Polycyclic Aromatic Hydrocarbons (PAHs); Skin.

Further Reading

Gao Y and Zhu L (2004) Plant uptake, accumulation and translocation of phenanthrene and pyrene in soils. *Chemosphere* 55: 1169–1178.

International Agency for Research on Cancer (IARC) (1983) *IARC Monographs* 32: 419–430.

Relevant Website

http://risk.lsd.ornl.gov – US Oak Ridge National Laboratory, Risk Assessment Information System. Toxicity Profile for Phenanthrene.

Phenazopyridine

Christopher P Holstege

- Chemical Abstracts Service Registry Numbers: CAS 94-78-0; CAS 136-40-3 (hydrochloride salt)
- Synonyms: 3-Phenylazopyridine-2,6-diyldiamine; Pyridium
- Chemical/Pharmaceutical/Other Class: Azo dye

Uses

Phenazopyridine is utilized as an analgesic for the urinary tract, thereby providing relief of urinary urgency and/or frequency.

Exposure Routes and Pathways

Ingestion is the exposure pathway.

Toxicokinetics

Phenazopyridine is absorbed through the gastrointestinal tract. It is metabolized rapidly, producing aniline, 2,3,6-triaminopyridine, *p*-aminophenol, and *N*-acetyl-*p*-aminophenol. Renal clearance is the major route of elimination. Approximately 90% of a therapeutic dose is excreted in the urine within 24 h, with 41% as phenazopyridine, 24% as *p*-aminophenol, 18% as *N*-acetyl-*p*-aminophenol, and 6.9% as aniline. The color of the urine changes to orange or red.

Mechanism of Toxicity

Phenazopyridine may induce methemoglobinemia. Erythrocytes possess four hemoglobin chains, each of which contains a heme moiety. Methemoglobin occurs when phenazopyridine induces oxidation of the heme moiety, changing the normal oxygen-carrying ferrous (Fe^{2+}) state to the ferric (Fe^{3+}) state. Ferric heme is incapable of binding oxygen. Ferric heme also shifts the hemoglobin dissociation curve to the left, thereby impairing the release of oxygen from the remaining ferrous heme groups on the same hemoglobin tetramer. Oxygen delivery to tissues is therefore impaired. Red blood cells with methemoglobin also become rigid and are unable to traverse the spleen, with resultant destruction and anemia. Renal failure may result either due to the phenazopyridine itself, as a result of hemolytic anemia, or secondary to rhabdomyolysis.

Acute and Short-Term Toxicity (or Exposure)

Animal

Phenazopyridine has been associated with methemoglobinemia, hemolysis, hepatic necrosis, muscle damage, and renal tubular necrosis in animals.

Human

Phenazopyridine-induced methemoglobinemia may manifest as dyspnea, tachycardia, cyanosis, dizziness, and syncope. Hemolysis may occur and result in anemia. Progressive oliguric renal failure may occur and is typically associated with methemoglobinemia and hemolysis, or with massive acute overdosage. Yellow discoloration of the skin and sclerae may occur due to deposition of this azo dye in the skin, primarily in patients with renal dysfunction. Rare cases of phenazopyridine-induced hypersensitivity hepatitis, rhabdomyolysis, and aseptic meningitis have been reported.

Chronic Toxicity (or Exposure)

Animal

Mice chronically fed phenazopyridine up to 1200 mg kg^{-1} 5 days a week for 80 weeks, survived. Animals receiving higher doses had greater rates of adenomas, adenocarcinomas, and carcinomas than the controls, and greater rates than those animals receiving lower doses.

Human

Patients have occasionally been treated with higher than therapeutic doses of phenazopyridine. Toxic effects (e.g., methemoglobinemia) are more commonly seen in patients with some degree of glucose-6-phosphate dehydrogenase deficiency. A man who took 600 mg phenazopyridine daily for 2 years developed pure phenazopyridine vesical calculi.

In Vitro Toxicity Data

Several carcinogenicity and mutagenicity studies have been performed on phenazopyridine. Mouse lymphoma studies have been mixed but rat hepatocyte studies have been positive.

Clinical Management

Symptomatic and supportive care is the mainstay of therapy. Adequate urine output should be assured. In acute overdose, charcoal may be considered. Methylene blue therapy may be considered for patients with methemoglobinemia. Dialysis has been used for phenazopyridine-induced renal dysfunction, but no studies have demonstrated an increased elimination of phenazopyridine with dialysis. In patients with hemolysis and marked anemia, transfusion may be necessary.

See also: Aniline; Carcinogenesis.

Further Reading

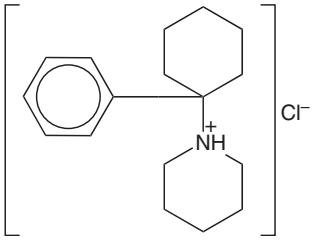
Mercieca JE, Clarke MF, and Phillips ME (1982) Acute haemolytic anaemia due to phenazopyridine hydrochloride in G-6-PD deficient subject. *Lancet* 2: 564.

Truman TL, Dallessio JJ, and Weibley RE (1994) Life-threatening pyridium plus(R) intoxication: A case report. *Pediatric Emergency Care* 10: 225–228.

Phencyclidine

Christopher P Holstege

- Chemical Abstracts Services Registry Numbers: CAS 77-10-1; CAS 956-90-1 (hydrochloride)
- Synonyms: 1-(1-Phenylcyclohexyl)piperidine; Angel dust; Busy bee; Crystal joint; Cyclones; DOA; Embalming fluid; Goon; Hog; Kay jay; Love boat; Lovely; Mint dew; Mist; Murder-1; Peace pill; PCP; Rocket fuel; Scuffle; Selma; Sernyl; Sernylan; Snorts; Soma; Star dust; Super grass; Super weed; Super kool; Surfer; Tranquilizer; Whacky weed; Zombie dust. Phencyclidine analogs with similar pharmacologic effects include phenylcyclohexylpyrrolidine (PHP), phenycyclopentylpiperidine (PCPP), thienycyclohexylpiperidine (TCP), and cyclohexamine (PCE)
- Chemical/Pharmaceutical/Other Class: Synthetic arylcyclohexylamine closely related to ketamine, a medically approved dissociative anesthetic
- Chemical Structure: Phencyclidine hydrochloride



Uses

Medical use in humans was discontinued in 1965 and veterinary use was discontinued in 1978. Today, phencyclidine is manufactured in illegal laboratories and utilized as a drug of abuse.

Exposure Routes and Pathways

Phencyclidine is sold illicitly as powder, tablets, liquid, rock crystal, or mixed with leaves (marijuana, oregano, mint, and parsley). Phencyclidine is ingested, smoked, snorted, and injected intravenously.

Toxicokinetics

When smoked or snorted, phencyclidine has an onset of action from 2 to 5 min; when ingested, effects are apparent within 30–60 min. Peak effects may be achieved 15–30 min after onset with effects persisting for as long as 24–48 h. Phencyclidine undergoes hepatic degradation by oxidative hydroxylation to two metabolites that have little psychotropic activity. The volume of distribution of phencyclidine is large, averaging $6\,l\,kg^{-1}$. Plasma protein binding is ~65%. Because of high lipid solubility, levels found in tissue far exceed those found in plasma. Phencyclidine follows first-order elimination kinetics. It undergoes enterohepatic recycling with subsequent excretion by the kidneys. The half-life of small doses is 1 h, increasing to 17.6 h (range, 7–50 h) in overdose.

Mechanism of Toxicity

The precise mechanisms by which phencyclidine causes its clinical effects have not been fully delineated. Phencyclidine blocks the *N*-methyl-*D*-aspartate (NMDA) receptors and thereby calcium influx into cells. Phencyclidine inhibits the biogenic amine reuptake complex and thereby inhibits norepinephrine and dopamine reuptake. Phencyclidine also increases adrenergic activity by indirectly releasing norepinephrine from presynaptic neurons. Phencyclidine in high doses stimulates sigma receptors.

Acute and Short-Term Toxicity (or Exposure)

Animal

Dose-dependent effects include depressed reflexes, tachycardia, twitching, dilated pupils, and hyperthermia. There are marked species differences in behavior. Mice primarily experience excitation. Dogs generally display depression at low doses and stimulation (e.g., seizures) at high doses.

Human

Phencyclidine use results in excitation with marked paranoid or aggressive behavior, which is often self-destructive. Effects include distortion of body image, diminished pain perception, illusions, and delusions, including a perception of superhuman strength and invulnerability. Miosis and nystagmus (horizontal, vertical, and rotary) may be seen in association with ataxia, bizarre behavior, and hallucinations. Tachycardia, hypertension, hyperreflexia, seizures, respiratory depression, and coma are reported with high doses. Dystonias and dyskinesias have been reported. Hypoglycemia can be seen. Rhabdomyolysis, acute renal failure, disseminated intravascular coagulation, liver necrosis, and traumatic injury are reported complications. The anesthetic dose of phencyclidine is $0.25\,mg\,kg^{-1}$ intravenously. Doses of 1–5 mg are purported to cause euphoria and numbness, 5–10 mg cause excitation and hallucinations, and 20 mg or more cause coma and serious toxicity or death. Plasma concentrations of phencyclidine vary widely after overdose. Phencyclidine crosses the placenta resulting in hyperirritability, tremors and hypertonia, depressed reflexes, and nystagmus in neonates.

Chronic Toxicity (or Exposure)

Animal

Rats demonstrate signs and symptoms of withdrawal after 7 days of receiving $45\,mg\,kg^{-1}\,day^{-1}$ phencyclidine.

Human

Cognitive decline, depression, anxiety, violent behavior, and weight loss are reported following chronic use of phencyclidine. Prolonged psychosis has been reported, which can mimic acute schizophrenia, and can persist for 4–6 weeks. Tolerance to the psychoactive effects can lead abusers to take increased doses. Psychological dependence has been noted, but no distinct withdrawal symptoms have been reported.

In Vitro Toxicity Data

Phencyclidine is extensively used in research because of its properties as a noncompetitive antagonist of NMDA glutamate receptors. *In vivo* and *in vitro* data have demonstrated that phencyclidine can produce apoptosis in the frontal cortex of rats.

Clinical Management

Adequate supportive care should be assured in the phencyclidine-intoxicated patient. There is no antidote for phencyclidine overdose. The patient should be isolated from all sensory stimuli as much as possible and protected from self-inflicted injury. Benzodiazepines should be administered liberally and titrated until the phencyclidine-intoxicated patient calms. Adequate hydration should be assured to maintain the urine output at $1–2\,cc\,kg^{-1}\,h^{-1}$. Although urine acidification theoretically enhances phencyclidine elimination, it is not recommended because of the high frequency of rhabdomyolysis and myoglobinuric renal failure seen with significant intoxication. Seizures should be treated with

benzodiazepines. Hypertensive crisis can be managed with nitroprusside. The patient's blood sugar, electrolytes, serum creatinine phosphokinase, urine myoglobin, and renal and hepatic function tests should be monitored.

See also: Benzodiazepines.

Further Reading

Aronow R, Miceli JN, and Done AK (1980) A therapeutic approach to the acutely overdosed PCP patient. *Journal of Psychedelic Drugs* 12: 259–266.

Patel R, Das M, and Palazzolo M (1980) Myoglobinuric acute renal failure in phencyclidine overdose: Report of observation in eight cases. *Annals of Emergency Medicine* 9: 549–553.

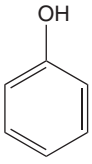
Phenelzine *See* Monoamine Oxidase Inhibitors.

Phenobarbital *See* Barbiturates, Long-Acting.

Phenol

Kathryn J Kehoe

- Chemical Abstracts Service Registry Number: CAS 108-95-2
- Synonyms: Carbolic acid; Hydroxybenzene; Phenic acid; Benzenol; Phenol alcohol
- Chemical/Pharmaceutical/Other Class: Phenol
- Chemical Formula: C_6H_5OH
- Chemical Structure:



Uses

Phenol is a product of the refining of oil and coal tar and is used industrially in the manufacture of pharmaceuticals, plastics, rubber, and plywood. It has antiseptic, germicidal, and anesthetic properties and may be used in disinfectants and preservatives. It is used in small amounts in many over-the-counter products with antiseptic properties. It is also a common reagent in nucleic acid/molecular biology research and is used to denature and remove protein from preparations of DNA and RNA.

Exposure Routes and Pathways

Phenol is readily absorbed from all surfaces of the body. Acute exposures by all routes (inhalation, skin contact, and ingestion) can be fatal. Inhalation exposure appears to be the most sensitive route of exposure, although limited, due to phenol's low volatility. Cigarette smoke contains phenol. Due to phenol's anesthetic properties initial exposure may not be painful; however in skin exposure, deep dermal damage progressing to gangrene is common.

Toxicokinetics

Phenol will undergo biotransformation to oxidation and conjugated products. Phenol and phenolic compounds can be oxidized by peroxidase-dependent prostaglandin H synthase to phenoxyl radicals. It is a reactive intermediate in the P450 oxidation of benzene to hydroquinone. Phenol that is not oxidized will undergo conjugation to etheral, sulfate, or glycuronate species. This is especially true of phenol introduced to the gastrointestinal tract. Conjugates appear less toxic than the parent compound and are subsequently excreted through the kidneys. A smaller amount may be eliminated through the lungs, as detected by an aromatic odor to the breath.

Mechanism of Toxicity

Phenol is a general protoplasmic poison. It can be oxidized to a reactive electrophile that combines with protein and DNA. The binding to hepatic or renal proteins leads to centrilobular and medullar damage, respectively.

Acute and Short-Term Toxicity (or Exposure)

Animal

Phenol shows high toxicity by all routes of exposure. The LD_{50} values in rats are as follows: 384 mg kg^{-1}

(oral), 669 mg kg^{-1} (skin), 250 mg kg^{-1} (interperitoneal), and 316 mg kg^{-1} (inhalation). The LD_{50} values for rabbits after dermal exposure is from 35 to 200 mg kg^{-1} body weight.

Human

Significant exposure to phenol by absorption through skin, by inhalation, or by ingestion can lead to death within minutes. Ingestion of even 1 g of phenol has been reported as lethal. It is extremely destructive to tissues of the mucus membranes and to the upper respiratory tract, skin, and eyes. Rapid death of nerve endings and tissue necrosis produces anesthesia and paralysis. Gastrointestinal, cardiovascular, and pulmonary symptoms will appear. Phenol effects on the gastrointestinal tract will result in pain, nausea, vomiting, and diarrhea. There may be cardiovascular collapse and subsequent shock. Pulmonary exposure can produce spasm, inflammation, and general edema. The central nervous system may have a transitory stimulation, followed by depression. Exposure to small amounts of phenol may result in a respiratory alkalosis similar to salicylate poisoning. This will be followed by an acidosis.

Chronic Toxicity (or Exposure)

Animal

Developmental studies performed in rats have shown decreased fetal weights and viability. Animal studies investigating long-term inhalation exposure to phenol have shown effects on the liver, kidney, respiratory, cardiovascular, and central nervous systems.

Human

Chronic exposure has been shown to damage the liver, kidney, and other major systems and has been correlated with an increased risk of ischemic heart disease. Literature reports of the human LD_{Lo} by the oral route range from 0.14 to 14 g kg^{-1}. Phenol is a known mutagen; however, conclusive carcinogenic data are not available. In laboratory experiments, it does show teratogenic and reproductive effects.

In Vitro Toxicity Data

Phenol had a direct toxic effect on human colonic epithelial cells *in vitro*. In this cell model glucuronidation was the preferred conjugation pathway involved in detoxification.

Clinical Management

Individuals exposed to phenol by inhalation should be removed to fresh air and given artificial respiration/cardiopulmonary resuscitation if necessary. Prompt transport to a medical facility is recommended with observation for up to 48 h. Treatment should be symptomatic, keeping in mind that effects such as pulmonary edema may be delayed. After ingestion, absorption should be delayed by giving milk, olive oil, castor oil, or polyethylene glycol 300, followed by repeated gastric lavage. Mineral oil or alcohol should not be administered because these can increase gastric absorption. Other therapy should be utilized as necessary noting edema and shock acidosis as predicted outcomes. After skin exposure, the affected area should be washed with soap and copious amounts of water for at least 10 min. Water alone may be harmful. Vegetable oil or polyethylene glycol should be applied with cotton swabs or dressings to assist in the removal of phenol from exposed skin.

Environmental Fate

Small, single releases of phenol into the air will be removed in less than a day. The oxidation of phenol will be accelerated by light and catalyzed by other atmospheric impurities. Phenol will persist up to 5 days in soil and even longer (9 days) in water.

Ecotoxicology

Phenol is biodegradable by both aerobic and anaerobic pathways. Little will accumulate in plants or animals and complete aerobic bacterial degradation will produce carbon dioxide. Still phenol is considered a potent insecticide, herbicide, and fungicide. The LC_{50} for aquatic organisms ranges from 12 to 68 mg l^{-1}.

Exposure Standards and Guidelines

Phenol is considered dangerous to life or health at 100 ppm. The permissible exposure limit – TWA (skin) is 5 ppm while the short-term exposure limit is 10 ppm. The odor threshold is 0.4–3.0 ppm.

See also: Skin.

Further Reading

Agency for Toxic Substances and Disease Registry (ATSDR) (1998) *Toxicological Profile for Phenol*. Atlanta, GA: US Department of Health and Human Services, Public Health Service.

Relevant Website

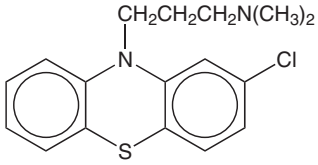
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Phenol.

Phenothiazines

Julie Weber

This article is a revision of the previous print edition article by Douglas J Borys, volume 2, pp. 515–516, © 1998, Elsevier Inc.

- Representative Chemicals: Chlorpromazine; Triflupromazine; Promazine; Promethazine; Acepromazine; Ethopropazine; Thioridazine; Mesoridazine; Piperacetazine; Fluphenazine; Perphenazine; Prochlorperazine; Trifluoperazine; Acetophenazine; Molindone; Pimozide; Haloperidol; Droperidol; Thiothixene; Chlorprothixene; Clozapine; Olanzapine; Risperidone; Quetiapine; Loxapine
- Chemical Abstracts Services Registry Number: Prototype compound, Chlorpromazine (CAS 50-53-3)
- Synonyms: Chlorpromazine, 2-Chloro-10-(3-dimethylaminopropyl)phenothizaine, Thorazine; Triflupromazine, 2-Trifluoromethyl-10-[3′-(1-methyl-4-piperazinyl)propyl]phenothiazine, Vesprin; Promazine, 10-(3-Dimethylaminopropyl)phenothiazine, Sparine; Promethazine, 10-(2-Dimethylaminopropyl)phenothiazine, Phenergan; Acepromazine, 1-[10-[3-(Dimethylamino)propyl]-10*H*-phenothiazin-2-yl]ethanone, Atravet; Ethopropazine, 10-(2-Diethylaminopropyl)phenothiazine, Parsidol; Thioridazine, 10-[2-(1-Methyl-2-piperidinyl)ethyl]-2(methylthio)-10*H*-phenothiazine, Mellaril; Mesoridazine, 10-[2-(1-Methyl-2-piperidinyl) ethyl]-2-(methylsulfinyl)-10*H*-phenothizaine, Serentil; Piperacetazine, 1-[10-[3-[4-(2-Hydroxyethyl)-1-piperidinyl]propyl]-10*H*-phenothizin-2-yl] ethanone, Quide; Fluphenazine, 4-[3-[2-(Trifluoromethyl)-10*H*-phenothiazin-10-yl]propyl]-1-piperazineethanol, Prolixin; Perphenazine, 4-[3-(2-Chloro-10*H*-phenothiazine-10-yl)propyl]-1-piperazineethanol, Trilafon; Prochlorperazine, 2-Chloro-10-[3-(4-methyl-1-piperazinyl)propyl]-10*H*-phenothiazine, Compazine; Trifluoperazine, 10-[3-(4-Methyl-1-piperazinyl)-propyl]-2-(trifluoromethyl)-10*H*-phenothiazine, Stelazine; Acetophenazine, 1-[10-[3-[4-(2-Hydroxyethyl)-1-piperazinyl]propyl]-10*H*-phenothiazin-2-yl]ethanone, Tindal; Molindone, 3-Ethyl-1,5,6,7-tetrahydro-2methyl-5-(4-morpholinylmethyl)-4*H*-indol-4-one, Moban; Pimozide, 1-[1-[4,4-Bis(4-fluorophenyl)butyl]-4-piperidinyl]-1,3-dihydro-2*H*-benzimidazol-2-one, Orap; Haloperidol, 4-[4-(4-Chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-1-butanone, Haldol; Droperidol, 1-[1-[4-(4-Fluorophenyl)-4-oxybutyl}1,2,3,6-tetrahydro-4-pyridinyl]-1,3-dihydro-2*H*-benzimidazol-2-one, Inapsine; Thiothixene, *N*,*N*-Dimethyl-9-[3-(4-methyl-1-piperazinyl) propylidene]thioxanthene-2-sulfonamide, Navane; Chlorprothixene, 3-(2-Chloro-9*H*-thioxanthen-9-ylidene)-*N*,*N*-dimethyl-1-propanamine, Taractan; Clozapine, 8-Chloro-11-(4-methyl-1-piperazinyl)-5*H*-dibenzo[*b*,*e*][1,4]diazepine, Clozaril; Olanzapine, 2-Methyl-4-(4-methyl-1-piperazinyl)-10*H*-thieno[2,3-*b*][1,5]benzodiazepine, Zyprexa; Risperidone, 3-[2-[4-(6-Fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4*H*-pyrido[1,2-*a*]pyrimidin-4-one, Risperdal; Quetiapine, 2-(2-(4-Dibenzo(*b*,*f*)(1,4)thiazepin-11-yl-1-piperazinyl)ethoxy)ethanol, Seroquel; Loxapine, 2-Chloro-11-(4-methyl-1-piperazinyl)-dibenz[*b*,*f*] [1,4]oxilapine, Loxitane
- Chemical/Pharmaceutical/Other Class: Neuroleptic agent, antipsychotic, major tranquilizer
- Chemical Formula: $C_{17}H_{19}ClN_2S$
- Chemical Structure: Chlorpromazine is the prototype phenothiazine



Uses

Phenothiazines are used to treat psychosis including schizophrenia; violent, agitated, disturbed behavior; and manic phase of bipolar disorder. Other uses include treatment of pain, headache, hiccups, acute severe anxiety, idiopathic dystonia, withdrawal, taste disorders, leishmaniasis, alleviation of nausea and vomiting, and acute intermittent porphyria. Phenothiazines permit smoother induction of anesthesia, potentiate anesthetic agents, and allow treatment of behavioral symptoms secondary to Alzheimer's disease and senile dementia. Some phenothiazines exert an antipruritic effect and are useful for the treatment of neurodermatitis and pruriginous eczema, and may relieve psychogenic itching.

Exposure Routes and Pathways

Phenothiazines are available in oral, parenteral, and rectal dosage forms. The principal exposure pathway is intentional ingestion in adults or accidental ingestion in small children.

Toxicokinetics

Phenothiazines are readily but incompletely absorbed due to first-pass metabolism. Oral bioavailability ranges from 10% to 69%. Peak serum levels are reached at 2–4 h after oral dosing and 0.5–1 h after immediate-release intramuscular injections. Phenothiazines are extensively metabolized in the liver through glucuronic acid conjugation, *N*-dealkylation, and sulfoxidation. Phenothiazines are widely distributed throughout the body, including the central nervous system (CNS). CNS levels may be up to 10 times greater than plasma levels. Phenothiazines are highly protein bound: 75–99% with a volume of distribution from 10 to 40 l kg^{-1}, with a mean of 20 l kg^{-1}. The main metabolites are excreted both in the urine and feces. Less than 1% is excreted in the urine unchanged. Elimination half-life ranges from 6 to 119 h, with an average of 18 h.

Mechanism of Toxicity

Phenothiazines primarily block postsynaptic neurotransmission by binding to dopamine (D_1 and D_2), muscarinic, histamine H_1, and serotonergic 5-HT_2 receptors. Phenothiazines also possess peripheral α-adrenergic receptor blockade and quinidine-like cardiac effects. Phenothiazines may also lower the seizure threshold.

Acute and Short-Term Toxicity (or Exposure)

Animal

Signs of toxicity reported in animals have included sedation, dullness, hypotension, respiratory depression, pulmonary edema, photosensitivity; photochemical reactions can cause acute keratitis and corneal ulceration in calves, weakness, anorexia, fever, icterus, colic, restlessness, seizures, anemia, and hemoglobinuria. Treatment consists of gastric decontamination and aggressive supportive care.

Human

Clinical signs of toxicity most frequently include sedation, coma, hypotension, extrapyramidal effects, and cardiac arrhythmias. Anticholinergic effects including blurred vision, decreased gastrointestinal motility, delirium, hallucinations, hyperthermia, and tachycardia have been seen. Cardiac effects include mild hypotension, prolonged Q–T interval, and ventricular dysrhythmias. Quinidine-like effects have rarely resulted in sudden cardiac death. The most commonly reported extrapyramidal symptoms include dystonia, akathisia, and parkinsonism. Respiratory depression, loss of gag reflex, and pulmonary edema may occur. Respiratory distress syndrome has been reported. Phenothiazines may interfere with the temperature regulating function of the hypothalamus; hyperthermia is seen more often in overdose, but hypothermia has been reported with haloperidol and thioridazine. Neuroleptic malignant syndrome has been reported after therapeutic use and acute intoxication.

Chronic Toxicity (or Exposure)

Animal

Phenothiazine is used as an antihelminthic in some animal species. Larger doses administered to sick animals have resulted in the development of neurologic effects. Horses seem more sensitive to phenothiazines than other animals and have been noted to develop hemolysis with phenothiazine exposure.

Human

Chronic dose-related exposure might cause tardive dyskinesia (lip smacking, tongue protrusion, grimacing, and chewing). Seizures are rarely seen, but are more common with loxapine and clozapine. The most commonly reported adverse reactions following therapeutic use include dry mouth, sedation, orthostatic hypotension, blurred vision, photosensitivity, anorexia, nausea, vomiting, constipation, diarrhea, and dyspepsia. Various hematologic changes have been reported. Clozapine has been linked to fatal agranulocytosis.

In Vitro Toxicity Data

Mutagenicity studies in Syrian hamster embryos have been positive but Ames Salmonella tests have been negative.

Clinical Management

Aggressive supportive care including airway management should be instituted when necessary. All patients with phenothiazine ingestion should have continuous cardiovascular monitoring and an electrocardiogram (EKG) performed. Emesis with syrup of ipecac is contraindicated due to the possible rapid onset of acute dystonic reaction and sedation. Lavage may be considered in massive, recent exposures, but is not routinely recommended. Phenothiazines readily bind to activated charcoal and it may be beneficial if given early after ingestion. Hypotension usually responds to intravenous fluids and placement of the patient in the Trendelenburg position. The vasopressor of choice is norepinephrine. Arrhythmias

should be treated with lidocaine and, if necessary, cardioversion and/or defibrillation. Quinidine, disopyramide, and procainamide are contraindicated. Benzodiazepines (diazepam or lorazepam) should be used to treat seizures, and if necessary use phenobarbital. Dystonic reactions respond well to intravenous benztropine or diphenhydramine. Oral therapy of diphenhydramine or benztropine should be continued for 1–2 days to prevent recurrence of the dystonic reaction. For patients suffering from neuroleptic malignant syndrome, dantrolene sodium and bromocriptine have been used in conjunction with cooling and other supportive measures. Hemodialysis and hemoperfusion have not been shown to be effective due to the high protein binding and large volumes of distribution. Fluids and electrolytes should be monitored closely. Baseline complete blood count (CBC), arterial blood gas (ABG) (if significantly CNS or respiratory depressed), and glucose should be obtained. Patient's temperature must be checked regularly. Creatine kinase (CK) must be monitored to detect elevation that may produce acute renal insufficiency or failure. Blood urea nitrogen (BUN), creatinine, and urinalysis should be monitored while looking for any symptoms of myoglobinuria, rhabdomyolysis, and renal insufficiency. Patients who have received adequate decontamination and remained asymptomatic with no vital-sign changes may be medically cleared after 4–6 h of observation.

Miscellaneous

Phenothiazines and metabolites have resulted in false positive results for tricyclic antidepressants using various screening methods. Unabsorbed phenothiazine may be radiopaque on abdominal X-ray. Use caution, as the absence of radiographic findings does not rule out ingestion.

See also: Benzodiazepines; Loxapine; Neurotoxicity; Quinidine.

Further Reading

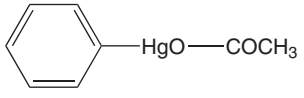
Buckley NA, Whyte IM, and Dawson AH (1995) Cardiotoxicity is more common in thioridazine overdose than with other neuroleptics. *Journal of Toxicology. Clinical Toxicology* 33: 199–204.

Henderson RA, Lane S, and Henry JA (1991) Life-threatening ventricular arrhythmia (torsade de pointes) after haloperidol overdose. *Human and Experimental Toxicology* 10: 59–62.

Phenylmercuric Acetate

Lynn Weber

- Chemical Abstracts Service Registry Number: CAS 62-38-4
- Synonym: Acetoxyphenyl mercury
- Chemical/Pharmaceutical/Other Class: Organomercurial
- Chemical Structure:



Uses

Phenylmercuric acetate is used as a seed dressing for the prevention of seed-borne diseases of vegetables, soybeans, cotton, peanuts, beets, and ornamental plants. Its use as a pesticide has been banned in the United States. Its use in paints was phased out in the United States in 1990–91. It was sometimes used as a food and cosmetic preservative as well as an antifungal agent. In paper, plastic, and fabric industries, this compound was also used as a preservative.

Exposure Routes and Pathways

Oral and dermal routes are the most common routes of exposure to phenylmercuric acetate.

Toxicokinetics

Phenylmercuric acetate is slowly absorbed through the skin; absorption is more efficient by the gastrointestinal tract. Relatively similar rates of absorption of phenylmercuric acetate and mercuric acetate were found in rat kidney slices. When absorption was studied in liver slices, however, the rate of absorption was found to be much higher (twice) for the organic form. Organic mercury has a greater affinity for the brain compared to inorganic mercury (probably because of its relative ease in crossing the blood–brain barrier).

Laboratory studies demonstrated that mercury from phenylmercuric acetate tends to distribute more

in the liver and kidneys compared to inorganic mercury. A chronic study with phenylmercuric acetate and mercuric acetate showed greater (10–20 times) distribution of the phenyl derivative into these tissues. Organomercury compounds usually undergo cleavage of the carbon–mercury bond in the body, releasing ionic inorganic mercury.

Phenylmercuric acetate is mainly excreted through urine. The excretion of phenylmercuric acetate in humans was reported to exhibit two phases. The first phase showed a transient increase in urinary mercury concentration followed by a second slower phase.

Mechanism of Toxicity

Toxic effects of phenylmercuric acetate are correlated with its rapid metabolic breakdown into the mercuric ion. Generally, mercury interferes with cellular enzymatic mechanisms by combining with sulfhydryl (–SH) groups of different enzymes and thereby produces nonspecific cell injury or death.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} of phenylmercuric acetate in rats was $60\,mg\,kg^{-1}$. In mice, the oral LD_{50} was $70\,mg\,kg^{-1}$. A single oral exposure to $2\,mg\,kg^{-1}$ phenylmercuric acetate was found to be genotoxic in murine bone marrow and male germline cells. Succinate dehydrogenase and alkaline and acid phosphatase activities in the renal epithelium were reported to be altered following intragastric administration of phenylmercuric acetate in rats.

Human

Phenylmercuric acetate can be lethal with oral doses as low as 100 mg. The principal manifestations of mercury salt poisoning are gastrointestinal, hepatic, and renal damage.

Ingestion of phenylmercuric acetate may cause metallic taste, thirst, severe abdominal pain, vomiting, and bloody diarrhea, which may persist for several weeks. Acute renal failure characterized by decreased urine output was reported 1 day to 2 weeks after ingestion.

Chronic Toxicity (or Exposure)

Animal

Dietary mercury ($2\,mg\,kg^{-1}\,day^{-1}$ for 2 years) in the form of phenylmercuric acetate did not affect rat growth, mortality, or organ weights. However, a dietary level of 160 ppm ($8\,mg\,kg^{-1}\,day^{-1}$) of phenylmercuric acetate was found to retard the growth of rats and shorten their survival time. Histochemical changes in the rat kidney have been observed following a dietary level of 0.5 ppm of phenylmercuric acetate.

Human

Ingestion of phenylmercuric acetate over a prolonged period may cause skin disorders (urticaria and stomatitis), salivation, diarrhea, anemia, leukopenia, and hepatic and renal damage. Prolonged dermal exposure to phenylmercuric acetate may cause mercurialism.

In Vitro Toxicity Data

Matrix metalloproteinases may be targeted by phenylmercuric acetate. Incidence of sister chromatid exchanges in human lymphocytes was increased at $1–30\,\mu m\,mol^{-1}$ concentrations.

Clinical Management

In case of acute poisoning, emergency measures should be taken by immediately removing the ingested poison using gastric lavage with tap water or using emesis or catharsis. Dimercaprol may be administered as an antidote for mercury poisoning with subsequent hemodialysis to accelerate the removal of the mercury–dimercaprol complex from the body. Penicillamine may also be administered as an antidote.

Environmental Fate

If released into air, phenylmercuric acetate is expected to be bound to particulates. If released into soil, its mobility may be high based on a K_{oc} of 60 of the undissociated form, but is likely to be much lower because it will dissociate and the cation will sorb to organic matter or clay. Water releases would result in quick dissociation of the salt and sorption of the cation to particulates or humics, with little bioconcentration in aquatic species. Photolysis of phenylmercuric acetate and subsequent loss through volatilization of inorganic mercury is expected in superficial soils and water.

Ecotoxicology

Pheasants and Japanese quail exhibit decreased egg production, decreased fertility, and increased embryonic mortality after oral phenylmercuric acetate exposure. Feeding, growth, oxygen consumption, swimming performance, and reproduction are

impaired in mosquitofish (*Gambusia affinis*) and rainbow trout (*Oncorhynchus mykiss*). Five-day oral LC_{50} values have been reported for Japanese quail (1028 ppm), ring-necked pheasant (2350 ppm), and mallard duck (1175 ppm). The 48 h aqueous LC_{50} value for rainbow trout has also been reported (1780 ppm).

See also: Mercury; Metals.

Further Reading

Clarkson TW (2001) Inorganic and organometal pesticides. In: Krieger R (ed.) *Handbook of Pesticide Toxicology*, 2nd edn., pp. 1357–1428. San Diego, CA: Academic Press.

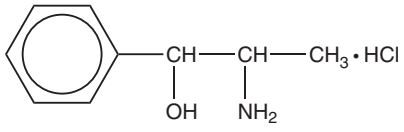
Relevant Website

http://www.inchem.org – International Programme on Chemical Safety.

Phenylpropanolamine

Brenda Swanson-Biearman

- Chemical Abstracts Service Registry Number: CAS 14838-15-4
- Synonyms: PPA; D,L-Norephedrine
- Chemical/Pharmaceutical/Other Class: A synthetic sympathomimetic drug structurally related to ephedrine and amphetamine
- Chemical Formula: $C_9H_{13}NO \cdot HCl$
- Chemical Structure:



Uses

Phenylpropanolamine (PPA) is used as a nasal decongestant and as an anorectic. As of November 2000, the US Food and Drug Administration (FDA) Nonprescription Drugs Advisory Committee (NDAC) determined that there is a significant association between PPA and hemorrhagic stroke and recommended that PPA not be considered safe for over-the-counter use. Products containing PPA such as Alka-Seltzer Plus®, Acutrim®, Contac®, Comtrex®, Dimetapp®, Triaminic®, Robitussin CF®, Dexatrim™ were reformulated. Although the risk of hemorrhagic stroke is very low, the FDA raised significant concerns because of the seriousness of a stroke and the inability to predict who is at risk. All drug companies have voluntarily discontinued marketing products containing PPA.

Exposure Routes and Pathways

PPA is available in liquid, tablet, and caplet dosage forms. Ingestion is the most common route of accidental and intentional exposure.

Toxicokinetics

Oral doses of PPA are rapidly and completely absorbed from the gastrointestinal tract, with maximal therapeutic effect in 1–3 h. In overdose, the peak toxic reaction is usually seen within 2 or 3 h following ingestion. In sustained release preparations, these effects may occur later and be prolonged. PPA is converted primarily to norephedrine. Small amounts of the drug are slowly metabolized in the liver to an active hydroxylated metabolite. PPA crosses the blood–brain barrier, resulting in central nervous system (CNS) effects. The brain-to-serum ratios are extremely close at 0.025 and 0.05 mmol kg^{-1}. The volume of distribution of PPA is 4.4þ1.2 l kg^{-1}. PPA is eliminated unchanged (80–90%) in the urine within 24 h, along with the metabolite norephedrine. PPA is a weak base and is eliminated more rapidly in acidic urine. Where the urine pH is normal (5.5–7.0), the plasma half-life is 3–7 h. In alkaline urine, the elimination half-life increased from a mean of 4.03 to 5.39 h.

Mechanism of Toxicity

The primary action of PPA is indirect alpha-adrenergic agonism, releasing norepinephrine at postganglionic sympathetic nerve terminals. PPA also possesses direct alpha-adrenergic agonist properties and, to a lesser degree, beta-adrenergic agonist activity. Hypertension results from alpha-adrenergic mediated vasoconstriction of peripheral blood vessels.

Acute and Short-Term Toxicity (or Exposure)

Animal

Following the ingestion of PPA, dogs and cats may exhibit hyperactivity, mydriasis, depression, vomiting, hyperthermia, disorientation, and bradycardia. Therapy is directed at prevention of absorption and control of tachyarrhythmias with lidocaine (dogs only) or procainamide (dogs only). Diazepam may be used for control of symptoms of CNS stimulation.

Human

Hypertension is the most common and most serious toxic effect of PPA. Hypertensive crisis, cerebral arteritis, cerebral hemorrhage, psychoses, seizures, and myocardial ischemia may result. Tachycardia is most often seen with PPA where it is combined with antihistamines in multi-symptom products. Bradycardia (as a reflex response to hypertension) is more common when the PPA is ingested exclusively. Concurrent substances that are prevalent in combination products and may contribute to the toxicological presentation of PPA exposures include analgesics, antihistamines, and antitussives. Consideration should be given to the alcohol component of liquid preparations. Caffeine may be added to PPA in illicit stimulant and weight-loss preparations. PPA has a low therapeutic index and adverse effects can occur at doses two or three times the normal daily dose. The recommended adult daily dose is 75–150 mg. An amount over $10\,mg\,kg^{-1}$ is toxic in children. Other neurological symptoms include anxiety, confusion, headache, hallucinations, and altered mental status.

Chronic Toxicity (or Exposure)

Animal

PPA has been used in veterinary practice as an agent to help with urinary continence, primarily in dogs. Dogs commonly develop signs and symptoms of CNS stimulation.

Human

The use of PPA has been associated with increases in blood pressure and an increased risk of hemorrhagic stroke. In November 2000, the US FDA issued a public health advisory on the use of PPA and asked drug companies to voluntarily withdraw PPA products from the market.

In Vitro Toxicity Data

PPA has been demonstrated to competitively and reversibly inhibit monoamine oxidase activity in both human brain and rat liver.

Clinical Management

Basic and advanced life-support measures should be instituted as indicated. Gastric decontamination may be performed depending on the patient's symptomatology and the history of the ingestion. Activated charcoal may be used to adsorb PPA. Most overdoses require observation only for a period of 4–8 h; sustained-release preparations may require a longer period of observation. Careful monitoring of the cardiac and hemodynamic status should be performed. Antidysrhythmics and antihypertensive agents may be necessary in severe exposures. Management of poisoning with concurrent drugs ingested should be appropriate to the agent(s) involved. Laboratory analysis of creatine phosphokinase and urinalysis should be performed in those with severe symptoms.

See also: Diazepam; Lidocaine; Procainamide.

Further Reading

Cantu C, Arauz A, Murillo-Bonilla LM, Lopez M, and Barinagarrementeria F (2003) Stroke associated with sympathomimetics contained in over-the-counter cough and cold drugs. *Stroke* 34: 1667–1672.

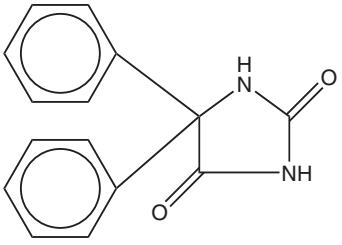
Edwards M, Russo L, and Harwood-Nuss A (1987) Cerebral infarction with a single oral dose of phenylpropanolamine. *American Journal of Emergency Medicine* 5: 163–164.

Lake CR, Gallant S, and Masson E (1990) Adverse drug effects attributed to phenylpropanolamine: A review of 142 case reports. *American Journal of Medicine* 89: 195–208.

Phenytoin

S Rutherfoord Rose

- Chemical Abstracts Service Registry Numbers: CAS 57-41-0; CAS 630-93-3
- Synonyms: Diphenylhydantoin (DPH); 5,5-Diphenylhydantoin, 5,5-Diphenylimidazolidine-2, 4-dione; Dilantin Infatabs®; Fenitoina; Phenantoinum; Phenytoin sodium (92% phenytoin); Diphenylhydantoin sodium; Diphenin; Phenytoinum natricum; Soluble phenytoin; Dilantin®; Epanutin®; Diphenylan®; Fosphenytoin is a water-soluble prodrug of phenytoin suitable for rapid intravenous administration
- Chemical/Pharmaceutical/Other Class: Hydantoin (a synthetic chemical that is structurally similar to barbituric acid)
- Chemical Formula: $C_{15}H_{12}N_2O_2$
- Chemical Structure:



Uses

Phenytoin is used as an anticonvulsant and rarely as an antidysrhythmic.

Exposure Routes and Pathways

Ingestion is the most common route of exposure. Phenytoin can also be administered intravenously.

Toxicokinetics

Oral absorption occurs in the small intestine and is dose dependent. Peak blood concentrations occur 2–4 h after single 100 mg doses but may be delayed by 4–12 h after a loading dose (600 mg). Peak levels may not occur for 2–7 days after an oral overdose. The oral bioavailability of phenytoin averages 90% (range, 70–100%). Intramuscular absorption is erratic and unpredictable.

The major pathway of biotransformation is via hepatic hydroxylation to *p*-hydroxyphenytoin, which is subsequently conjugated to glucuronide. Minor metabolites include *m*-hydroxyphenytoin and 3,4-dihydro-dihydroxyphenytoin. All metabolites are inactive. Phenytoin biotransformation is capacity limited, with linear (first-order) kinetics observed at low (therapeutic) doses, and zero-order (Michaelis–Menton) elimination observed at toxic and even high therapeutic doses. Phenytoin and its metabolites undergo enterohepatic recirculation prior to elimination.

The volume of distribution averages 0.5–0.8 l kg^{-1} and binding to plasma proteins is normally ~90%. Protein binding is altered in neonates, the elderly, and under many conditions including aremia, nephrotic syndrome, hypoalbuminemia, hyperbilirubinemia, and hepatic disease. Alterations in protein binding will result in variations in the amount of unbound (free) drug that is the active component. Thus, free phenytoin levels (therapeutic = 1–2 μg ml^{-1}) rather than total levels (therapeutic = 10–20 μg ml^{-1}) may correlate better with clinical efficacy and toxicity in the presence of these conditions. Phenytoin crosses the placenta and is excreted in breast milk.

Small amounts of phenytoin are excreted unchanged in the urine (2–4%) and feces (5%). Most is eliminated renally as inactive conjugated metabolites. The elimination half-life at linear doses averages 20–30 h (12–20 h in children) but may be as long as 60 h, and as high as 200 h after overdose, due to saturation of hydroxylation pathways. The maximum rate of metabolism is estimated at 6 mg kg^{-1} day^{-1}.

Mechanism of Toxicity

Phenytoin possesses anticonvulsant activity without significant central nervous system (CNS) depression. At various concentrations, phenytoin has been shown to inhibit inward Na^+ currents, outward K^+ currents, and Ca^{2+}-mediated action potentials. The ability to inhibit sodium channels is responsible for the antidysrhythmic action (class II-B) of phenytoin. Phenytoin can induce enzymes of the hepatic cytochrome P450 system.

Acute and Short-Term Toxicity (or Exposure)

Animal

Toxicity in animals has been similar to that observed in humans (e.g., effects are primarily neurologic).

Human

Clinical effects after overdose are generally dose related and primarily involve the peripheral and central nervous systems; nystagmus (>20 μg ml^{-1}),

ataxia ($>30\,\mu g\,ml^{-1}$), and lethargy ($>40\,\mu g\,ml^{-1}$) are most characteristic. Nausea, tremor, dysarthria, and confusion are also relatively common. Coma or significant cardiac dysrhythmias are unusual. Hypotension or dysrhythmias may be encountered with very rapid intravenous infusion. Paradoxical CNS excitation has been reported, but the potential of phenytoin to actually cause seizures at very high serum concentrations is unclear. This phenomenon has typically occurred in patients with a preexisting seizure disorder on chronic phenytoin therapy.

Chronic Toxicity (or Exposure)

Animal

Chronic feeding studies have demonstrated increased development of tumors in mice compared with untreated controls.

Human

Chronic toxic effects are dose related and typically involve cerebellar and vestibular functions (nystagmus and ataxia). Nausea, dizziness, diplopia, behavioral changes, gingival hyperplasia, hirsutism, hyperglycemia, osteomalacia, pancytopenia, and skin eruptions are reported complications of chronic therapy. Hypersensitivity (idiosyncratic) reactions, including hepatic necrosis and Stevens–Johnson syndrome, can occur and are potentially fatal.

Phenytoin use during pregnancy has been associated with intrauterine growth retardation, mental retardation, craniofacial abnormalities, and digital hypoplasia (e.g., fetal hydantoin syndrome).

In Vitro Toxicity Data

Studies of sister chromatid exchange have been positive, Ames *Salmonella* tests have been negative, mouse lymphoma tests have been negative, and Chinese hamster ovary assays have been negative for mutagenicity.

Clinical Management

The basis of treatment is supportive care. Hypotension is usually associated with rapid infusion of injectable phenytoin and should respond to slowing the infusion rate and intravenous fluid therapy. Seizures should be treated with intravenous doses of diazepam or lorazepam and discontinuation of phenytoin. Assessments of toxicity should be based on serum drug levels rather than the amount of drug ingested. Serum phenytoin concentrations should be determined in all symptomatic patients or patients with ingestions exceeding $20\,mg\,kg^{-1}$. Serial levels are needed to determine peak (highest measured) concentration. Serum levels of electrolytes, glucose, hepatic enzymes, blood urea nitrogen, and bilirubin should be determined in hospitalized patients. Activated charcoal is useful to prevent gastrointestinal absorption and to enhance the elimination of the absorbed drug (i.e., gastrointestinal dialysis). Multiple oral doses of charcoal are indicated to facilitate the lowering of toxic blood levels that possibly would require days to decline in conditions of zero-order metabolism. However, care must be taken to ensure that patients have gastrointestinal motility before using multiple doses of activated charcoal. Other measures to enhance phenytoin elimination are not warranted. Continuous cardiac monitoring is not necessary in the absence of preexisting cardiac disease or massive overdose with hemodynamic compromise. Patients should be monitored until serum levels are (near) normal and they are neurologically competent. Death resulting from oral ingestion is rare.

See also: Charcoal; Diazepam.

Further Reading

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Mellick LB, Morgan JA, and Mellick GA (1989) Presentations of acute phenytoin overdose. *American Journal of Emergency Medicine* 7: 61–67.

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Phorbol Esters

Samantha E Gad and Shayne C Gad

Tumor promotion is described as 'the process by which an agent brings about the selective expansion of initiated cells which increase the probability of malignant transformation'. Such promotion can also be considered nongenotoxic or epigenetic carcinogenesis. The concept of tumor promotion came from studies which found that a single application of coal tar or a polycyclic hydrocarbon to the skin of rabbits or mice in subcarcinogenic amounts would initiate the process of skin carcinogenesis if followed by a promotional event.

The characteristic of a promoter in the mouse skin model can be described as follows:

1. That it should not be carcinogenic *per se*.
2. That it should not increase tumor yield if administered before the initiating carcinogen.
3. That when applied after an initiating, subcarcinogenic dose of the carcinogen, it should accelerate the rate of development of tumors and thus increase the total, time-related tumor incidence.
4. That the yield of tumors produced should be related to the dose of the initiator, not to dose of the promoter, providing the promoter is used in excess of the minimum amount required to promote all initiated cells.
5. That, unlike initiation, which can take place rapidly during a single exposure to the initiator and which is a permanent event, promotion requires long exposure to the promoter before the changes induced become irreversible.

Phorbol esters were first detected in oil prepared from seeds of *Croton tiglium*, and are the most widely studied skin tumor promoters; however, many other chemical compounds have been shown to possess skin tumor-promoting properties, for example, phenobarbitol, DDT, and the peroxisomal proliferators. Within a few hours after application of a single effective dose of phorbol 12-myristate 13-acetate (also known as TPA and 12-*O*-tetradecanoyl-phorbol-13-acetate, CAS 16561-29-8) to mouse skin, localized edema and erythema characteristic of inflammation and irritation are evident, and within 24 h there is leukocytic infiltration of the dermis. Within 1 or 2 days after a single promoter treatment, stimulation of mitotic activity in the basal cell layer of the epidermis is evident and continues for several days. This results in an increased number of nucleated cell layers, and is followed by a phase of increased keratinization of the upper layers of the epidermis. Without additional promoter treatments, these responses to the promoter gradually subside and the epidermis regains its normal appearance within ~2 or 3 weeks of treatment. Repeated promoter treatment, however, prevents this decrease in response, and the skin appears to be in a chronic state of irritation and regenerative hyperplasia. Phorbol esters have been shown to transform cultured fibroblasts and embryonic cells that have been previously exposed to polycyclic aromatic hydrocarbons *in vitro*.

The best known receptors for phorbol esters and their derivatives are the isozymes of protein kinase C (PKC), which bind phorbol esters and the physiological second messenger diacylglycerol (DAG) by cysteine-rich domains, the C1 domains. The exact functions of the different PKC isozymes is not known at present; however, they have been shown to be involved in synaptic transmissions, the activation of ion fluxes, secretion, cell cycle control, differentiation, proliferation, tumorigenesis, metastasis, and apoptosis.

Phorbol esters also target numerous C1-containing receptors unrelated to PKC. Identifying and understanding the complete set of key mediators for the physiological DAG responses and phorbol ester-induced tumorigenesis will help in the understanding of signal integration, and can also help in the development of new strategies for therapeutic cancer intervention. For example, individual PKC isozymes appear to have opposite effects on skin carcinogenesis despite being all activated by phorbol esters in the mouse skin chemical carcinogenesis model, and a better understanding of the different epidermal expression patterns and substrate proteins are needed to explain their opposing effects on skin carcinogenesis.

See also: Carcinogenesis; DDT (Dichlorodiphenyltrichloroethane); Peroxisome Proliferators; Polycyclic Aromatic Hydrocarbons (PAHs); Skin; Toxicity Testing, Dermal.

Further Reading

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Hofmann J (2004) Protein kinase C isozymes as potential targets for anticancer therapy. *Current Cancer Drug Targets* 4: 125–146.

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Newton AC (2004) Diacylglycerol's affair with protein kinase C turns. *Trends in Pharmacological Sciences* 25: 175–177.

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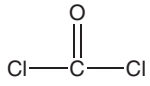
Phosgene

Samantha E Gad

- Chemical Abstracts Service Registry Number: CAS 75-44-5
- Synonyms: Carbonyl chloride; Chloroformyl chloride; Carbon oxychloride
- Chemical/Pharmaceutical/Other Class: Haloform
- Chemical Formula: $COCl_2$
- Chemical Structure:



Uses

Phosgene is widely used as a chemical intermediate. It is used in metallurgy and in the production of pesticides, herbicides, and many other compounds. It is a by-product of chloroform biotransformation and can be generated from some chlorinated hydrocarbon solvents under intense heats. Phosgene has been used as a chemical warfare agent.

Exposure Routes and Pathways

Inhalation and exposure to skin and mucous membranes are possible exposure routes. Potential for toxicity depends on concentration, route of exposure, and length of time exposed.

Toxicokinetics

Phosgene is absorbed by the lungs and excreted via the liver and kidneys.

Mechanism of Toxicity

Rapid-onset ocular, nasal, and airway irritations from high levels of phosgene are caused by hydrochloric acid released during hydrolysis. The carbonyl group (C = O) participates in acylation reactions with amino ($-NH_2$), hydroxyl (–OH), and sulfhydryl (–SH) groups. These reactions may account for some toxic effects of phosgene. At alveolar-capillary membranes, these reactions can cause fluid leakage into the interstitial lung. Leakage of fluid into the pulmonary interstitium is opposed by lymphatic drainage, but as fluid accumulates, this drainage is overwhelmed. After a latent period, fluid reaches alveoli and peripheral airways, leading to increasingly severe dyspnea and pulmonary edema.

Acute and Short-Term Toxicity (or Exposure)

Animal

In animals, phosgene exposure has resulted in liver or kidney damage, skin irritation, and respiratory damage. The inhalation LC_{50} is 1400 mg m^{-3} in rats, 1800 mg m^{-3} in mice, 4200 mg m^{-3} in dogs, 600 mg m^{-3} in monkeys, 1000 mg m^{-3} in rabbits, and 1300 mg m^{-3} in guinea pigs. The lowest observed lethal concentration in cats was 190 mg m^{-3} for 15 min. Acute (4 h) exposure of male rats to phosgene (0.125–1 ppm) led to changes in lung weights (wet and dry) and increased protein in lavage fluid. Total number of cells in lavage fluid was higher in phosgene-exposed rats. Increase in polymorphonuclear leukocytes was a sensitive indicator of phosgene toxicity. All parameters returned to near control levels within 3 days, indicating repair of damage and reversible lung damage.

Human

Exposure to high levels may cause death. Phosgene causes irritation to skin, eyes, nose, throat, and lungs. Phosgene exposure may be asymptomatic in the short term, with effects delayed for up to 48 h. High concentrations may cause accumulation of fluids in the lungs or pneumonia, and can produce choking, chest

constriction, pain in breathing, coughing, blood in sputum, and heart failure. Exposure to eyes and mucous membranes can be very irritating. Buildup of phosgene in the liver or kidneys may produce damage.

Chronic Toxicity (or Exposure)

Animal

Relatively little information is available on the long-term effects of chronic exposure to phosgene in animals. Studies in male rats exposed (6 h day^{-1}) to 0.1, 0.2, 0.5, or 1 ppm of phosgene, either acutely or repeatedly, for up to 12 weeks suggested that high concentrations with long exposure intervals led to more chronic pulmonary damage (increased bronchoalveolar lavage protein, hydroxyproline, and collagen). Chronic pneumonitis and fibrinous pneumonia was reported in one study with long-term phosgene exposure.

Human

Chronic inhalation to low levels of phosgene can lead to some degree of tolerance to acute effects noted in humans, but can also cause irreversible pulmonary changes, for example, emphysema and fibrosis. There appears to be no increased incidence of cancer in workers chronically exposed to phosgene.

Clinical Management

The exposed individual should be removed from exposure. Clothing should be removed carefully avoiding further exposure. The body should be washed rapidly with soap and water, and eyes flushed if needed Centers for Disease Control and Prevention (CDC). Individuals should be given immediate medical attention and monitored for 48 h for delayed effects (CDC). There is no antidote (CDC).

Environmental Fate

In air, the major route of phosgene degradation in air is hydrolysis. Even with very high humidity, however, phosgene is slowly degraded and is likely to persist and be transported long distances. In water, phosgene is efficiently degraded to hydrochloric acid and carbon dioxide. Phosgene is not likely to be detected in soil or vegetation.

Ecotoxicology

Relatively little is known regarding ecotoxicity of phosgene. It is estimated that common environmental levels would have little effect on aquatic or terrestrial species. Some damage to plants and aquatic organisms could occur, however, with accidental releases from release of hydrochloric acid upon hydrolysis.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration permissible exposure limit for phosgene is 0.1 ppm. The National Institute for Occupational Safety and Health immediately dangerous to life and health value is 2 ppm. The California Environmental Protection Agency chronic inhalation reference exposure level is 0.0003 mg m^{-3}. The US Army general population limit is 0.0025 mg m^{-3}.

See also: Pesticides; Respiratory Tract; Sensory Organs.

Further Reading

Cucinell SA (1974) Review of the toxicity of long-term phosgene exposure. *Archives of Environmental Health* 28: 272–275.

Gosselin RE, Smith RP, and Hodge HC (eds.) (1984) *Clinical Toxicology of Commercial Products*, 5th edn. Baltimore: Williams and Wilkens.

Hatch G, Kodavanti U, Crissman K, Slade R, and Costa D (2001) An 'injury-time integral' model for extrapolating from acute to chronic effects of phosgene. *Toxicology and Industrial Health* 17: 285–293.

Relevant Websites

http://www.inchem.org – International Programme on Chemical Safety.

http://chppm-www.apgea.army.mil – US Army Center for Health Promotion and Preventive Medicine. Detailed Facts About Choking Agent Phosgene (CG) (accessed September 3, 2004).

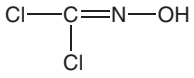
http://www.bt.cdc.gov – Centers for Disease Control (CDC) (2004) Facts about phosgene (accessed September 3, 2004).

http://www.epa.gov – US Environmental Protection Agency (EPA) (accessed September 3, 2004).

Phosgene Oxime

David R Wallace

- Chemical Abstracts Service Registry Number: CAS 1794-86-1
- Synonyms: Dichloroformoxime; CX
- Chemical/Pharmaceutical/Other Class: Urticant or nettle agent
- Chemical Formula: CCl_2NOH
- Chemical Structure:



Uses

Phosgene oxime was originally developed as a chemical warfare agent. It is sometimes grouped with the vesicant agents, but it is not a true vesicant in that it does not induce blisters. Phosgene oxime is an urticant or nettle agent that causes corrosive-type injuries. There is no evidence that this agent has ever been used as a chemical warfare agent.

Exposure Routes and Pathways

Phosgene oxime is a colorless solid or yellowish-brown liquid that can vaporize at room temperature. Due to its ability to rapidly change physical state, phosgene oxime can be absorbed through inhalation, dermal/ocular contact, or oral ingestion.

Toxicokinetics

Phosgene oxime appears to act directly and there have been no reported studies that have determined if any metabolism of phosgene *in vivo*.

Mechanism of Toxicity

The molecular mechanism of phosgene oxime toxicity is unknown.

Acute and Short-Term Toxicity (or Exposure)

Most studies on the action of phosgene oxime have utilized animal studies. Human information has been obtained from accidental exposure to the chemical. Health effects following phosgene oxime exposure are dependent on the route of exposure.

Since phosgene oxime is an urticant/nettle agent, common physical effects include erythema, wheals, and urticaria. Phosgene oxime is a highly corrosive agent and the response resembles wounds caused by strong acids. Ocular contact results in severe pain, conjunctivitis, and keratitis. Direct dermal exposure to phosgene oxime causes immediate pain and blanching with an erythematous ring. In ~ 0.5 h a wheal will form followed by tissue necrosis. Extreme pain can persist for days. Absorption of phosgene oxime through the skin can result in pulmonary edema. Inhalation of phosgene oxime vapor will produce immediate irritation to the airways. Pulmonary edema, necrotizing bronchiolitis, and pulmonary thrombosis can also occur following inhalation or systemic absorption of phosgene oxime. There has been no human data on effects of phosgene oxime following ingestion, but animal studies suggest that hemorrhagic inflammatory lesions may occur throughout the gastrointestinal tract.

Chronic Toxicity (or Exposure)

There have been no studies on chronic exposure to phosgene oxime. Thus, there is no data regarding the carcinogenicity or teratogenicity of phosgene oxime.

In Vitro Toxicity Data

No *in vitro* toxicity studies have been reported. The mechanism of phosgene oxime toxicity is unknown and long-term exposure effects have not been determined.

Clinical Management

Individuals who come in contact with phosgene oxime liquid or solid can contaminate those around them by release of vapor. Individuals who have been exposed to the vapor will not be able to contaminate others. Patients who come in contact with phosgene oxime will experience immediate pain and develop necrotic lesions. Since there is no antidote for phosgene oxime exposure, only supportive measures can be given. Patients arriving to the triage area must first be decontaminated to prevent cross-contamination. For inhalation exposures, the individual should be removed from the source of exposure. Oxygen must be administered to patients with significant respiratory symptoms. Artificial respiration should be given if necessary. For ocular treatment, eyes

should be flushed with copious amounts of water. Topical antibiotics should be applied to reduce the risk of infections and adhesions. Topical anticholinergics should be applied to reduce the risk of future synechiae formation. Skin contact will require decontamination with large amounts of water. Treatment should be in the same fashion as with a chemical burn. If phosgene oxime has been ingested, emesis should not be induced. Parental analgesics such as morphine or meperidine may be administered to reduce pain.

Environmental Fate

Phosgene oxime does not accumulate in the soil. Small amounts that may be present can vaporize into the air or be degraded by soil bacteria. Once in vapor form, phosgene oxime remains in vapor form and will be inactivated by compounds in the atmosphere or broken down by bacteria. There is no evidence that phosgene oxime will accumulate in groundwater.

Ecotoxicology

Phosgene oxime is rapidly cleared from the environment and poses little threat unless animals come directly in contact with the gas or liquid/solid.

Exposure Standards and Guidelines

No exposure standards and guidelines have been established.

See also: Diphosgene; Phosgene.

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Phosgene Oxime.

http://www.biochemhazard.com – Biochemhazard.com, Dominion Research Center (2000) Chemical Weapon Agent: Phosgene Oxime (CX) – Blister Agent.

http://www.emedicine.com – eMedicine, CBRNE: Urticants, Phosgene Oxime (2003).

Phosphine

Danny Villalobos

- Chemical Abstracts Service Registry Number: CAS 7803-51-2
- Synonyms: Hydrogen phosphide; Phosphorus hydride; Phosphorus trihydride; Phosphoretted hydrogen; Aluminum phosphide (Celphos, Phostoxin, Quick Phos)
- Chemical/Pharmaceutical/Other Class: Fumigant
- Chemical Formula: PH_3

Uses

Phosphine is used as an insecticide for the fumigation of grains, animal feed, and leaf-stored tobacco, and as a rodenticide. Phosphine is also used as an intermediate in the synthesis of flame retardants for cotton fabrics, as a doping agent for *n*-type semiconductors, as a polymerization initiator, and as a condensation catalyst. Phosphine is used in the semiconductor industry to introduce phosphorus into silicon crystals.

Exposure Routes and Pathways

Inhalation is the major route of phosphine exposure. Phosphides may be absorbed through broken skin causing systemic toxicity. Phosphine gas produces little adverse effects on the skin or eyes. Contact with liquefied or compressed phosphine gas may cause frostbite. Ingestion of phosphine is rare due to its volatility. However, metallic phosphide ingestion can lead to systemic toxicity.

Toxicokinetics

Phosphine is absorbed readily through the lungs and produces early symptoms in the brain and liver, suggesting that it is rapidly distributed at least to these organs. After peak exposure, phosphine is excreted unchanged in expired air and some is oxidized to phosphite and hypophosphite ions, which are excreted in the urine. Metal phosphides may hydrolyze to produce phosphine, which may be absorbed through the intestine after ingestion. Some zinc phosphide has been shown to reach the liver and kidneys intact after ingestion and to hydrolyze slowly in the tissues to phosphine and zinc salts. Hydrolysis of metal phosphides on the skin could lead to the evolution of gaseous phosphine, which could then be absorbed by inhalation. Little percutaneous absorption of metal phosphides occurs.

Mechanism of Toxicity

Metallic salts can cause severe gastrointestinal irritation. Phosphine may be an *in vivo* inhibitor of

oxidative phosphorylation, via inhibition of cytochrome oxidase. As with other fumigants, sufficient phosphine in the atmosphere can lead to oxygen starvation, apnea, and cardiac arrest.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute animal tests in rats have demonstrated phosphine to have extreme acute toxicity via inhalation. Signs include early hypoactivity followed by restlessness, escape behaviors, ataxia, convulsions, and death within 30 min with high concentrations. Concentration–time studies demonstrated evidence of Haber's law, that is, within certain limits, the product of concentration and time of exposure to elicit lethality was remarkably constant. The lowest lethal concentration in rats was $7.5\,mg\,m^{-3}$. The acute oral LD_{50} for metallic salts (e.g., aluminum phosphide) is typically quite low ($\sim 10\,mg\,kg^{-1}$). In rabbits acutely exposed to high levels of phosphine via inhalation, dyspnea, paralysis, convulsions, hepatotoxicity and renal toxicity, and damage to the spleen were reported.

Human

Phosphine is a respiratory tract irritant that attacks primarily the cardiovascular and respiratory systems causing peripheral vascular collapse, cardiac arrest and failure, and pulmonary edema. Acute (short-term) inhalation exposure to phosphine may cause headaches, dizziness, fatigue, drowsiness, burning substernal pain, nausea, vomiting, cough, labored breathing, chest tightness, pulmonary irritation, pulmonary edema, and tremors in humans. In severe exposure, lung irritation with persistent coughing, ataxia, paraesthesia, tremor, diplopia, and jaundice may also occur. Very severe cases may progress to acute pulmonary edema, cardiac dysrhythmias, convulsions, cyanosis, and coma. Oliguria, proteinuria, and anuria may be induced. Delayed pulmonary edema with an onset of 72 h or more postexposure can occur. Convulsions may ensue after an apparent recovery. Ingestion of phosphine is unlikely because it is a gas at room temperature. Ingestion of metallic phosphides (e.g., aluminum phosphide) can produce phosphine intoxication when the solid phosphide contacts gastric acid. Deliberate ingestion of phosphides causes nausea, vomiting, and sometimes diarrhea, retrosternal and abdominal pain, tightness in the chest and coughing, headache, and dizziness. In severe cases, gastrointestinal hemorrhage, tachycardia, hypotension, shock, cardiac arrhythmias, hypothermia, metabolic acidosis, cyanosis, pulmonary edema, convulsions, hyperthermia, and coma may occur. Clinical features of renal insufficiency and hepatic damage including oliguria and jaundice may develop later, if the patient survives.

Chronic Toxicity (or Exposure)

Animal

There is relatively little information on effects of prolonged exposure to phosphine. Decreased body weight and kidney and liver effects have been reported in animals exposed repeatedly to phosphine via inhalation. Male and female rats exposed to phosphine ($1.5–15\,mg\,m^{-3}$) exhibited marked lethality (4/10) in females only with the highest dosage. Significant reductions in body weight and food consumption were noted across all treatment groups and sexes. Dose-related changes in blood urea nitrogen and other clinical parameters were also seen across exposure groups. Histopathological examinations revealed renal cortical lesions with the highest dosage, $15\,mg\,m^{-3}$, but not at lower exposure levels. All effects were apparently reversible within a month of termination of exposure. Phosphine does not appear to be a reproductive or developmental toxicant.

Human

Chronic (long-term) occupational exposure of workers to phosphine may cause inflammation of the nasal cavity and throat, weakness, dizziness, nausea, gastrointestinal, cardiorespiratory, and central nervous system symptomology, jaundice, liver effects, and increased bone density. Chronic exposure to very low concentrations may result in anemia, bronchitis, gastrointestinal disturbances, and visual, speech, and motor disturbances. Chronic exposure may be more serious for children because of their potential longer latency period. There is no evidence of cumulative effect in grain workers exposed for long periods to phosphine. Intermittent exposures for months led to headaches but no other symptoms.

The US Environmental Protection Agency has determined that phosphine is not classifiable as to its human carcinogenicity.

In Vitro Toxicity Data

Studies on isolated rat liver showed that mitochondrial oxygen uptake is inhibited by phosphine due to its reaction with cytochrome C and cytochrome C oxidase. Phosphine inhibits insect catalase, though this appears to be an indirect effect and might be a consequence, not a cause, of toxicity.

Clinical Management

Management depends on the route of exposure and proper first aid treatment must be performed. There is no antidote available.

First Aid

In case of phosphine inhalation, the patient must be removed from the exposure site and rested. Rescuers should follow full safety procedures. If a patient is unconscious, place him in the semiprone recovery position, otherwise maintain the airway and give oxygen if required. If breathing stops, immediately ventilate the patient artificially (mouth-to-mouth/nose or mechanically with oxygen if available). If the heart stops, begin cardiopulmonary resuscitation. The patient must then be referred to the nearest medical center for further treatment.

In case of ingestion of a metal phosphide, do not give milk, fats, or saline emetics by mouth. If the patient is conscious, consider induction of emesis. After vomiting, administer activated charcoal (50 g in water by mouth) if available.

Medical Treatment

1. Gastric lavage, endotracheal intubation to protect the airway, followed by activated charcoal.
2. Monitor and support vital functions, particularly cardiovascular, respiratory, hepatic, and renal functions.
3. Treat shock conventionally with appropriate vasopressors as needed.
4. Perform arterial blood gas analysis and correct respiratory dysfunction by clearing the airways, giving oxygen and perform artificial (mechanical) respiration if required. Metabolic acidosis must also be treated by giving sodium bicarbonate according to the results of arterial pH and blood gas analyses.
5. Hepatic and renal failure should be treated as required, with consultation with an experienced hepatologist and nephrologist.

There are no specific blood or urine tests for phosphine itself. Breakdown products of phosphine can be measured in urine, but the result of this test is generally not useful in the clinical management of patients.

Environmental Fate

In the air, phosphine will exist solely as a gas. Phosphine gas reacts with substances commonly found in the air. Half of the phosphine in the air degrades in ~ 1 day. At high concentrations, phosphine vapors may spontaneously combust in air. Phosphine is expected to react with water and be broken down into other products. Some of the phosphine that is not broken down may evaporate into air. When released to soil, phosphine is broken down very quickly. Phosphine does not accumulate in the food chain.

Ecotoxicology

Little information is available on the ecotoxicity of phosphine. Turkeys and chickens exposed to 211 and 224 mg m^{-3} for 74 and 59 min, respectively, showed dyspnea, convulsions, and death. Aluminum phosphide is very toxic to rainbow trout, with an acute LC_{50} of 4.1 μg l^{-1}.

Other Hazards

Phosphine reacts with air, oxidizers, chlorine, acids, moisture, halogenated hydrocarbons, and copper.

Exposure Standards and Guidelines

The reference dose for aluminum phosphide is 0.004 mg kg^{-1} day^{-1}.

The Occupational Safety and Health Administration permissible exposure limit for phosphine is 0.3 ppm (averaged over an 8 h work shift).

The National Institute for Occupational Safety and Health immediately dangerous to life or health value for phosphine is 50 ppm.

ERPG-2 (emergency response planning guideline) (maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to 1 h without experiencing or developing irreversible or other serious adverse health effects or symptoms that could impair an individual's ability to take protective action) = 0.5 ppm.

Miscellaneous

Phosphine is a colorless gas with odor of garlic or decaying fish (1–3 ppm threshold). It is slightly soluble in water (0.3% at 68°F). Phosphine is extremely flammable and explosive; it may ignite spontaneously on contact with air.

See also: Aluminum Phosphide; Phosphorus.

Further Reading

Brautbar N and Howard J (2002) Phosphine toxicity: Report of two cases and review of the literature. *Toxicology and Industrial Health* 18: 71–75.

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Phosphine.

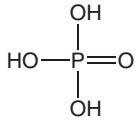
http://www.intox.org – Phosphine (Poisons Information Monograph 865 from the International Programme on Chemical Safety).

http://www.epa.gov – Phosphine (from the US Environmental Protection Agency's Air Toxics Website).

Phosphoric Acid

Samantha E Gad and Russell Barbare

- Chemical Abstracts Service Registry Number: CAS 7664-38-2
- Synonyms: Orthophosphoric acid; Hydrogen phosphate
- Chemical/Pharmaceutical/Other Class: Inorganic acids; Corrosive mineral acids
- Chemical Formula: H_3PO_4
- Chemical Structure:



Uses

Phosphoric acid is a component of fertilizers (80% of total use), detergents, and many household cleaning products. Dilute solutions have a pleasing acid taste; thus, it's also used as a food additive, lending acidic properties to soft drinks and other prepared foods, and in water treatment products. It is also used in rust proofing, engraving, and metal coating and is an intermediate or reagent in many manufacturing processes. Phosphoric acid also occurs naturally in many fruits and their juices. Apart from use of phosphoric acid itself, the greatest consumption of phosphoric acid is in the manufacture of phosphate salts. Taking advantage of its ability to lower blood pH, phosphoric acid has been used therapeutically to treat lead poisoning.

Exposure Routes and Pathways

Inhalation of mist, ingestion, and dermal, ocular, and mucous membrane contact are possible routes of exposure. Because phosphoric acid has a low vapor pressure, it must be aerosolized somehow and become airborne in order to affect the respiratory tract.

Toxicokinetics

Phosphoric acid is rapidly absorbed from the gastrointestinal tract and through the skin.

Mechanism of Toxicity

Because of its acidic properties, phosphoric acid produces toxicity much like any other acid. Excessive exposure causes corrosion on contact and disruption of internal pH balance (acidosis) when large concentrations are distributed systemically.

Acute and Short-Term Toxicity (or Exposure)

Animal

Phosphoric acid is irritating to the skin and eyes of rabbits. In rats the oral LD_{50} is $1530\,mg\,kg^{-1}$, the inhalation $LC_{50} > 850\,mg\,m^{-3}$, and the no-observed-adverse-effect level is $180\,mg\,m^{-3}$.

Human

Exposure to highly concentrated solutions can irritate the skin and mucous membranes. Phosphoric acid is highly corrosive. If ingested, corrosion damage may occur to the gastrointestinal tract and nausea and vomiting are possible. The inhalation of acid mist may cause irritation to the throat and lungs leading to reactive airway dysfunction and respiratory failure in extreme cases.

Chronic Toxicity (or Exposure)

Animal

Chronic inhalation studies have been conducted with combustion products of phosphorus with plastics or felt. These products produce phosphoric acid on contact with the water in tissue but also have effects from the other combustion products. Deaths and respiratory damage occurred in all species studied if the dose was high enough. Fetal effects were seen in rats exposed *in utero*. Effects included increased mortality and decreased pup body weights.

Human

Bronchiolar fibrosis has been reported with chronically high exposures. However, chronic inhalation exposure at the low levels most exposed individuals are likely to experience, produces no changes in pulmonary function, or in other effects occasionally

reported, including reduced leukocyte count, or reduced hand bone density.

Clinical Management

Gastric lavage and emetics should be avoided after exposure to phosphoric or other acids, should not be induced. An exposed area should be washed with copious amounts of water and a neutralizer such as magnesium oxide, lime water, or aluminum hydroxide gel. Eyes should be irrigated with large amounts of water.

Environmental Fate

Phosphoric acid quickly disperses in natural water sources. The acidity of this compound is eventually reduced but phosphate may persist indefinitely.

Ecotoxicology

Phosphoric acid percolates through soil and is harmful to aquatic life due to its acidity. If undiluted, it will destroy vegetation. When entering the water table, phosphate remaining from the reduction of phosphoric acid can stimulate marine and fresh water algae and plant growth, leading to algae blooms and eutrophication.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value (TLV) and the Occupational Safety and Health Administration permissible limit value is $1\,mg\,m^{-3}$ of air; TLV – STEL (short-term exposure limit) is $3\,mg\,m^{-3}$. Phosphoric acid is listed by the US Food and Drug Administration on the 'Generally Recognized as Safe' list when used according to good manufacturing practices. The Food and Agriculture Organization considers less than $30\,mg\,kg^{-1}$ of body weight safe when ingested in foods.

See also: Acids; Corrosives.

Further Reading

Bingham E (2001) Phosphorus, selenium, tellurium, and sulfur. In: Bingham E, Cohrssen B, and Powell CH (eds.) *Patty's Toxicology*, 5th edn., vol. 3, pp. 465–466. New York: Wiley.

Relevant Websites

http://www.osha.gov – Chemical Sampling Information: Phosphoric Acid. US Department of Labor, Occupational Safety and Health Administration.

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Phosphoric Acid.

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Phosphorus

Heriberto Robles

- Chemical Abstracts Service Registry Number: CAS 7723-14-0
- Synonyms: Elemental phosphorus; White phosphorus; Red phosphorus; Yellow phosphorus
- Chemical Formula: P

Uses

Phosphorus is used in the manufacture of weapons, insecticides, fertilizers, and rodenticides.

Background Information

Phosphorus is found in rocks, soil, plants, and animal tissues. Commercial preparations of phosphorus are either white or yellow. Yellow phosphorus is white phosphorus that contains small quantities of red phosphorus. Heating white phosphorus in the presence of an oxygen-free and inert atmosphere produces red phosphorus.

Phosphorus is an essential mineral element. Phosphorus homeostasis in the body is controlled by hormonal and renal control systems. Phosphorus intoxication from excessive consumption in food is not known. Toxic exposures have been reported to occur from its industrial use or from suicidal ingestion of phosphorus-containing materials. Phosphorus is highly toxic to humans and animals. The acute lethal dose in humans is $\sim 1\,mg\,kg^{-1}$.

Phosphorus has a garlic-like odor and, when exposed to air, it produces a white smoke and a greenish light. These physical properties can help the clinician in the diagnosis of phosphorus poisoning as

the vomitus and feces of patients who have ingested phosphorus may have a garlic-like odor, be luminescent, and give off what appear to be white fumes.

Toxicokinetics

Phosphorus can be absorbed into the systemic circulation from the skin, lungs, and intestinal tract. For all practical purposes, white and yellow phosphorus are readily absorbed while red phosphorus is not. The target organs of toxicity include the gastrointestinal tract, liver, kidney, bone, and the cardiovascular and central nervous systems.

Mechanism of Toxicity

Phosphorus is an oxidizing agent that, when exposed to air, may burn spontaneously. Thus, direct contact may result in both thermal and chemical burns. Second- and third-degree burns can be seen at the point of contact. When absorbed, phosphorus will act as a cellular poison by uncoupling oxidative phosphorylation.

Acute and Short-Term Toxicity (or Exposure)

Human

The major hazard associated with direct exposure to phosphorus is direct irritation and severe damage to skin, eye, or mucosal surfaces. Systemic absorption of high doses (normally $>0.2\,\mathrm{mg\,kg^{-1}}$) results in acute poisoning. Phosphorus poisoning presents three distinct phases: during the first phase, a painful burning sensation in the throat and stomach is present. Intense thirst, nausea, vomiting, and diarrhea accompany the abdominal pain. Breath, vomitus, and excreta may present the characteristic garlic odor. Feces and vomitus may be luminescent and appear to give off fumes. Severe poisoning may be accompanied by shock and death. During the second phase, poisoning symptoms disappear and the patient appears to be recovering. The second phase may last a few days. During the third phase, the gastric symptoms reappear with nausea, vomiting, and diarrhea. In addition, symptoms indicative of blood, liver, and kidney damage appear. Some of the symptoms include liver tenderness and enlargement, jaundice, oliguria, hematuria, albuminuria, anuria, skin itching and hemorrhages, inhibited blood clotting, and cardiovascular collapse. During advanced stages, the presence of convulsions, delirium, and coma are indicative of central nervous system damage. Death may occur within 4 to 8 days. Prognosis is good if the patient survives for more than 1 week after exposure.

Chronic Toxicity (or Exposure)

Human

Chronic ingestion and/or inhalation of phosphorus may result in osteomyelitis and bone necrosis. Signs and symptoms of this condition include bone inflammation, spontaneous bone fractures, anemia, and weight loss. A typical example of this condition is 'phossy jaw'. This condition is caused by the absorption of phosphorus fumes through teeth cavities. Once absorbed, phosphorus attacks and destroys the bones of the mandible and maxilla. The extent of facial bone loss can be so severe that the bone necrosis may extend from the maxilla to the eye orbits. Phossy jaw is an irreversible and usually fatal condition.

Clinical Management

Basic life-support measures should be implemented and further absorption should be prevented by removing contaminated clothing and washing the affected area. If ingested, the esophagus and digestive tract may be irritated and may be burned. Therefore, a careful examination should be performed and gastric lavage should be instituted only if the esophagus is not damaged and it is believed that lavage may be effective at removing the ingested material.

Medical examination should look for signs of skin, eye, gastric, liver, and kidney damage. Patients should be monitored and treated in an intensive care unit. Monitor vital signs and blood chemistry at least once a day. Life support should be instituted as needed.

Exposure Standards and Guidelines

Phosphorus is listed as a hazardous pollutant under the Clean Air Act and the Clean Water Act. Federal Drinking Water Guidelines: Environmental Protection Agency (EPA) $0.1\,\mu\mathrm{g\,l^{-1}}$ (white phosphorus); Occupational Safety and Health Administration: permissible exposure limit: Table Z – 1 8 h time-weighted average (TWA): $0.1\,\mathrm{mg\,m^{-3}}$; threshold limit values: 8 h TWA: $0.1\,\mathrm{mg\,m^{-3}}$ (yellow phosphorus).

See also: Gastrointestinal System; Organophosphates.

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http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Phosphorus.

Photoallergens

Shayne C Gad

Introduction

Photoallergy is an acquired immunologically mediated reaction to a chemical that is initiated by the formation of photoproducts. A photoallergen is a chemical that leads to this response. The occurrence of a photoallergic response to a chemical is sporadic and highly dependent on the specific immune reactivity of the host. Photoallergic responses are thought to be cell-mediated hypersensitivity reactions involving two distinct mechanisms.

In the first reaction type, light initiates the conversion of the hapten (synonymous with photosensitizer) to a complete allergen. Animal studies suggest that the photoreactive chemical in the skin absorbs light and is converted to a photoproduct that subsequently binds to tissue proteins producing a complete antigen:

$$\underset{}{\mathrm{PS}} \rightarrow \underset{(\mathrm{Hapten})}{\mathrm{Photoproduct}} + \mathrm{Tissue\ \ Protein} \rightarrow \underset{(\mathrm{Antigen})}{\mathrm{Allergen}}$$

Halogenated salicylanilide photoproducts are believed to be formed in this fashion.

In the second type of reaction, light absorbed by the photosensitizer results in its conversion to a photoproduct that is a more potent allergen than the parent compound:

$$\underset{(\mathrm{weak\ \ allergen})}{\mathrm{PS}} \rightarrow \underset{(\mathrm{strong\ \ allergen})}{\mathrm{Photoproduct}}$$

The photoproduct of sulfanilamide is thought to be formed by this second pathway in which the parent sulfanilamide compound is converted by ultraviolet (UV) light to the potent allergic sensitizer *p*-hydroxyaminobenzene sulfonamide. Patients with this type of photoallergy have demonstrated an allergic reaction to sulfanilamide in the dark.

Background

The first basic experimental work in photoallergy was done more than 50 years ago, when Epstein, in a straightforward and very perceptive study, demonstrated that sulfanilamide was both a phototoxin and a photoallergen. He and others had observed patients receiving sulfanilamide who developed dermatitis in sun-exposed areas. Six naive subjects (one of whom was himself) were chosen and skin sites were injected intradermally with sulfanilamide (0.1 ml of a 1% saline solution). Then these areas were irradiated with UV light from a mercury arc lamp (UVA and UVB). In all six subjects, the procedure induced a mild erythema leading to hyperpigmentation at the injected sites; that is, a sulfanilamide-mediated phototoxic reaction. Repetition of the protocol (intradermal sulfanilamide and then UV irradiation) at a different site, some days later, caused a marked dermatitis in two of the six individuals. These two subjects had been photosensitized to sulfanilamide, and with further phototesting they continued to show an altered reactivity to sulfanilamide followed by UV radiation (but not to sulfanilamide alone); their photoallergy persisted. In later work, Epstein induced photoallergic contact dermatitis to chlorpromazine in human subjects, utilizing the topical application of chlorpromazine for photosensitization and photochallenge. Those results paralleled his findings with sulfanilamide: Chlorpromazine was both a phototoxin and a photoallergen. Biopsies of positive chlorpromazine photoallergic reaction sites showed a histopathological picture consistent with that of delayed-type hypersensitivity; that is, reactions similar to those of classical experimental allergic contact dermatitis in humans.

Over the ensuing years, a considerable number of compounds have been tested in humans for their possible photoallergenicity, and many of the larger dermatology units have photo testing sections for evaluating patients for possible photoallergy to materials with which they come into contact. Experimental

work in humans sometimes followed the lead of clinical impressions, as was the case with chlorpromazine and tetrachlorosalysilanilide. Kaidbey and Kligman designed a prospective testing scheme in humans for evaluating possible photocontact allergens. Their method requires repeated photosensitizing exposures; that is, application of the test chemical to the skin followed by UV light, for photosensitization; photochallenge is done 10–14 days after the last photosensitization at an untreated skin site. This routine, which is a variant of the 'maximization' test in humans for classical contact allergens, has proven very useful for identifying the photoallergenicity of suspect materials.

Schwartz and Speck were the first to demonstrate photoallergy in an experimental animal, the guinea pig. Their initial investigation was with sulfanilamide and derivatives, and later experiments were with chlorpromazine. The common theme in tests is to photosensitize by the repeated successive application of prospective allergen, followed by UV radiation, to a clipped area (sometimes with the injection of complete Freund's adjuvant into the photosensitization site). Photochallenge is done at a different skin site some weeks later and reactions are evaluated by eye, as for classic allergic contact dermatitis in the guinea pig. The technique successfully identifies most known photocontact allergens, although the substance bisphenol-A, by clinical report a photosensitizer of humans, does not appear to photosensitize guinea pigs.

Test procedures designed to identify potentially photosensitizing chemicals evolved in the wake of the photosensitivity outbreak caused by the antimicrobial halogenated salicylanilides in the early 1960s. Photocontact allergy, although relatively uncommon, proved to be particularly troublesome. A minority of affected patients developed a persistent photodermatitis for many years despite avoidance of further contact with the offending chemical. While removal of the photosensitizing phenolic compounds from the marketplace reduced the incidence of photosensitivity, it quickly became apparent that other, chemically unrelated substances were also capable of inducing this adverse reaction. There was a clear need for a laboratory test to detect potentially photosensitizing agents.

Testing for photoallergy is similar to patch testing for allergic contact dermatitis. Duplicate allergens are placed on the back under occlusion with stainless steel chambers. Approximately 24 h later, one set of patches is removed and irradiated with UVA. All patches are removed and clinical assessments of patch test sites are made 48 h and then 1 week following placement. A reaction to an allergen solely on the irradiated side is deemed photocontact dermatitis. Reactions occurring simultaneously on the irradiated and nonirradiated sides are consistent with an allergic contact dermatitis. There is disagreement about the likelihood of coexisting allergic contact and photocontact dermatitis to the same agent since a photopatch test may occasionally exhibit greater reactivity on the irradiated side compared to the nonirradiated side. **Table 1** lists potential photoallergens used in photopatch testing.

Phototoxicity versus Photoallergenicity

From a mechanistic standpoint, light-induced dermatopathologic changes can be divided into phototoxic and photoallergic categories. Phototoxic skin damage results from the direct interaction of irradiation with subcellular targets, while photoallergic reactions involve immunomodulation of cutaneous photoreactivity. Both variants require initiation by exogenous light, but subsequent cytopathologic mechanisms may be substantially different.

With phototoxicity, light may originate directly from exogenous sources, such as the sun, artificial lighting, or photodynamic topical chemicals, or it

Table 1 Photoallergen series for photo-patch testing

p-Aminobenzoic acid
Bithionol (thiobis-dichlorophenol)
Butyl methoxydibenzolymethane
Chlorhexidine diacetate
Chlorpromazine hydrochloride
Cinoxate
Dichlorophen
4,5-Dibromosalicylanide
Diphenhydramine hydrochloride
Eusolex 8020 (1-(4-isopropylphenyl)-3-phenyl-1,2-propandione)
Eusolex 6300 (3-(4-methylbenzyliden)-camphor)
Fenticlor (thiobis-chlorophenol)
Hexachlorophene
Homosalate
Menthyl anthranilate
6-Methylcoumarin
Musk ambrette
Octyl dimethyl *p*-aminobenzoic acid
Octyl methoxycinnamate
Octyl salicylate
Oxybenzone
Petrolatum control
Promethazine
Sandalwood oil
Sulfanilamide
Sulisobenzone
Tetrachlorocarbanilide
Thiourea
Tribromosalicylanilide
Trichlorocarbanilide
Triclosan

may emanate from endogenous sources such as photodynamic drugs or chemicals following activation or excitation by percutaneous irradiation. Subcellular targets have not been completely characterized but may include the formation of thymine dimers, DNA–protein cross-links, or photodependent oxidations. Immunologic processes are not involved in this form of photosensitivity.

With photoallergic reactions, cytopathologic events are believed to be even more complex than with direct phototoxicity. Although many mechanistic features remain obscure, fundamental concepts include the photoactivation of endogenous or xenobiotic haptens so that they combine with cellular proteins and form a complete antigen. Subsequent immunologic reactions, especially cell-mediated hypersensitivity, complete the sensitivity process.

In contrast to phototoxicity, photoallergy represents a true type IV delayed hypersensitivity reaction. Hence, while phototoxic reactions can occur with the first exposure to the offending chemical, photoallergy requires prior sensitization. Induction and subsequent elicitation of reactions may result from topical or systemic exposure to the agent. If topical, the reactions are termed photocontact dermatitis, while systemic exposures are termed systemic photoallergy. In many situations, systemic photoallergy is the result of the administration of medications. Generally, the mechanisms of photocontact dermatitis and that of systemic photoallergy are the same as those for allergic contact dermatitis. In the context of photocontact dermatitis, however, UV light is necessary to convert a potential photosensitizing chemical into a hapten that elicits an allergic response.

Although precise cytopathologic mechanisms have not been established for many photosensitivity reactions, clinical and pathological features have been extensively documented. The following outline describes key diagnostic findings that serve to differentiate photosensitivity reactions from other dermatologic phenomena.

Photoallergy versus Contact Allergy

Photocontact allergic reactions are often compared with contact allergic reactions. Four pathogenetic features are present in both reaction types:

1. Compounds with a low molecular weight can act as haptens.
2. The antigen is produced by covalent binding of the hapten to skin components.
3. The immunological reactions are T cell dependent.
4. The histological pictures of contact and photocontact allergic reactions are similar.

Photoactivation of Molecules

The main difference between the two pathogenetic mechanisms is that in photoallergy light energy is necessary for the activation of the hapten or skin components to form covalent allergenic adducts. Besides photoactive exogenic or endogenic heteromolecules, the following skin components can be activated by photon energy: amino acids and proteins, blood components, lipoproteins, DNA, RNA, and so on. The reaction possibilities between hapten, light, and skin components can be classified in six different groups depending on the activated molecule:

1. Through the absorbed light quantum the prohaptene is transformed into the haptene.
2. Through the absorbed light quantum the active protein carrier is formed from the protein in the skin.
3. The haptene formed by irradiation combines with a skin protein to form an antigen.
4. The haptene combines with the protein changed by light to form an antigen.
5. The haptene altered by light combines with the protein changed by light, thus forming an antigen.
6. The haptene and light catalyze a chemical reaction on the protein, which leads to an autoantigen.

Distinct photochemical processes are now known for molecular photoactivation. Most of the photoactive molecules have X electrons. If a molecule is activated by light, two different energy levels can be attained. The molecule can be activated from the ground state to the singlet energy level to the triplet energy level. In the case of the singlet-state level, an electron reaches a higher orbital while the original spin configuration is maintained. In the case of the triplet-state level, the electron in the higher orbital changes the spin configuration so that the two electrons in the different orbitals have parallel spin configurations.

The activated singlet-state molecules are short-lived and return to their ground state in time periods of 10^{-1} to maximal 10^{-6} s. Fluorescence is one of the observed manifestations of the nascent energy. Triplet states are of longer duration, their lifetime can reach the range of 10^{-1}–100 s. Phosphorescence may be observed. Besides fluorescence and phosphorescence, nascent energy of activated molecules can also be released in the form of heat; electrical charges can be transferred to other molecules, and radicals can be formed or the molecule itself transformed.

In the case of photoallergic reactions, the formation of heteroadducts plays an important role. It comprises the combination of exogenous molecules

with autologous tissue or cell components. This is the main process for the formation of the complete antigen. The formation of heteroadducts is also the most important factor in the treatment of psoriasis with 8-methoxypsoralen (8-MOP) and UVA. The binding of 8-MOP to thymine molecules in the DNA is important not only for clinical treatment but also for possible late side effects (carcinogenicity).

An important complication of some of the chemicals inducing photoallergic responses is the development of persistent light reactions in which a marked sensitivity to light persists despite the apparent termination of exposure. Removal of the offending photoallergen in these cases does little to abate the condition and the action spectrum broadens to include the UVB as well as the UVA bands. As the phrase implies, this condition is long-lived and troublesome. This particular problem validates the importance of developing and utilizing screening tests for photoallergenicity to prevent exposure of a susceptible population of people to chemicals with this potential.

Clinical Findings

Usually, but not invariably, dermatologic lesions are restricted to light-exposed areas. Changes may vary from urticaria to papular and eczematous eruptions with subsequent exfoliation and lichenification. Microscopically, it is very difficult to distinguish photoallergic reactions from nummular eczema, atop dermatitis, eczematous drug eruptions, and, especially, allergic contact dermatitis.

Histopathologic Findings

Generally, microscopic findings do not provide an adequate basis for separating photoallergic reactions from the eczematous drug eruptions and allergic contact dermatitis previously discussed. Salient features include spongiosis with lymphocytic exocytosis, mild dermal edema, and mild to moderate dermal perivascular cuffing consisting of lymphocytes, histiocytes, and varying numbers of eosinophils. A feature that may distinguish photoallergy from contact allergy in human skin is that inflammatory cell infiltrations in light-induced allergic reactions may be both superficial and deep within the dermis, whereas with contact allergy they tend to be limited to the superficial dermis.

Assessment of Photosensitization

Photosensitivity reactions account for a very small percentage of the total number of undesirable effects from environmental chemicals. However, the increasing incidence and severe disability resulting from these types of skin changes suggest that additional photobiologic research efforts are needed, particularly when the photosensitivity response is of the persistent light reactor mechanism. Predictive testing is an obvious approach used to assess the photosensitizing potential of new chemicals entering the commercial market. These methods make it possible to identify and possibly minimize or eliminate exposures to those compounds demonstrating risk-benefit ratios that are undesirable for the general population or especially sensitive individuals.

In vitro and *in vivo* methods with predictive value for estimating the photosensitizing potential of new compounds have developed rapidly to meet the demanding requirements of today's society. *In vitro* methods for assessing photosensitization are desirable because they are usually rapid and inexpensive and therefore allow screening of a large number of compounds. Many of these methods are not very specific, however, and will generate a greater percentage of false positive results than *in vivo* tests using animal or human models. Complex *in vitro* test systems appear to be useful in identifying the site and mechanism of action in certain situations. Continued evolution of *in vitro* methodologies will add to the understanding of the photosensitization mechanism as better correlation is established with *in vivo* studies.

See also: Skin; Toxicity Testing, Dermal.

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Photochemical Oxidants

Shayne C Gad

Introduction

The oxidant of critical importance in the photochemical atmosphere is ozone (O_3). Several miles above the Earth's surface, in the troposphere, there is sufficient shortwave ultraviolet (UV) light to directly split molecular O_2 to atomic O to combine with O_2 to form O_3. These UV wavelengths do not reach the Earth's surface. In this region, nitrogen dioxide efficiently absorbs longer wavelength UV light, which leads to the following simplified series of reactions:

$$NO_2 + UV \rightarrow O + NO$$

$$O + O_2 \rightarrow O_3$$

$$O_3 + NO \rightarrow NO_2$$

This process is cyclic, with NO_2 regenerated by the reaction of NO and O. In the absence of hydrocarbons, this series of reactions would approach a steady state with no excess or buildup of O_3.

However, near the Earth's surface, the hydrocarbons, especially olefins and substituted aromatics, are attacked by the free atomic O, and with NO, produce more NO_2. Thus, the balance of the reactions shown in the above reactions is upset so that O_3 levels build up, particularly when the Sun's intensity is greatest at midday. The reactions with hydrocarbons are very complex and involve the formation of unstable intermediate free radicals that undergo a series of changes. Aldehydes are major products in these reactions. Formaldehyde and acrolein account for $\sim$50% and 5%, respectively, of the total aldehyde in urban atmospheres. Peroxyacetyl nitrate (CH_3COONO_2), often referred to as PAN, and its homologs, also arise in urban air, most likely from the reaction of the peroxyacyl radicals with NO_2.

Short-Term Exposures to Smog

The complexity of photochemical air pollution challenged toxicologists early on to ascertain its potential to affect human health adversely. Although ozone was quickly suspected as a primary toxicant because of its reactivity and abundance, a number of studies were undertaken with actual (outdoor-derived) smog or synthetic (photolyzed laboratory-prepared atmospheres) smog in an attempt to assess the potency of a more realistic pollution mix. When human subjects were exposed to actual photochemical air pollution (Los Angeles ambient air pumped into a laboratory exposure chamber), they experienced changes in lung function similar to those described in controlled clinical studies of ozone (i.e., reduction in spirometric lung volumes; see below), thus supporting the notion that ozone is of primary concern.

Acute animal studies utilized more easily controlled synthetic atmospheres (usually irradiated automobile exhaust) where the ozone target levels could be made to mimic high air pollution levels: <0.5 ppm. Again, very much like ozone alone, just a few hours of exposure to irradiated exhaust resulted in deep lung damage, primarily within the alveolar or small airway epithelium. In some of these studies, early evidence of edema appeared in the interstitium, particularly in older animals. Additionally, similarly exposed mice were found to be more susceptible to bacterial challenge and lung pneumonias. With time after the termination of exposure, the end-airway lesions recovered and the susceptibility to infection waned, although some of the pathology in the distal lung persisted for more than 24 h. While ozone appeared to be the prime toxicant in these studies, that was not always the case. When guinea pigs were exposed to irradiated automobile exhaust, airway resistance increased, indicating that a more soluble irritant probably was active, presumably reactive aldehydes. Thus, the array of effects of a complex atmosphere may be more diverse than would be predicted if it were assumed that ozone alone was responsible.

Chronic Exposures to Smog

Studies of both humans and animals exposed to smog have attempted to link chronic lung defects with photochemical air pollution. Cross-sectional and retrospective field studies have suggested an accelerated loss of lung function in people living in areas of high pollution compared to those living in area of low pollution, but most of these studies have been imprecise because of confounding factors (meteorological factors, exposure measurement imprecision, and population variables). Recently, there has been a rejuvenation of interest in what are sometimes called sentinel studies, which allow a detailed study of animals exposed to the same highly polluted urban air to which people are exposed. This approach has had a troubled past, but newer studies have attempted to minimize or at least control for the problems of infection, animal care, and lack of control of the exposure atmosphere.

Synthetic smog studies in animals were undertaken to eliminate some of the concerns about ambient smog exposure. The most extensive effort to evaluate the potential long-term health effects of synthetic smog was undertaken at the Cincinnati US EPA laboratory in the mid-1960s. Beagle dogs were exposed to synthetic atmospheres on a daily basis (16 h) for 68 months, followed by a clean air recovery period of ~3 years. The lungs of exposed dogs then underwent extensive morphological examination to correlate physiological and morphological observations. While the study did not show time-related lung function changes, all exposure groups had abnormalities, most of which persisted or worsened over the 3 year recovery period in clean air. Enlargement of air spaces and loss of interalveolar septa in proximal acinar regions were most severe in dogs that were exposed to oxides of nitrogen, oxides of sulfur, or oxides of sulfur with irradiated exhaust. Oxidants such as ozone arising from the irradiated exhaust would be expected to act on the distal lung. These studies elucidated a morphological lesion that was degenerative and progressive in nature, not unlike that of chronic obstructive pulmonary disease, the condition most often noted in the epidemiological studies.

See also: Nitric Oxide; Ozone; Pollution, Air; Respiratory Tract.

Further Reading

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Relevant Website

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Phthalate Ester Plasticizers

Shayne C Gad

- Representative Chemicals: Di-(2-ethylhexyl) phthalate (DEHP); Diisononyl phthalate (DINP); Diethyl phthalate (DEP); Di-*N*-butylphthalate; Dimethylphthalate; Methyl-glycol phthalate; Phthalic acid; Bis(2-methoxyethyl) ester. For the purposes of this article, the focus will be on DEHP, which is the most widely used phthalate, with some discussion of DINP since it has been the subject of controversy with regards to its possible adverse effects on children's health.
- Chemical Abstracts Service Registry Number: CAS 131-11-3 (Di-(2-ethylhexyl) phthalate)
- Synonyms: *o*-Dicarboxylic acid esters; Phthalic acid ester (PAE)

Uses

Phthalate esters are widely used in the production of plastics, particularly vinyl plastics, to add flexibility to products made with these materials. DEHP is commonly used in medical devices, including cardiac catheters, endotracheal tubes, and certain implanted devices, while DINP is more often found in wires and cables, hoses, and plastic toys. DEHP is also used in plastic containers, such as those used for food. These phthalates are not bound chemically to the plastic but are physically dissolved in it.

Exposure Routes and Pathways

The routes of exposure of most concern for DEHP are leaching into liquids administered intravenously in the course of medical treatment and leaching into foodstuffs (particularly lipophilic foods) stored in plastic containers, although exposures through the latter route are much smaller. For DINP, the main exposure concern is leaching as a result of children mouthing plastic toys.

Toxicokinetics

All phthalate esters are readily absorbed, but toxicokinetics vary based on the route of exposure. Once absorbed, they are quickly distributed to organs and other body tissues such as the liver (bile) or kidneys. Phthalate esters metabolize quickly to a monoester but do not progress further. From 4.5% to 15% of single doses of 10–30 g of DEHP are excreted as metabolites in the urine of man.

Mechanism of Toxicity

The mechanism by which DEHP causes at least some of its adverse effects appears to be through peroxisome proliferation in the liver of rodents. There is some controversy as to whether this effect would also occur in humans since they seem much less sensitive to this type of toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

Phthalates are not irritating or slightly irritating to eye or skin, and are not sensitizers. For DEHP, the intraperitoneal LD_{50} is 14–75 $g\,kg^{-1}$ and the oral LD_{50} is $>30\,g\,kg^{-1}$ in mice. In rats, the intraperitoneal LD_{50} is 30.7 $g\,kg^{-1}$ and the oral LD_{50} is $>25\,g\,kg^{-1}$.

Human

Phthalate esters may irritate eyes, skin, or mucous membranes.

Chronic Toxicity (or Exposure)

Animal

Repeat oral high doses of DEHP result in hepatomegaly (peroxisome proliferation) and tumorgenesis in rats. While DEHP appears to cause reproductive toxicity, similar effects have not been seen with DINP exposure. As a result of these and other effects, there has been concern that DEHP can cause endocrine effects. It appears that neither DEHP nor DINP are genotoxic.

Human

There are reports from patients receiving hemodialysis that DEHP may cause toxicity to the heart, lungs, and reproductive system. DEHP has been classified as a probable human carcinogen by the US Environmental Protection Agency (EPA).

Clinical Management

If ingested, gastric lavage should be performed and respiratory therapy administered, if needed. Emesis should be avoided. Treatment should be symptomatic.

Ecotoxicology

Phthalates are of low toxicity to aquatic life. For example, the LC_{50} for bluegill is $>770\,000\,\mu g\,l^{-1}$ per 96 h and the LC_{50} for *Daphnia magna* is 1000–5000 $\mu g\,l^{-1}$ per 48 h. Phthalates tend to persist to some degree in the fat of organisms in the environment.

Exposure Standards and Guidelines

The US Occupational Safety and Health Administration has established a permissible exposure limit of 5 $mg\,m^{-3}$. The US EPA has established a drinking water standard (maximum contaminant level) of 0.006 $mg\,l^{-1}$.

See also: Endocrine System; Polymers.

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Phthalates *See* Phthalate Ester Plasticizers.

Physical Hazards

Gene Rider

Introduction

Toxicologists are often required to assess the overall chemical- and design-related risks of reasonably foreseeable exposures to consumer products. Such a risk assessment would include the potential physical hazards associated with consumer products. Examples of potential physical hazards associated with products include designs that could lead to asphyxiation, aspiration, choking, strangulation, or suffocation incidents. Other examples of potential physical hazards include products that could create light (specifically, certain wavelengths of electromagnetic radiation), products creating very cold or hot temperatures, products with a high potential for flammability, products with an ability to create loud noise, products capable of forceful impact or with a sharp component that could lead to trauma, and products capable of generating strong vibration.

Analysis of the etiology of the physical injuries from consumer products includes three components: determination of the at-risk population (exposure to hazard), mechanism of injury (consequences of hazard), and characteristics of products (mitigation of hazard).

In order to understand the potential physical hazard-type risks associated with consumer products, it is important to utilize a multidisciplinary approach. In order to identify the population that is at risk, it is necessary to investigate injury and fatality incidents with similar products. Following this identification, characterization of the physical interaction of a consumer and a product reveals hazard and associated severity levels. Characterization of product attributes allows for the development of strategies that may mitigate product hazard and therefore reduce the probability of injury.

At-Risk Populations (Exposure to Hazard)

If a consumer can gain access or become exposed to hazardous product characteristics, the probability of this event must be determined. Probable exposure to the hazard may be determined using injury and fatality data analysis.

Learning from history through the analysis of variables associated with real-life injuries and fatalities allows for an understanding of the connections between product characteristics, child behavior, and injury. Statistical analysis and modeling reveal the critical characteristics associated with the risk of product-related injury.

Mechanism of Injury (Consequences of Hazard)

If a consumer is exposed to hazardous product characteristics, the severity level or potential consequence of this exposure must be evaluated. Human factors analysis is conducted to determine the consequences (i.e., potential product-related injuries) based on the foreseeable behaviors consumers will use when interacting with products. Virtual and physical models of the human anatomy are used to effectively diagnose and demonstrate hazardous product characteristics. (In contrast to a physical hazard such as those noted above, 'physical' in this human context relates to the usage of three-dimensional (3D) models of various parts of humans relevant to the exposures associated with use and/or misuse of a product.)

Human factors analysis utilizes accurate virtual and physical simulations of the human anatomy to identify the potential hazards posed by consumer products.

In order to determine the potential magnitude of a product-related physical hazard, both product characteristics and anatomical characteristics of likely consumers are examined. Virtual and physical human factor tools are used to conduct this research.

Characteristics of Products (Mitigation of Hazard)

The severity level of a physical hazard may be reduced by design characteristics that lead to reduced consequence or decreased time to effective treatment, and possibly by product labeling and/or usage instructions that could impact consumer behavior to help eliminate the hazardous condition, or at least mitigate the consequences of the exposure.

Airway Obstruction

At-Risk Populations

Children are driven to mouth objects as an inevitable part of their normal developmental process. This behavior has the potential to lead to choking, aspiration, insertion, or ingestion injuries. Small part injuries involve the unintentional entry of a foreign body into the aerodigestive system through the mouth or nose.

Children under the age of 4 represent the majority of airway obstruction injuries (**Figure 1**). Individuals of advanced age are also at greater risk for such injuries as they may be edentulous and suffer from

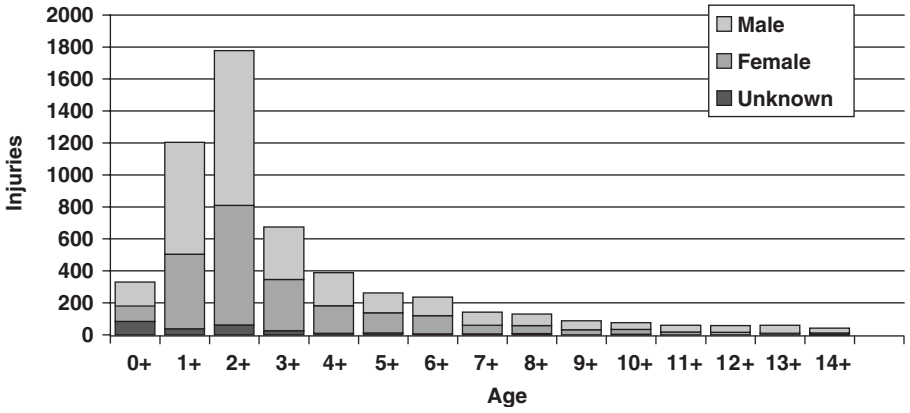

Figure 1 Age of victims of airway obstruction injury.

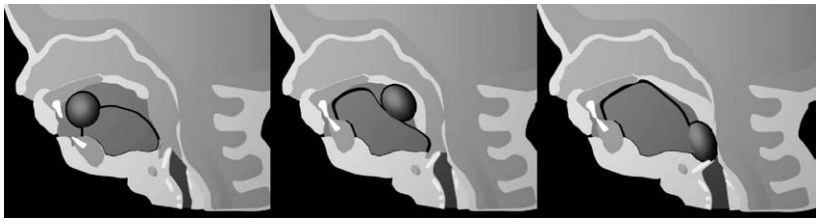

Figure 2 Compressible object lodging in pharynx.

decreased oral sensation. Alcohol or drug use and aging decrease the sensation of the nerves in the oral cavity increasing the likelihood of airway obstruction injuries.

Neurologically impaired people (estimated at 2–4% of the population often have greater difficulty during feeding and swallowing increasing the likelihood of airway obstruction. The term neurologically impaired is a blanket description that covers a multitude of different disorders. Persons suffering from these disorders may have them to widely varying degrees. Oral airway dysfunction in this group may present as diminished control, sensation, or comprehension. While most neurologically impaired persons have a normal airway, a segment of this population has abnormal airway anatomy altering their risk of airway obstruction.

Mechanism of Foreign Object Airway Obstruction

Direct Obstruction Several characteristics influence a foreign object's chance of penetrating the defenses of the mouth and pharynx. Foreign objects that are small, thin, smooth, or slick when wet may inadvertently slip through and enter the pharynx. Foreign objects that are round or cylindrical and pliable or compressible most effectively form a plug in the airway (**Figure 2**). A large bolus or foreign object mass is more likely to block the airway at the pharynx and cause asphyxia. When a premature or an inadvertent foreign object penetration occurs, a gag and or cough reflex may be triggered. Smooth, slick, and pliable food-like objects are less likely to trigger a timely gag reflex than textured or sharp objects. Gagging and coughing is frequently followed by rapid deep inhalation as the victim attempts to regain breath. This action may draw the object downward leading to physical impaction and obstruction. This consequence is facilitated by the temporary expansion of the pharyngeal and laryngeal chambers that occurs during vigorous inspiratory effort. This reaction is more intense in infants than in older children or adults. Pliable conforming objects (**Figure 3**) are less likely to be expelled from the airway than rigid objects.

Esophageal Wall Protrusion Foreign objects that are ingested and lodge beyond the upper esophageal sphincter may distend the wall of the esophagus into the volume of the airway along the length of the trachea resulting in asphyxia (**Figure 4**).

Oral Nasal Occlusion Rounded 3D objects that reach the rear of the oral cavity, posterior to the hard palate, may obstruct the flow of air into the lungs, leading to asphyxia (**Figure 5**). The mechanism of these injuries includes the following actions: these

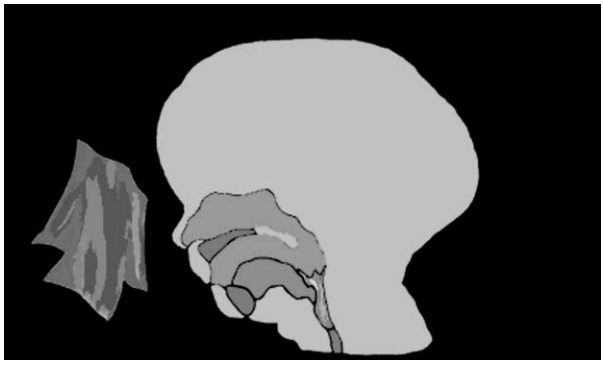

Figure 3 Conforming object lodging in pharynx.

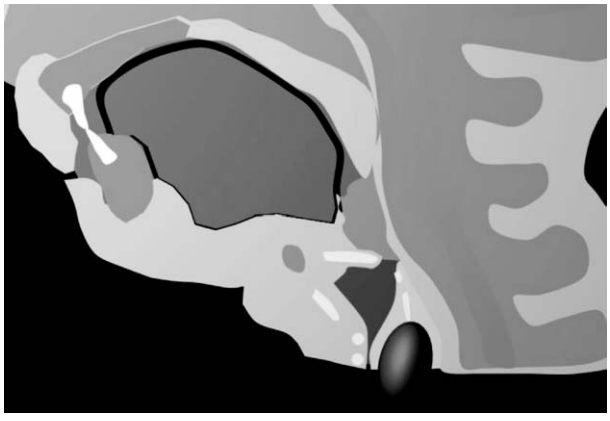

Figure 4 Esophageal wall protrusion.

objects may elevate the soft palate and prevent air passage through the nasopharynx (A). This action may diminish the size of the nasopharynx at this point, preventing the passage of air while simultaneously creating a seal against the soft tissue of the rear of the oral cavity (B). Objects that pass beyond the hard palate may be difficult to extricate due to the mechanical resistance to anterior motion created by the interference with the edge of this skeletal structure (C).

Object Characteristics Associated With Airway Obstruction

Size Airway (pharynx, larynx, trachea) sized objects will occlude the airway if other conditions are present. Small, lightweight nonwettable objects may be entrained in the inspiratory flow of air and consequently aspirated. Oral sensors can discriminate the size of solid materials, but children, the elderly, and the neurologically impaired may make poor decisions as to what they can successfully swallow.

Shape Previous studies of airway obstruction have classified foreign objects by shape. Square and

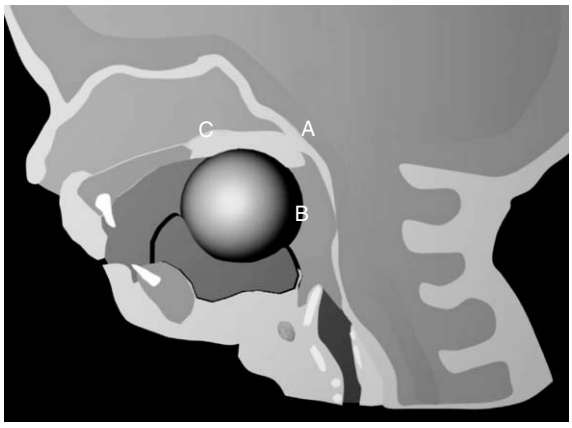

Figure 5 Oral nasal occlusion.

rectangular shapes make up less than 6% of the objects in this database, while a significant number, 22%, are spheres and ellipsoids. This illustrates that round objects make effective airtight seals against the flexible, rounded interior walls of the aerodigestive system. As expected, most of the incidents involving two-dimensional (2D) objects, such as coins, resulted in less severe symptoms, while 3D and conforming objects are more likely to produce more severe symptoms.

Consistency – Food Foreign Objects Penetration of the airway by food foreign objects is possible due to the combined oral airway functions of the oral cavity and pharynx. Humans must interrupt respiration during the pharyngeal phase of the swallow cycle. An asphyxiation hazard for a foreign object is a function of the degree of difficulty in processing the foreign objects into a bolus suitable for successful swallowing. Foreign objects that are difficult to process create a condition where the hazard of asphyxiation is present.

Compressible and nonfriable foods resist bolus formation. Compressible foods that do not break apart do not become well mixed with saliva. Compressible foods require transverse and rotational movement of the mandible to be effectively masticated. Compressible foods will deform to the shape of the airway.

Consistency – Nonfood Foreign Objects Conforming objects will, with certain flexibility and surface characteristics, adhere closely to the surface topographic features of the airway, making an effective seal. Expiratory effort will allow air to leave the lungs but inspiration will create a negative pressure on the distal surface of the conforming object that draws it further into the airway. Deformable materials (i.e., materials or objects whose shapes can be altered by

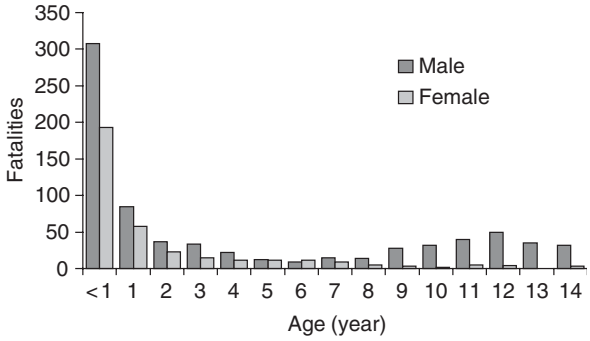

Figure 6 Age and gender of victims of strangulation fatalities.

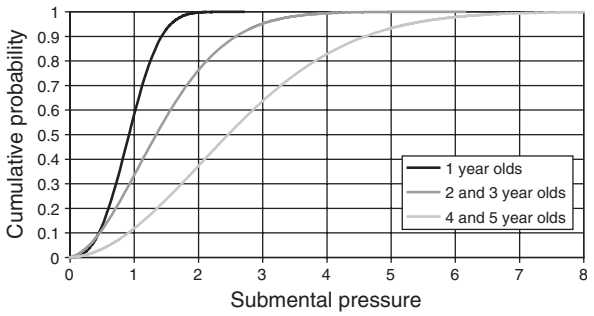

Figure 7 Strangulation force.

applying forces similar in magnitude to those which would be experienced in a child's mouth) also account for a significant number of serious injuries.

Strangulation

At-Risk Populations

There are 1160 strangulation fatalities involving children under age 15 documented in US Consumer Product Safety Commission (CPSC) death certificate files between 1994 and 2003. These 1160 strangulation fatalities constitute 14.1% of the total childhood fatalities (8224) (**Figure 6**).

Age: The number of strangulation fatalities was greatest among children under one, who accounted for approximately half (45%) of the childhood strangulation fatalities. The fatality trend declined until age seven, after which it rose until dropping again at age 13.

Gender: The majority (68%) of the incidents occurred to male children. The discrepancy in gender is more noticeable after the age of eight.

Mechanism of Injury

The neck region is a complex passageway for communication between the head and the trunk. The bony components in the neck are the vertebra, which enclose the spinal cord. The most important vessels are the carotid artery and the interior jugular vein that are contained in the carotid sheath along with the vagus nerve. The neck also contains the airway (larynx and the trachea); in children they are cartilaginous and highly mobile.

Strangulation is due to constriction of the neck causing direct airway closure. This often occurs as a result of suspension of all or a portion of the body weight by an object around the neck resulting in asphyxia. The constriction generally occurs above the larynx but below the angle of the jaw. The most common scenario in children is partial hanging, occluding the airway but not the jugular vein or carotid artery. Airway obstruction is currently believed to occur as the base of the tongue is pushed against the posterior wall and the epiglottis folds over the larynx.

In a previous study by the author, submental pressure (**Figure 7**) was noted to elevate the larynx, but occlusion occurred at the level of the nasopharynx and oropharynx. The soft tissues of the submental region pushed the tongue against the soft palate.

Suprahyoid pressure brought the epiglottis up and posterior compressing it against the posterior pharyngeal wall. The arytenoids compressed by the posterior wall of the hypopharynx overrode the true vocal cords to occlude the airway. The thyroid cartilage supported by its attachments to the hyoid was lifted superiorly and posteriorly (**Table 1**).

Characteristics of Objects Causing Strangulation

Apparel of children that contain components that can be caught on doorknobs, play ground equipment, or protrusions.

Continuous loops with a circumference greater than 13.94 in (35.4 cm) can be placed over a child's head.

Loose ends of strings, cords, or straps capable of forming a loop greater than 8.66 in (22.0 cm) can be placed around a child's neck.

Suffocation

At-Risk Populations

Approximately 300 infants have died over the past 4 years as a result of accidental suffocation associated with a variety of consumer products (plastic films, toys, and packing materials). Suffocation injuries and fatalities are most common to children under the age of 2 years (**Figures 8** and **9**).

There are two potential mechanisms involved in suffocation incidents, suffocation caused by mechanical

Table 1 Force required to occlude airway: Summary by percentile

Percentile	*Force(lbs)*					*Weight of child*			
	1 year old	*2 year old*	*3 year old*	*4 year old*	*5 year old*	*⩽10 kg*	*12.5 kg*	*15.0 kg*	*>15 kg*
10%	0.7	0.7	1.0	1.1	1.4	0.4	0.6	0.7	1.1
20%	0.9	0.9	1.2	1.4	1.6	0.6	0.8	0.9	1.4
30%	1.1	1.1	1.4	1.6	1.9	0.8	1.0	1.1	1.7
40%	1.3	1.3	1.6	1.8	2.1	1.0	1.2	1.3	2.0
50%	1.5	1.5	1.8	2.1	2.4	1.2	1.4	1.5	2.4
60%	1.7	1.8	2.1	2.3	2.8	1.6	1.8	1.8	2.9
70%	2.1	2.2	2.5	2.7	3.2	2.1	2.2	2.1	3.5
80%	2.6	2.9	3.0	3.3	3.9	3.0	3.0	2.7	4.6
90%	3.6	4.3	4.1	4.3	5.1	5.4	4.9	3.9	6.7
95%	4.8	6.1	5.3	5.5	6.4	9.3	7.6	5.4	9.6

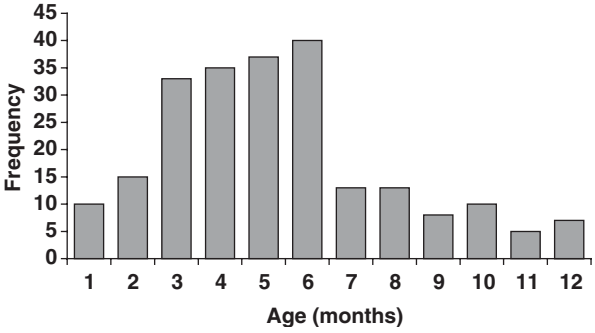

Figure 8 Age distribution in months – films.

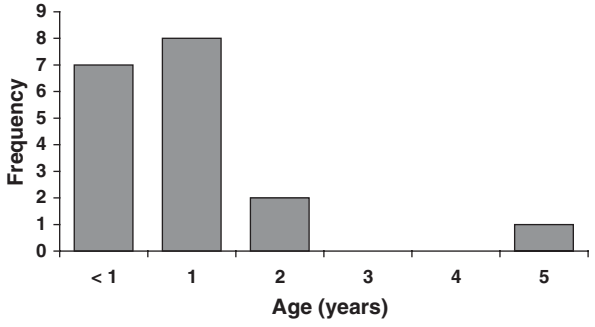

Figure 9 Age distribution in years – rigid container mechanisms of suffocation.

resistance to the passage of air and suffocation caused by physical responses to carbon dioxide (CO_2) rebreathing.

Mechanical Resistance Suffocation

Objects placed externally on the face of a child may lead to suffocation incidents. These objects are most commonly plastic films or bags, but may be any product that makes a seal against the face of a child, obstructing airflow to the mouth or nose. The faces of children in this age group have greater amounts of fat and undeveloped prominent bony structures and are consequently more likely to provide an effective seal, leading to suffocation injuries.

Respiration

Infants usually breathe through the nasal passages. However, during crying or in the event their nasal passages are blocked, infants may breath through their oral cavities. One-year-old children can produce a respiratory pressure up to 30 cm H_2O (positive for expiration and negative for inspiration) for a brief period of time. Young children can produce pressures of 15 cm H_2O for a more extended period of time.

Suffocation

Mechanical resistance suffocation takes place when the passage of air to the oral cavity and nasopharynx are both blocked externally by an object. When respiration is interrupted, CO_2 levels in the blood rise. The body's response to this elevation in CO_2 level is to attempt respiration. If the mechanical blockage is complete and the agent of suffocation is not removed, the incident will be fatal after 2–3 min. Partial blockage may be survived for longer periods of time, depending on the level of resistance and the strength of the child.

Protective Mechanisms

Very young children do not have effective defense mechanisms to protect themselves from suffocation injuries. In adults, raised CO_2 levels incite more and more strenuous attempts at respiration. In infants and young children, this response is not present. During suffocation incidents in infants, if the initial attempts at respiration fail, an increase in respiration effort and agitation is not observed.

Characteristics of Objects Causing Suffocation

Table 2 lists the respiration characteristics for both 6-month-old and 1-year-old children.

Table 2 Respiratory characteristics

Characteristics	*6 Month old*	*12 Month old*
Weight (lbs)	13	20
Flow resistance, R (cm H_2O per liter per second)	21	13
Peak flow rate (liter per minute)	22	34
Pressure for airway flow resistance, 'Peak flow rate $\times$ R' (cm H_2O)	7.7	7.4
Pressure for elastic recoil (cm H_2O)	4	4
Long-period sustainable pressure (cm H_2O)	14	18
Six-hour sustainable pressure (cm H_2O)	25	30
One-hour sustainable pressure (cm H_2O)	30	35

Table 3 Computed allowable pressure P_a

Time	*Pressure*	*6 Month old*	*12 Month old*
Long period	Allowable pressure (using peak flow rate)	2.3 (cm H_2O)	6.6 (cm H_2O)
Six hours	Allowable pressure (using peak flow rate)	13.3 (cm H_2O)	18.6 (cm H_2O)
One hour	Allowable pressure (using peak flow rate)	18.3 (cm H_2O)	23.6 (cm H_2O)

As a part of the sustainable pressure must be used to overcome normal airway flow resistance and elastic recoil of lung and chest, the amount of pressure (P_a) allowed to be used to overcome abnormal airway blockage, such as plastic film blockage, is smaller and can be found by

$$\begin{aligned} P_a = {} & \text{Sustainable pressure} \\ & - (\text{Pressure for flow resistance} \\ & + \text{Pressure for elastic recoil}) \end{aligned}$$

Table 3 gives the computed allowable pressure P_a for different time duration for both 6-month and 12-month-old children.

Thermal Burn Injury

Thermal burns are classified by the amount of damage done to the skin and other body tissue. The surface area of the skin ranges from 0.2 to 0.3 m^2 in an average newborn, and 1.5–2.0 m^2 in an adult. The skin consists of two layers: the epidermis, ranging from 0.05 mm thickness (in such areas as the eyelids) to over 1 mm thickness on the soles; and the dermis, usually at least 10 times thicker than the associated epidermis. An average total skin depth is 1–2 mm. Males generally have thicker skin than females. Skin is very thin in infants, increasing in thickness until age 30–40, and then progressively thinning with age.

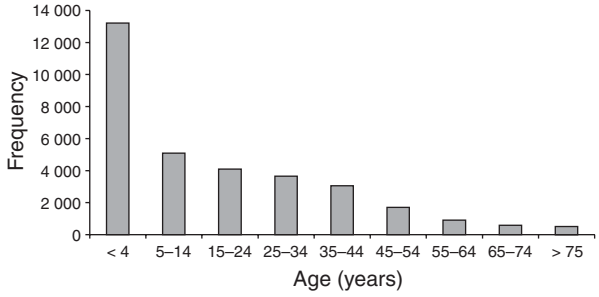

Figure 10 Age of victims of thermal burn injury.

Burn injury is tissue damage caused by thermally induced irreversible chemical reactions. Burns are often classified according to their severity as first degree, second degree, or third degree. First-degree burns, often referred to as surface burns, are minor burns that heal quickly. Second-degree burns, also referred to as partial thickness burns, are more serious injuries, which may require medical attention and possibly skin grafts to prevent permanent scarring. Third-degree burns, also referred to as full-thickness burns, extend deeply into tissue and are characterized by charring of the skin.

At-Risk Populations

Children constitute the most at-risk population for thermal burn injuries (**Figure 10**).

Mechanism of Injury

Thermal injuries to skin are assessed by measuring the amount of energy transferred to the skin by radiation, convection, and conduction.

These factors, and duration of exposure, which may be expressed as a dose function, are the primary determinants of the severity of thermal injury.

From an anatomical viewpoint, it is common to classify burns according to the depth of penetration into various skin layers. As seen in **Figure 11**, skin is composed primarily of a (usually) thin outer layer called the epidermis followed by a thicker dermis. Below the dermis is a layer of subcutaneous fat followed by skeletal muscle. Burns can be characterized according to the deepest layer of tissue penetrated and the depth to which that layer has been affected. In this scheme, two numbers are used to classify a burn: the first designates the lowest layer of tissue damaged, and the second the fractional depth to which that layer has been penetrated. Therefore, a 1.5-degree burn is one affecting only the epidermis (skin layer 1) in which half of the epidermis (0.5) has

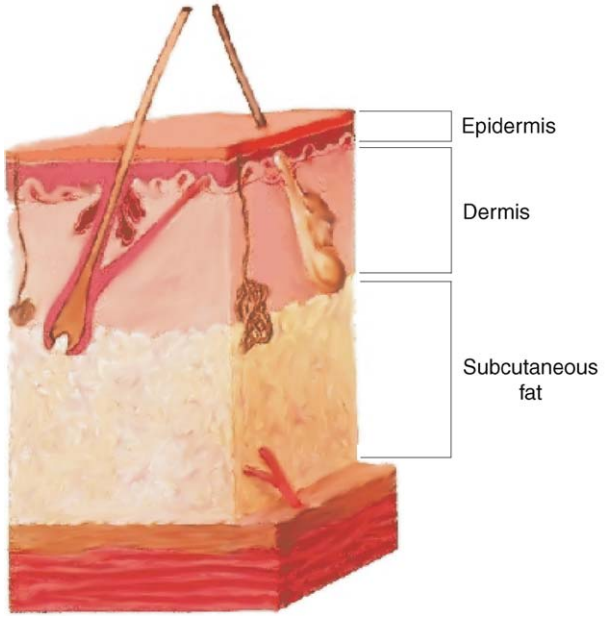

Figure 11 Diagram of skin showing epidermis, dermis, and fat.

been penetrated. By comparison, a 2.8-degree burn is one in which at least some portion of the injury extends 8/10ths of the way through the dermis (second skin layer), and a 3.4-degree burn is one in which the deepest portion of the injury extends through the epidermis and dermis until it has reached 4/10ths of the way through the subcutaneous fat (third layer).

The science of burn modeling effectively began in the late 1940s with a series of studies conducted at Harvard medical school by A.R. Moritz and F.C. Henriques. Motivated by the events of World War II, Henriques and Moritz conducted experimental studies of burn injuries from various heat sources to pigs and human volunteers. The studies of greatest relevance to modern-day burn modeling involved the use of flowing constant-temperature water to produce burn injuries on the backs of both pigs and humans. One hundred seventy-nine experiments were conducted on pigs at water temperatures between 44°C and 100°C and times ranging from 1 s to 7 h, and 33 exposures performed on volunteer soldiers at temperatures ranging from 44°C to 60°C and times between 3 s and 3 h. Although other experimental studies of contact or scald burn injury were conducted both before and after Henriques and Moritz, the combination of extent, high-temperatures/short times, and applicability to humans make their work unique.

One of Henriques' and Moritz' primary goals was to develop 'dose–response' curves designating the minimum exposure times at given temperatures yielding second-degree burns and the maximum exposure times at the same temperatures resulting in only first-degree burns. To obtain this information, they waited for up to a week following exposure and then medically examined each injury to determine its severity. Henriques subsequently developed a model based on this dose–response information that remains the basis for the vast majority of burn injury calculations performed today.

Characteristics of Objects Causing Thermal Burns (The Henriques Model)

Henriques developed a two-step method to calculate burn injury. The first step is a calculation of temperature distribution within the skin, while the second determines burn injury based on the time–temperature history. This general approach remains in use although modern burn modeling techniques generally involve sophisticated computer models of temperature distribution that were unavailable to Henriques and Moritz.

Henriques treated the skin as a semi-infinite body in which all skin layers have the same thermal properties, and the total skin thickness is far greater than that heated by the thermal source. Based on this assumption, he obtained the following formula for the temperature of the basal epidermal layer as a function of time:

$$T_t = T_s - (T_s - T_0)\mathrm{erf}(\gamma/\sqrt{t})$$

where t is time (seconds) after the start of heat exposure, T_t is the temperature of the basal epidermal layer at time t, T_s is the surface temperature of the skin during heat exposure, T_0 is the initial skin temperature (assumed constant 35°C throughout prior to heat exposure), erf is the error function, and γ is given by

$$\gamma = \frac{L}{2\sqrt{k/c_p\rho}}$$

where L, k, c_p, and ρ are, respectively, the thickness of the epidermis and the thermal conductivity, heat capacity, and density of the skin. Henriques used $\gamma = 0.15\ \mathrm{s}^{1/2}$ in his calculations.

Having approximated the temperature of the basal epidermal layer as a function of time, Henriques assumed that the burn injury process follows the kinetics of irreversible unimolecular reactions constant and obtained the following expression:

$$\Omega = P\int_0^t \exp\left(\frac{-\Delta E}{RT}\right)\,\mathrm{d}t$$

where Ω is a burn injury function indicative of the extent of burn injury. He arbitrarily set $\Omega = 1$ for

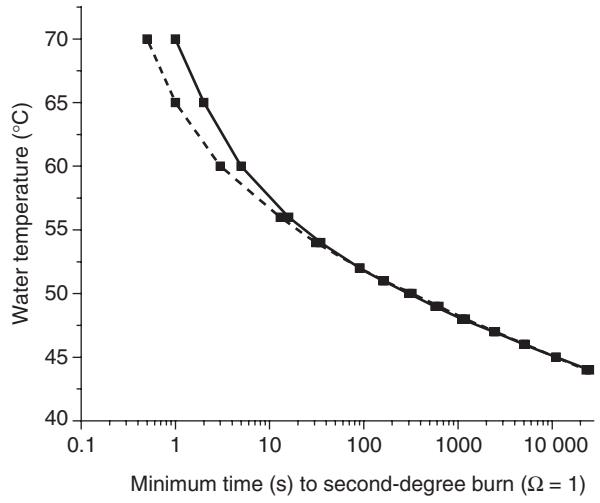

Figure 12 Henriques calculations (dashed line) vs. data (solid line) for minimal second-degree burn.

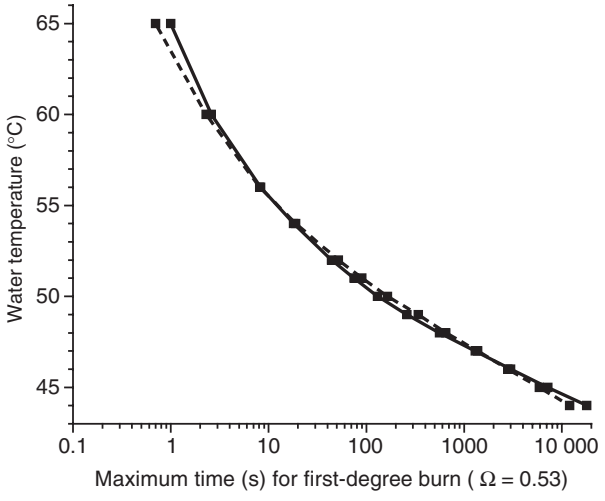

Figure 13 Henriques calculations (dashed line) vs. data (solid line) for maximal first-degree burn.

minimal second-degree injury (burn injury corresponding to the shortest time yielding a second-degree burn at a given temperature) and determined the values of the constants $\Delta E = 150\,000\,\text{cal mol}^{-1}$ ($\Delta E/R = 75\,000\,\text{K}$) and $P = 3.1 \times 10^{98}\,\text{s}^{-1}$ by fitting his equations to the burn injury data. Graphs showing Henriques' calculations along with Moritz and Henriques' data are shown in **Figures 12** and **13**.

Hazard Level

The data shown in **Figures 12** and **13** are based on measurements made on the backs of adult males having epidermal thickness of $\sim 80\,\mu\text{m}$. The time to produce burns can be significantly shorter for areas having thinner epidermis (e.g., eyelids), and for children and older adults. To prevent burns in at-risk groups, the American Burn Association recommends setting water heaters to 120°F and taking measures to prevent children and other at-risk populations from coming in contact with heat sources such as hot drinks, cooking surfaces, and hot appliances.

Light Toxicity

Introduction

This article reviews direct and indirect (e.g., afterimage, flash blindness) light hazards from common incoherent light sources. For direct hazards specific to lasers and other specialized coherent sources, the reader is referred to organizations such as the Laser Institute of America and the International Electrotechnical Commission.

Light is a form of electromagnetic radiation that is distinguished from other regions of the electromagnetic spectrum, such as radio waves, microwaves, X-rays, by its wavelength. From the standpoint of hazard, light is usually divided into three regions: ultraviolet (UV), visible, and infrared (IR). UV light ranges in wavelength from ~ 400 to 100 nm and is hazardous due to photochemical action. (A nanometer or nm is 1 billionth of a meter.) The most common source of UV is the sun. However, tanning lamps, black lights, and very hot objects such as welding torches can emit a significant amount of UV radiation. Visible light ranges in wavelength from ~ 700 to 400 nm. IR light ranges in wavelength from ~ 700 nm to 1 mm and is perceived by the body as heat. Common IR emitters include hot objects, heat lamps, light-emitting diodes in remote controls and other electronic equipment, and the sun. The hazards associated with light are reviewed by a number of international and national bodies, including the International Commission on Non-Ionizing Radiation Protection (ICNIRP), the International Electrotechnical Commission, the American Conference of Governmental Industrial Hygienists (ACGIH), the American National Standards Institute, and the National Radiological Protection Board.

Mechanism of Injury (UV Radiation)

Of the three regions of light discussed here, UV is most often associated with hazards. UV light primarily affects the skin and eyes. Sunburn (erythematic), premature skin aging, and skin cancer, are the best known effects of UV light; however, the skin incorporates part of the immune system, and UV exposure can accordingly decrease immune response to skin cancer, infectious agents, and other antigens. UV light can damage external ocular tissues including the cornea, iris, and conjunctiva, resulting in photokeratitis

Table 4 ICNIRP/ACGIH recommended limiting exposure limits

Exposure per day	*Effective irradiance E_{eff} ($W m^{-2}$)*
8 h	0.001
4 h	0.002
2 h	0.004
1 h	0.008
30 min	0.017
15 min	0.033
10 min	0.05
5 min	0.1
1 min	0.5
30 s	1
10 s	3
1 s	30
0.5 s	60
0.1 s	300

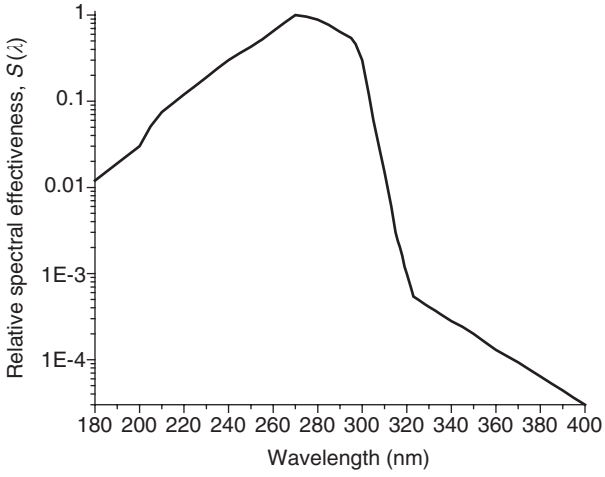

Figure 14 Relative spectral effectiveness for UV exposure.

or photokeratoconjunctivitis (more commonly known as snow blindness or welder's flash). UV light that penetrates through the cornea can result in opacities of the lens (cataracts) as well as retinal damage if not absorbed in the aqueous or vitreous humor. While it is difficult to quantify the extent of UV-related health problems, the World Health Organization believes solar UV exposure to be a significant contributor to the 2–3 million nonmelanoma skin cancers and 130 000 melanoma skin cancers occurring globally each year.

The nature and extent of UV toxicity is both wavelength and intensity dependent. In order to simplify wavelength dependence, the UV is divided into three sub regions: UV-A (315–400 nm), UV-B (280–315 nm), and UV-C (100–280 nm). ICNIRP and ACGIH recommend that skin and eye exposure to UV-A radiation be limited to $10\,W\,m^{-2}$ for periods exceeding 1000 s, and $10\,kJ\,m^{-2}$ for periods less than 1000 s. For UV-B and UV-C, recommended exposures are given in **Table 4**. The effective irradiance E_{eff} is calculated as

$$E_{eff} = \sum E_\lambda \cdot S_\lambda \cdot \Delta_\lambda$$

where E_λ is spectral irradiance ($W\,m^{-2}\,nm^{-1}$) and S_λ is the relative spectral irradiance given in **Figure 14**.

The exposure limits in **Table 4** are meant to be absolute limits for the eye and advisory limits for skin. They are based on consideration of the Caucasian population, which has the greatest UV sensitivity. These limits do not apply to at-risk populations such as highly photosensitive adults, aphakic individuals, young children, or persons exposed to photosensitizing agents. A large number of agents can cause hypersensitivity to UV radiation including antibiotics such as tetracycline and sulfathiazole, antidepressants such as imipramine and sinequan, and some antipsychotic drugs, diuretics, dyes, cosmetics, and coal tar products. Further, intense UV light produced by lasers must be considered differently from more common incoherent light sources.

Mechanism of Injury (Visible Light)

Visible light is most commonly associated with damage to the eye. However, skin injury is possible, particularly in the presence of endogenous (e.g., bilirubin) or exogenous (e.g., phenothizine) photosensitizers. The ocular tissue most susceptible to visible light injury is the retina. (In the absence of cataracts, ocular structures anterior to the retina transmit visible light.) Visible-light-induced eye injury can occur through either a photochemical or a thermal mechanism. The photochemical mechanism is commonly known as 'blue light' hazard. The relative hazard level as a function of wavelength is known as the blue light hazard function, $B(\lambda)$, and is shown in **Figure 15**. The peak in this function at 440 nm is due, in large part, to absorption of shorter wavelength radiation by the lens and cornea. In the case of aphakic individuals, $\sim$80% of UV-A radiation reaches the retina. For these individuals and children under 2, whose lenses have enhanced UV transmission, the aphakic hazard function shown in **Figure 16** is more appropriate.

In order to assess blue light hazard, ICNIRP and ACGIH recommend calculating an average source radiance, L_B

$$L_B = \sum L_\lambda \cdot B(\lambda) \cdot \Delta\lambda$$

where L_λ is the spectral radiance in the wavelength interval $\Delta\lambda$, and $B(\lambda)$ is the blue light hazard function

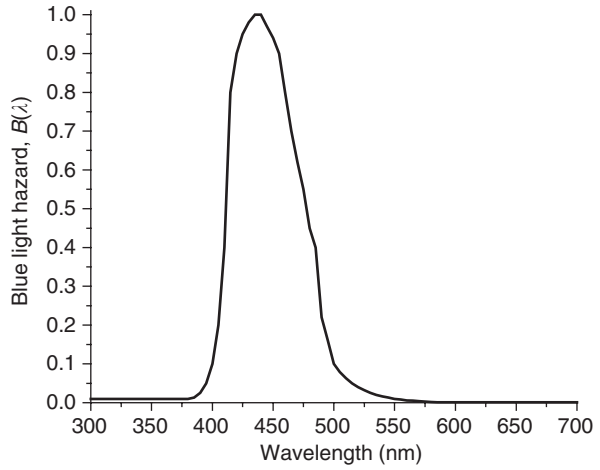

Figure 15 Blue light hazard function.

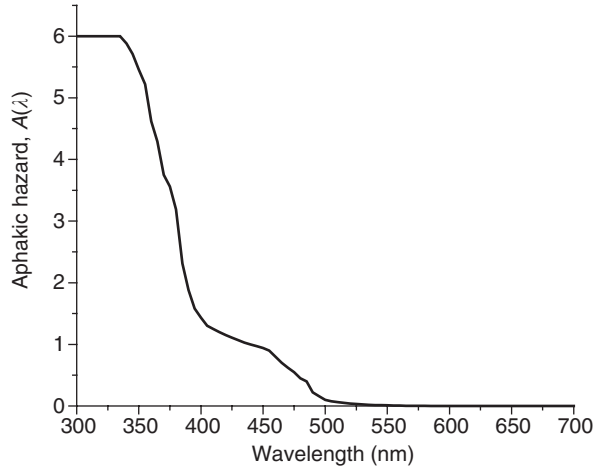

Figure 16 Aphakic hazard function.

shown in **Figure 15**. (The aphakic hazard function should be used for aphakic individuals and small children.)

For blue light and other photochemical hazards, the threshold dose is a product of dose rate and time. That is, injury can result from a short exposure to a very bright light or a longer exposure to a less intense light.

For exposures less than 10 000 s, the recommended exposure limit is

$$L_B \cdot t \leqslant 1 \text{ MJ m}^{-2} \text{ sr}^{-1}$$

(megajoule per square meter per steradian)

and for longer exposures

$$L_B \leqslant 100 \text{ W m}^{-2} \text{ sr}^{-1}$$

In addition to direct tissue damage, visible light can create indirect hazard through effects such as glare, flash blindness, and afterimage. The extent of this hazard depends not only on the qualities of the light source, but also on ambient light conditions and the nature of the activities in which individuals may be engaged. Perhaps the most stringent regulations concerning indirect light hazard are those developed to protect civilian pilots conducting terminal operations from hazards such as laser pointers. Here four flight safety exposure limits have been established: laser free zone ($0.0005\,\text{W m}^{-2}$), critical flight zone ($0.05\,\text{W m}^{-2}$), sensitive flight zone ($1\,\text{W m}^{-2}$), and normal flight zone ($25\,\text{W m}^{-2}$). (By contrast, normal direct terrestrial visible solar irradiance is in the range of several hundred watts per meter square.) Indirect light hazards from artificial light sources have been observed in other environments as well, with increasingly powerful light emitting diodes becoming a particular concern.

Mechanism of Injury (IR Light)

The most common effects to skin and ocular tissue associated with IR light are: (1) thermal injury to the retina; (2) thermal injury to the lens; (3) thermal burns to skin; and (4) thermal burns to cornea (1400 nm–1 mm).

Unlike photochemical injury, thermal injury does not exhibit reciprocity between intensity and length of exposure. Injury only occurs if the light intensity is sufficient to raise tissue temperature above $\sim 45^{\circ}\text{C}$. In the case of less intense light and longer exposure, normal heat transfer mechanisms within the body serve to cool the exposed tissue.

In the case of exposure to sources such as welding torches and arc welding equipment producing both IR and visible light, a small temperature rise caused by IR can work synergistically to increase blue light damage.

For sources that include visible light (wavelengths between 380 and 1400 nm) and viewing times between 10 μs and 10 s, ICNIRP and ACGIH recommend a maximum weighted source radiance of

$$\sum_{380}^{1400} L_\lambda \cdot R(\lambda) \cdot \Delta\lambda < 50/(\alpha \cdot t^{0.25}) \text{ kW m}^{-2} \text{ sr}^{-1}$$

where α is the angle subtended by the light source at the viewing distance, t is the viewing time, and $R(\lambda)$ is the retinal thermal hazard function shown in **Figure 17**. For times greater than 10 s, the 10 s value is used. For sources such as heat lamps that produce little visual light and therefore do not trigger pupillary contraction, ICNIRP recommends limiting the weighted radiance for exposures greater than 10 s to

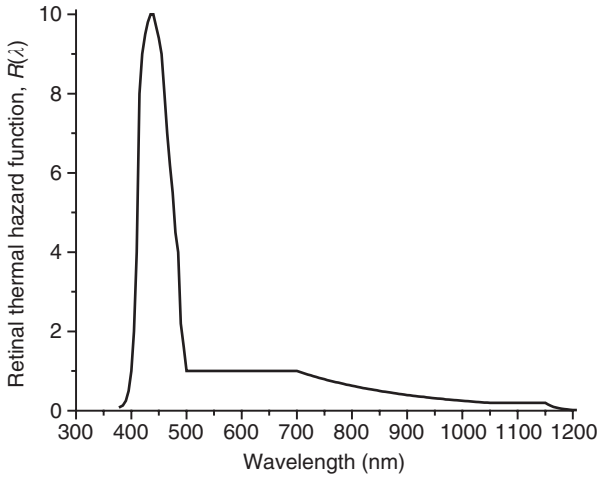

Figure 17 Retinal thermal hazard function.

$$\sum_{780}^{1400} L_\lambda \cdot R(\lambda) \cdot \Delta\lambda \leqslant 6000/\alpha \text{ W m}^{-2} \text{ sr}^{-1}$$

For protection of the cornea and lens, ICNIRP recommends limiting exposure to IR radiation (between 780 nm and 3 μm) to irradiances of less than $100\,\text{W m}^{-2}$ for long exposures (greater than 1000 s). For shorter exposures, ICNIRP recommends limiting irradiances given by

$$E_{\text{IR}} \leqslant 18t^{-3/4}(\text{kW m}^{-2})$$

where t is the exposure time in seconds. ICNIRP does allow relaxation of this limit in cold environments.

For short duration (less than 10 s) thermal injury to the skin, ICNIRP recommends limiting visible and IR exposure to below

$$20\,000t^{0.25} \text{ J m}^{-2}$$

Noise-Induced Hearing Loss

At-Risk Populations

Noise-induced hearing loss is a problem of epidemic proportion in modern society, and is currently the second most common form of hearing impairment in the United States (after age-related hearing loss). Although it is difficult to accurately assess the extent of the problem, the US Centers for Disease Control and Prevention has estimated that 12.5% of US children aged 6–19 years have some form of noise-induced hearing loss in one or both ears, and a 1990 Consensus Statement issued by the US National Institutes of Health estimated that over one-third of the 28 million people in the United States suffering from hearing impairment could attribute this problem at least in part to noise.

Sound Measurement

From the standpoint of sound toxicity, the most important properties of sound are power (or loudness) and frequency (or pitch). Sound power is usually expressed in terms of a logarithmic scale known as the decibel scale and is given by

$$L = 10 \log \frac{W}{W_0} \text{ dB}$$

where L is the sound power level in decibels (dB), W is the intensity of the sound in W m^{-2} and W_0 is a reference level equal to $10^{-12}\,\text{W m}^{-2}$ (approximately the softest audible sound). This somewhat unusual scale was chosen to accommodate human loudness perception. Increasing sound intensity by 10 dB is perceived as an approximate doubling in loudness even though the sound power is actually increased by a factor of 10.

Sound frequency is usually expressed in units of cycles per second or hertz (Hz). Humans have a useful hearing range of ~20–20 000 Hz, but are most sensitive to frequencies between about 1000 and 6000 Hz. (For reference, the lowest and highest notes on the piano are 27.5 and 4186 Hz, respectively.) This increased sensitivity is due in part to the shape of the external portion of the ear and the ear canal, which serve to amplify frequencies in this range.

Human loudness perception depends in a complex manner on both frequency and the overall loudness of sound. (For example, bass is more difficult to hear in music played at low volume than in the same music played at high volume.) To capture this behavior, two weighting scales have been developed for use in sound hazard analysis. The most common of these is the A weighting scale, which is commonly used to assess occupational and environmental noise. The A scale weights sounds in the 1000–6000 Hz range much more heavily than low-frequency sounds. The A-weighted intensities (dBA) of some common sounds are listed in **Table 5**. By contrast, the C weighting scale is used for very loud sounds and is a much flatter function of frequency.

Mechanism of Injury

The human hearing apparatus is commonly considered in three sections: the outer ear, middle, and inner ear. The outer ear consists of the pinna (generally called the ear) and the external auditory canal, which terminates in the tympanic membrane or eardrum. The outer ear collects sound, amplifying some frequencies and attenuating others. The eardrum

Table 5 Intensity and response for some common sounds

Sound	*Intensity (dBA)*	*Response*
	0	Threshold of hearing
Normal breathing	10	
Rustling leaves	20	
Soft whisper or ticking clock	30	
Quiet street at night	40	Quiet
Quite office	50	
Normal conversation	60	
Vacuum cleaner	70	Moderately loud
Loud speech or radio	80	
Heavy truck (50 ft)	90	
Pile driver (50 ft), ambulance siren (100 ft)	100	Very loud
Loud thunder	110	
Jet takeoff (200 ft), rock concert	120	Threshold of feeling and pain
Machine gun at close range	130	Painful
Aircraft carrier deck operations	140	

Table 6 Exposure times recommended by NIOSH and CDC

Noise level (dBA)	*Recommended permissible exposure time*
85	8 h
88	4 h
91	2 h
94	1 hr
97	30 min
100	15 min
103	$7\frac{1}{2}$ min
106	$3\frac{3}{4}$ min

transfers vibration to three small bones in the middle ear known as the ossicles, which in turn transfer vibration to the inner ear. The inner ear contains a helical organ called the cochlea in which sound vibrations are converted into nerve impulses by a series of small cells known as hair cells. It is the hair cells that are damaged by sound. Noise-induced hearing loss is usually divided into three classes. Noise-induced temporary threshold shift is a reversible loss of sensitivity over a range of frequencies. Noise-induced permanent threshold shift has a similar manifestation but is permanent. Both types of threshold shift generally result from relatively long exposure to loud noise. By contrast, acoustic trauma is hearing impairment associated with short-term exposure at extremely high levels.

Hazard Level

Numerous international standards have been developed to regulate noise exposure. Permissible exposure times recommended by the US National Institute for Occupational Safety and Health and the US Centers for Disease Control and Prevention are shown in **Table 6**.

The halving of recommended permissible exposure time with each 3 dBA increase in noise level reflects the doubling of sound power with each 3 dB increment.

See also: American Conference of Governmental Industrial Hygienists; American Industrial Hygiene Association; Consumer Product Safety Commission; National Institute for Occupational Safety and Health; Occupational Exposure Limits; Occupational Safety and Health Administration.

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http://www.icphso.org – International Consumer Product Health and Safety Organization (ICPHSO).
http://www.cpsc.gov – US Consumer Product Safety Commission (CPSC).
http://www.census.gov – US Department of Commerce, Bureau of the Census, Statistical Abstract of the United States 1998.
http://www.nsc.org – US National Safety Council (NSC).

Picloram

Richard A Parent

This article is a revision of the previous print edition article by Richard A Parent, T R Kline, and R E Sharp, volume 2, pp. 530–531, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 1918-02-1
- Synonyms: 4-Amino-3,5,6-trichloro-2-pyridinecarboxylic acid; 3,5,6-trichloro-4-aminopicolinic acid; 4-amino-3,5,6-trichloro-2-picolinic acid
- Chemical/Pharmaceutical/Other Class: Chlorinated amino-pyridine herbicide
- Chemical Formula: $C_6H_3C_{13}N_2O_2$

Uses

Picloram and its salts are systemic herbicides produced by chlorination of 2-methylpyridine followed by hydrolysis and reaction with ammonia. Most broadleaf crops, except crucifers, are sensitive; most grasses are resistant. Picloram is effective in controlling annual weeds, is used alone or in combination with 2,4-D against deep-rooted perennials on noncropland, and is used typically as pellets or in combination with 2,4-D or 2,4,5-T for brush control.

Exposure Routes and Pathways

Picloram is either a colorless powder or crystalline solid having very low vapor pressure, making inhalation exposure unlikely unless the dust is inhaled. Exposure to picloram occurs mainly through its manufacture and its use as a herbicide in forests. Environmental exposures in humans occur when forest visitors or others not directly involved in spray operations come in contact with spray or sprayed foliage, inhale spray mist, eat plants or animals contaminated with the herbicide, or drink water containing the herbicide. A suggested no-adverse-effect level is $1.05\,mg\,l^{-1}$.

Toxicokinetics

Picloram is readily translocated from foliage or from roots to other plant parts, accumulating primarily in the areas of most rapid growth. Picloram is rapidly absorbed from the gastrointestinal tract and is excreted virtually unchanged in the urine and feces of male Fischer 344 rats within 48 h. The fate of picloram was defined in six healthy male volunteers following single po doses of 5.0 and $0.5\,mg\,kg^{-1}$ and a dermal dose of $2.0\,mg\,kg^{-1}$. Picloram was administered orally as the sodium salt in grape juice. The dermal dose was applied to the backs of volunteers as the free acid dissolved in ethanol. The resulting data indicated that the compound was rapidly absorbed from the gastrointestinal tract and rapidly excreted unchanged in the urine. Over 90% of the oral dose was recovered as unchanged picloram in the urine excreted through 72 h. Most of the dose ($\geqslant$75%) was excreted within 6 h. By comparison, picloram was slowly absorbed through the skin, and only a small fraction (0.2%) of the picloram applied to the skin was absorbed.

Picloram is not readily metabolized and is rapidly excreted unchanged in the urine and feces of treated rats. Following a $10\,mg\,kg^{-1}$ [^{14}C]picloram intravenous dose, the isotope was cleared biophysically and excreted in the urine. Balance studies in rats indicated that 98.4% of the dose was recovered. Urinary excretion resulted in an 80–84% recovery, fecal excretion resulted in $\sim$ 15% recovery; less than 0.5% was recovered in the bile, and virtually no radioactivity was recovered as trapped $^{14}CO_2$ or as other volatile compounds. Studies with [^{14}C]picloram showed that 90% of the compound fed in the diet to dogs was excreted within 48 h in the urine, with small amounts appearing in the feces.

Mechanism of Toxicity

Little is known about the mechanism of toxicity of picloram.

Acute and Short-Term Toxicity (or Exposure)

Animal

Picloram is a relatively nontoxic pesticide. Rat LD_{50} (oral) is given as $8200\,mg\,kg^{-1}$. Other reports of oral LD_{50} values include $500\,mg\,kg^{-1}$ in rats, 200–$4000\,mg\,kg^{-1}$ in mice, and $\sim 2000\,mg\,kg^{-1}$ in rabbits. The reported dermal LD_{50} in rabbits is $\sim 4000\,mg\,kg^{-1}$. The technical grade of picloram is moderately toxic by inhalation with a reported 4 h LC_{50} of greater than $0.35\,mg\,l^{-1}$.

Signs of intoxication from acute oral administration may include skin rashes, eye irritation, hair loss, tachycardia, diarrhea, ataxia, leukopenia, vaginal bleeding, prostration, and, in cases of very high exposure, seizures. Liver and kidney lesions have also been reported.

Human

Picloram causes a mild skin irritation, although it is not a skin sensitizer in humans. Picloram is not likely to be absorbed readily through the skin. Contact with exposed eyes causes moderate irritation which heals readily; corneal injury is unlikely. Inhalation of contaminated dusts may be somewhat irritating but is not likely to cause illness. Possible nausea may result from ingestion of massive amounts. Some believe that picloram may have the ability to damage the central nervous system.

Chronic Toxicity (or Exposure)

Animal

Picloram administered orally at three dose levels (20, 200, and $2000\,mg\,kg^{-1}$ body weight) induced no cytogenic aberrations in bone marrow cells. B6C3F1 mice and Osborne–Mendel rats were fed picloram for 80 weeks. After treatment, mice were observed for 10 weeks. Upon death or sacrifice, major organs were examined. In rats there was a high incidence of follicular hyperplasia, C cell hyperplasia, and C cell adenoma of the thyroid. There was an increased incidence of hepatic neoplastic nodules considered to be benign in female and male rats. Both male and female rats showed lesions of the liver diagnosed as foci of cellular alteration. It was concluded that picloram was not carcinogenic in mice or male rats but did possess the ability to induce benign tumors in the livers of female Osborne–Mendel rats.

Lifetime daily exposure of rats and dogs to diets containing $150\,mg\,kg^{-1}$ body weight doses of picloram resulted in no observable gross or microscopic signs of toxicity. A 6 month dog study at doses as high as $175\,mg\,kg^{-1}\,day^{-1}$ did result in weight loss, increased relative and absolute liver weights with a calculated NOEL of $7\,mg\,kg^{-1}\,day^{-1}$. A 90 day feeding study in B6C3F1 mice also produced increases in absolute and relative liver weights in female mice at doses starting at $1000\,mg\,kg^{-1}\,day^{-1}$.

Human

Because of a lack of information for humans and animals, picloram is not classifiable with regard to its carcinogenicity in humans according to the IARC and the ACGIH. Little or no data is available relating to the chronic toxicity of picloram.

In Vitro Toxicity Data

Picloram was not mutagenic in gene mutation assays in bacteria and yeast, with or without metabolic activation. Using the forward mutation spot test picloram was mutagenic in *Stertomyces coelicolor*, which is not a widely accepted screen for mutagens.

Clinical Management

Emesis is not recommended after oral ingestion of picloram because of potential for seizures. Gastric lavage may be considered but a patent airway must be maintained. Activated charcoal may be administered but treatment should be supportive of symptomatology. For seizures, benzodiazepine should be considered with subsequent phenobarbital if seizures persist. Hypotension, dysrhythmias, respiratory depression, and need for endotracheal intubation should be monitored. Evaluation must be done for hypoglycemia, electrolyte disturbances, and hypoxia. If inhaled, patient should be removed to clean air and respiratory distress should be monitored. If cough or difficulty breathing occurs, evaluation for respiratory tract irritation, bronchitis, or pneumonitis should be done. Bronchospasm should be treated with inhaled β-2 agonist and oral or parenteral corticosteroids. For dermal contact, contaminated clothing should be removed and the skin washed with soapy water.

Environmental Fate

Picloram is a herbicide and is introduced directly into the earth. It does not absorb on soil, and does not hydrolyze or evaporate from soils or groundwater. It is subject to leaching and may biodegrade in soils and groundwater. In groundwater, it is not expected to adsorb on sediment, to bioconcentrate, to evaporate or hydrolyze significantly. Near surface photolysis is possible and as a result its half-life ranges from 2.3 to 41.3 days. Since it is an amine, its degradation could be accelerated through contact with oxidizing agents.

Release to the atmosphere would result in significant deposition and washout due its low vapor pressure. In soil, the half-life of picloram could exceed 5 years depending on the conditions.

Other Hazards

No teratogenic or embryotoxic effects have been found in rats fed up to 1000 mg kg^{-1} on gestational days 6–15. Similar findings were noted in rabbits. A multigeneration study in which rats were exposed to picloram from gestation through reproductive cycles to levels as high as 3000 ppm diet produced no evidence of effects on fertility, gestation, viability of pups, lactation, or skeletal development. Pregnant rats receiving doses of 1000 mg kg^{-1} day^{-1} during organogenesis were normal, but there was a slight increase in embryo resorption. A dose of 2000 mg kg^{-1} day^{-1} was toxic to the mothers but did not induce malformations in the pups. A negative response to an effort to induce embryotoxic and teratogenic effects in New Zealand white rabbits at doses as high as 400 mg kg^{-1} day^{-1} also failed to provide any indication of a dose–response relationship to the sporadic findings.

Multigeneration studies in rats dosed orally at 15 mg kg^{-1} day^{-1} did not have any effect on fertility, whereas rats showed no effects when dosed up to 180 mg kg^{-1} day^{-1}. Picloram does not appear to cause reproductive toxicity.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV) 8 h time-weighted average (TWA) is 10 mg m^{-3}.

ACGIH exclusion limit for 30 min day^{-1}: 30 mg m^{-3}.

Environmental Protection Agency's (EPA's) permissible exposure limit (PEL) is 5 mg m^{-3} for the respirable fraction (8 h TWA).

ACGIH cannot classify picloram relative to human carcinogenicity.

EPA's Federal Drinking Water Standard is 500 μg l^{-1} while Arizona has a standard of 49 μg l^{-1}.

EPA's maximum contaminant level goal (MCLG) has been set at 0.5 ppm.

Picloram is a slightly toxic compound in EPA toxicity class III and products containing it must bear the signal word CAUTION on the label.

Miscellaneous

Picloram has a molecular weight of 241.48 and is a white crystalline solid at room temperature. It has a chlorine-like odor but has a very low vapor pressure (6.16×10^{-7} mmHg) at 35°C. It has some solubility in water (450 mg l^{-1}) but is a lot less soluble in nonpolar solvents such as benzene or ether.

See also: Pesticides; Pollution, Water.

Relevant Websites

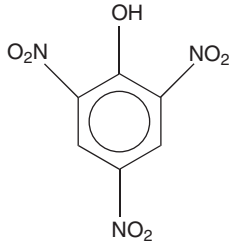
http://www.epa.gov – US Environmental Protection Agency.
http://ace.orst.edu – National Pesticide Information Center, Oregon State University, Corvallis, OR, USA.
http://infoventures.com – Information Ventures, Inc., Philadelphia, PA, USA.

Picric Acid

Samantha E Gad

This article is a revision of the previous print edition article by Jayne E Ash, volume 2, pp. 531–532, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 88-89-1
- Synonyms: 2,4,6-Trinitrophenol; Piconitric acid; Carbazotic acid; Nitroxanthic acid
- Chemical/Pharmaceutical/Other Class: Nitrogen-substituted phenols, a trinitro phenol
- Chemical Formula: $C_6H_3N_3O_7$
- Chemical Structure:



Uses

Picric acid is used in the production of explosives, matches, and electric batteries. It is also used in

etching copper and manufacturing colored glass, in the leather industry, and in the synthesis of dyes. Picric acid is very unstable and is a flammmable/combustible material. It may be ignited by heat, sparks, or flames. Dried-out picric acid may explode if expose to heat, flame, friction, or shock, and should be treated as an explosive. Picric acid can react vigorously with oxidizing materials, and it can form unstable salts with concrete, ammonia, bases, and metals.

Exposure Routes and Pathways

The most likely exposure to picric acid is in the workplace from use in explosives, matches, electric batteries, from etching copper, and making colored glass. These activities could lead to dermal contact or the inhalation of dust of picric acid or its salts.

Toxicokinetics

Picric acid is readily absorbed through the skin or through the respiratory tract. It is eliminated from humans as picric acid and as picramic acid.

Mechanism of Toxicity

Picric acid is an uncoupler of mitochondrial metabolism.

Acute and Short-Term Toxicity (or Exposure)

Animal

In animals, picric acid is a dermal sensitizer and strong eye irritant. The oral LD_{50} in rats is $200\,mg\,kg^{-1}$, and the oral LD_{Lo} is $500\,mg\,kg^{-1}$ in cats, $120\,mg\,kg^{-1}$ in rabbits, and $100\,mg\,kg^{-1}$ in guinea pigs. Dogs receiving an acute lethal dose of picric acid die from respiratory paralysis, and necropsy found yellow staining of the subcutaneous fat, lung, intestines, and blood vessels. Liver swelling and glomerulitis were also observed. Sublethal doses in dogs, $\leqslant 50\,mg\,kg^{-1}$, caused transient changes in the kidneys, including glomerulitis and other changes in ultrastructure.

Human

In occupational exposures, for example, the manufacture of explosives, the main health issue has been the occurrence of skin disease. Systemic poisoning is rare. Picric acid is irritating to eyes and skin. Dermal exposure may cause local or generalized allergic reactions. It causes yellow staining of skin. Absorption into the skin or ingestion may cause nausea, vomiting, diarrhea, abdominal pain, olguria, anuria, staining of skin, pruritus, sudden acne, stupor, convulsions, and death. The CDC revised the IDLH (documentation for Immediately Dangerous to Life or Health concentrations) after ingestion of 1–2 g caused severe poisoning in man. During the 1920s and 1930s, picric acid was used alone and in combination with butyl aminobenzoate as an antiseptic surgical dressing for the treatment of burns; however, this was reported to be capable of leading to serious central nervous system problems. An outbreak of hematuria among US Navy personnel based in Japan was attributed to picric acid in drinking water that had been contaminated by confiscated Japanese ammunition; $2–20\,mg\,l^{-1}$ picric acid were found in the drinking water.

Chronic Toxicity (or Exposure)

Animal

Picric acid also causes liver and kidney damage and produces central nervous system effects. It has been a mutagen in some, but not all, studies.

Human

Liver, kidney, and blood are affected by prolonged or repeated exposure. Hair, skin, and conjunctiva of eye may become yellow, with matching yellow vision (symptoms not from jaundice). Delayed cataract formation may occur, as well as intravascular hemolysis.

In Vitro Toxicity Data

Picric acid has shown mutagenic properties *in vitro* in some, but not all, studies.

Clinical Management

The victim should be removed from exposure. Gastric lavage with water should be performed. Activated charcoal is also recommended.

Ecotoxicology

Juvenile rainbow trout (*Salmo gairdneri*) and American oyster (*Crassostrea virginica*) were exposed to sublethal doses of picric acid for 42 days. No significant inhibition of growth was observed for rainbow trout exposed to 0.45 and $0.05\,mg\,l^{-1}$ picric acid. American oysters exposed to 0.45 and $0.05\,mg\,l^{-1}$ picric acid showed significant inhibition of shell deposition during exposure period. Discoloration of the nacre layer of the shell and body mass was observed in oysters by the end of 42 days.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value, as an 8 h time-weighted average (TWA), is 0.1 mg m^{-3}. The same value (0.1 mg m^{-3}) has also been recommended by the (US) Occupational Safety and Health Administration permissible exposure limit (PEL), 8 h TWA, with an added skin designation, and the (US) National Institute for Occupational Safety and Health (NIOSH) recommended exposure level (REL), averaged over a 10 h work day. The NIOSH short-term exposure limit (STEL), for a 15 min exposure, is 3 mg m^{-3}.

See also: Acids.

Further Reading

Gosselin RE, Smith RP, and Hodge HC (1984) *Clinical Toxicology of Commercial Products*, 5th edn. Baltimore: Williams and Wilkins.

Nipper M, Carr RS, Biedenbach JM, *et al.* (2001) Development of marine toxicity data for ordinance compounds. *Archives of Environmental Contamination and Toxicology* 41: 308–318.

Nipper M, Carr RS, Biedenbach JM, Hooten RL, and Miller K (2002) Toxicological and chemical assessment of ordinance compounds in marine sediments and porewaters. *Marine Pollution Bulletin* 44: 789–806.

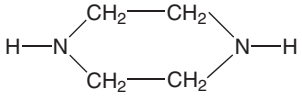
Relevant Website

http://www.cdc.gov – 2,4,6-Trinitrophenol (International Chemical Safety Card).

Piperazine

David Brandwene

- Chemical Abstracts Service Registry Number: CAS 110-85-0
- Synonyms: 1,4-Diazacyclohexane; 1,4-Piperazine; Diethylenediamine; Hexahydropyrazine; Piperazidine; Pyrazine hexahydride
- Chemical/Pharmaceutical/Other Class: Ethyleneamines
- Chemical Formula: $C_4H_{10}N_2$
- Chemical Structure:



Uses

Piperazine is an intermediate in the manufacture of insecticides, rubber chemicals, corrosion inhibitors, and urethane foam production catalysts. It is also used in gas-washer liquids to absorb carbon dioxide and as a hardener in prepolymers for adhesives. Piperazine is an intermediate in the production of human and veterinary pharmaceuticals and is also an active ingredient in veterinary antihelminthics. It is prescribed as a human antihelminthic in the United States.

Exposure Routes and Pathways

Occupational exposure may occur in industries where piperazine is manufactured or used as an intermediate. Routes of occupational exposure are primarily dermal and inhalation.

Consumer exposure from the diet may occur from residues present in eggs of treated hens.

Toxicokinetics

Animal studies show that piperazine is readily absorbed from the gastrointestinal tract, excreted primarily in the urine with the peak plasma concentration reported 1 h after dosing. Most of the parent compound is excreted unchanged during the first 48 h. *N*-Mononitrosopiperazine has been identified as the primary urinary metabolite. Limited human data indicate a similar toxicokinetic profile to animals. There are no data available on the toxicokinetics of piperazine following dermal or inhalation exposure.

Mechanism of Toxicity

The mechanism of the neurotoxicity induced by piperazine in mammals is unknown. Piperazine acts as a GABA agonist in invertebrates. The mechanism of liver and kidney toxicity seen in subchronic oral studies in laboratory animals has not been determined.

Acute and Short-Term Toxicity (or Exposure)

Piperazine is corrosive to skin and eyes and causes skin and respiratory sensitization. Piperazine has not been shown to be teratogenic or mutagenic.

Animal

Oral LD_{50} values in rodents range from 2.4 to 4.9 g kg^{-1}. The dermal LD_{50} in rabbits was reported

to be $4\,g\,kg^{-1}$. In skin irritation studies in rabbits, a 50% concentration of piperazine was corrosive following a 4 h exposure period. In an eye irritation study in rabbits, a 1–5% concentration of piperazine was corrosive.

Piperazine was evaluated in the local lymph node assay (LLNA) and the guinea pig maximization test. In both studies, piperazine was a mild sensitizer. In the LLNA, piperazine did not induce markers indicative of respiratory sensitization. In laboratory studies, piperazine has exhibited cross-sensitization reactions with diethylenetriamine.

Piperazine phosphate at dose levels of 250, 1000, or $5000\,mg\,kg^{-1}$ was orally administered to pregnant rats during days 6–15 of gestation. There were no teratogenic effects in any dose group. In a developmental toxicity study in rabbits, piperazine phosphate was orally administered during days 6–18 of pregnancy at dose levels of 100, 225, or $500\,mg\,kg^{-1}$. An increased incidence in embryotoxicity and malformations were seen at the highest dose. These effects were considered to be secondary to maternal toxicity.

In a micronucleus study, administration of piperazine phosphate orally to mice at doses up to $5000\,mg\,kg^{-1}$ did not result in an increase in the level of micronuclei in bone marrow erythrocytes.

Human

Transient side effects from therapeutic use of piperazine via the oral route include headaches, nausea, vomiting, lethargy, tremor, and vague ocular disturbances. EEG changes were observed in a study of children treated with piperazine hexahydrate. Although the mechanism of toxicity for EEG changes and several other case reports of neurotoxicity is unknown, it may be related to GABA agonism. This conclusion is based on laboratory data in invertebrates.

Piperazine causes primary dermal irritation and skin burns at high concentrations. Piperazine also causes eye irritation in humans.

Many case studies have shown that exposure to piperazine results in allergic contact dermatitis and occupational asthma. A recent study of 93 patients exposed dermally to a 1% piperazine solution showed 3.2% positive reactions. At a piperazine production facility, ~10% of the current and former factory workers were diagnosed with occupational asthma.

Chronic Toxicity (or Exposure)

In repeated dose studies, systemic toxicity was seen at dose levels of piperazine above $50\,mg\,kg^{-1}$. The results of a reproductive toxicity study suggest that piperazine can impair fertility.

Animal

In a 13 week oral study, piperazine dihydrochloride was administered to dogs (four per sex per dose) at concentrations up to 3692 ppm in the diet. Clinical chemistry changes indicative of mild liver effects were the only sign of systemic toxicity. The no-observed-adverse-effect level in the study was 1477 ppm, which is $\sim 25\,mg\,kg^{-1}$ of piperazine base. In another 13 week oral study, piperazine was administered to rats (10 per sex per dose) at concentrations of ~50, 150, or $500\,mg\,kg^{-1}$ in the diet. Histopathological changes were seen in the liver and kidneys in the two higher dose groups. The no-effect level in the study was $50\,mg\,kg^{-1}$.

In a two generation reproductive toxicity study, piperazine dihydrochloride was administered in the diet of rats at doses of 250, 600, or 1250 mg $kg^{-1}\,day^{-1}$. A dose–response effect for decreased litter size was seen in the 600 and 1250 mg kg^{-1} groups suggesting that piperazine exposure at these dose levels can impair fertility.

Human

There are no reports of long-term repeated exposure to piperazine. Therapeutic uses of piperazine via the oral route for ~1 week have resulted in symptoms of neurotoxicity in children and adults.

In Vitro Toxicity Data

Piperazine was not mutagenic in the Ames assay and did not produce chromosome aberrations in Chinese hamster ovary cells.

Clinical Management

If exposure occurs, medical attention should be sought. In general, the following are recommended. For ingestion, water should be provided but emesis must not be induced. For skin and eye exposure, the affected area should be flushed with water for at least 15 min but no attempts must be made to neutralize with chemical agents. For inhalation, the exposed individual should be removed to fresh air and artificial respiration given if necessary.

Environmental Fate

Piperazine is not readily biodegradable, does not rapidly hydrolyze, and has a low potential for bioaccumulation. The octanol–water partition coefficient is -1.24. Piperazine released to the environment would be expected to distribute primarily to soil and water.

Ecotoxicology

The acute 96 h LC_{50} in guppies and 72 h EC_{50} in algae are greater than 1000 ppm (relatively nontoxic). Piperazine is more hazardous to invertebrates than fish or algae. In *Daphnia magna*, the 48 h EC_{50} is 26 mg l^{-1} and adverse effects were seen in a 21 day study at concentrations of 25 mg l^{-1} and above with a no-adverse-effect level of 12.5 mg l^{-1}.

Exposure Standards and Guidelines

No US exposure standards or guidelines for piperazine were identified. The 8 h occupational exposure limit in Europe is 0.1 mg m^{-3}.

See also: Kidney; Liver; Neurotoxicity.

Further Reading

American Conference of Governmental Industrial Hygienists Inc. (1991) Piperazine dihydrochloride. In: *Documentation of the Threshold Limit Values and Biological Exposure Indices*, 6th edn., vol. 3, pp. 1276–1277.

EU Final Draft Risk Assessment on Piperazine, November 2003.

Trochimowicz HJ, *et al.* (1994) Heterocyclic and miscellaneous nitrogen compounds. In: Clayton GD and Clayton FE (eds.) *Patty's Industrial Hygiene and Toxicology*, 4th edn., vol. 2, Part E, pp. 3315–3319. New York: Wiley.

Relevant Websites

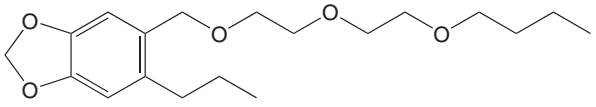
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Piperazine.

Piperonyl Butoxide

Marilyn Weber

This article is a revision of the previous print edition article by Sushmita M Chanda, volume 2, pp. 532–533, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 51-03-6
- Synonyms: Butacide; Butocide; Butoxide; Nusyn-noxfish; Prentox; Pybuthrin; Pyrenone; 5-[[2-(2-Butoxyetoxy) ethoxy]methyl]-6-propyl-1,3-benzodioxole
- Chemical/Pharmaceutical/Other Class: Methylenedioxyphenyl insecticide synergist
- Chemical Structure:



Uses

Piperonyl butoxide is a synergist for carbamates, pyrethrins, pyrethroids, and rotenone.

Exposure Routes and Pathways

Dermal exposure is the most common exposure pathway. Piperonyl butoxide is available as an aerosol, dust, emulsion, and solution.

Toxicokinetics

Piperonyl butoxyl inhibits detoxification of pesticides by insects. The synergists inhibit cytochrome P-450 dependent monooxygenases (cyp450 s), detoxifying enzymes found in both mammals and insects. These cyp450 s degrade selected foreign substances such as pyrethrum, allethrin, or resmethrin. Synergists simply bind the oxidative enzymes and prevent them from degrading the toxicant.

Mechanism of Toxicity

Piperonyl butoxide exerts toxicity by inhibiting mixed function oxidases. These enzymes are responsible for detoxifying pyrethrins and pyrethroids; their toxicity is therefore increased by piperonyl butoxide.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute toxicity in laboratory animals following exposure to piperonyl butoxide is low (the oral LD_{50} in rats is >6 g kg^{-1}). Repeated doses may also cause delayed onset of these same signs. A no-observed-adverse-effect level in dogs was 3 mg kg^{-1} day^{-1}.

Human

Piperonyl butoxide has a low incidence of acute toxicity. A single oral dose of piperonyl butoxide (50 mg, $\sim$0.71 mg kg^{-1} body weight) in adult volunteers did not elicit any signs of toxicity. A likely oral lethal dose

in humans was estimated at 5–15 g kg^{-1}, or between a pint and a quart for a 150 pound person. Laboratory findings in animal studies indicate that piperonyl butoxide may cause various anemias, hepatic changes, liver injury, and increased metabolic enzymes. No eye injuries have been reported with piperonyl butoxide/pyrethrin combination.

Chronic Toxicity (or Exposure)

Animal

Piperonyl butoxide was reported to increase liver tumor incidence at high exposure levels in mice but not rats. The primary target organ for chronic piperonyl butoxide exposures is the liver.

Human

Very little is known about the chronic effects of piperonyl butoxide. The US Environmental Protection Agency has categorized piperonyl butoxide as a group C carcinogen based on limited evidence of cancer in laboratory animals.

Clinical Management

Dermal decontamination should be accomplished by repeated washing with soap. Exposed eyes should be irrigated with copious amounts of water for at least 15 min. Piperonyl butoxide may be mixed with hydrocarbons; thus, emesis should be avoided. Activated charcoal can be administered following oral exposure. Treatment is symptomatic. No antidote is available.

Environmental Fate

Piperonyl butoxide is short-lived in the environment. It has a low to moderate potential for leaching into groundwater.

Ecotoxicology

Piperonyl butoxide has low to very low toxicity in birds. Researchers consider piperonyl butoxide moderately toxic to fish, although it is unlikely to accumulate. Some aquatic invertebrates may be highly sensitive to this compound.

Exposure Standards and Guidelines

The acute dermal and oral reference doses (RfDs) for piperonyl butoxide are 10 and 2 mg kg^{-1} day^{-1}, respectively. The chronic oral RfD is 0.0175 mg kg^{-1} day^{-1}.

See also: Pesticides; Pyrethrins/Pyrethroids.

Further Reading

Jones DG (1998) *Piperonyl Butoxide – The Insecticide Synergist*. San Diego, CA: Academic Press.

Moretto A (1995) Piperonyl butoxide. In: *Pesticide Residues in Food – 1995. Joint FAO/WHO Meeting on Pesticide Evaluations 1995; Part II – Toxicological and Environmental*, pp. 277–306. Geneva, Switzerland: International Programme on Chemical Safety, World Health Organization.

Osimitz TG and Breathnach R (2001) The safety assessment of piperonyl butoxide. In: Krieger R (ed.) *Handbook of Pesticide Toxicology*, pp. 1461–1480. San Diego, CA: Academic Press.

Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Piperonyl Butoxide.

Plants, Poisonous

Teresa Dodd-Butera and Molly Broderick

Edible and nonedible plants contain numerous naturally occurring chemical substances that can be toxic if exposures are excessive. Many of these chemicals act as natural 'pesticides' that help protect the plant from insects and other predators. As far as the edible plants are concerned, eating a varied diet containing fresh fruits and vegetables with beneficial properties usually avoids significant risks of poisoning by naturally occurring plant toxins.

The toxins selected for discussion in this section are found in plants that are not consumed as part of normal diets. The plants discussed are those most commonly identified in acute, systemic poisonings. These poisonings can be quite severe, although variability in the concentration of toxic chemicals may occur between and within plant species due to factors such as age and part of plant, season, and growth conditions. Historically, the use of plants has ranged from medicinal to homicidal purposes. The list of poisonous plants is extensive and varied, and can not be fully covered in this text. Human

exposures to many plants do not result in appreciable toxicity; however, some of the more notable toxic exposures will be mentioned.

Ackee (*Blighia sapida*)

Description

Ackee is a fruit-bearing tree distributed throughout Africa, the Caribbean, and tropical areas.

Uses

When ripe, the fruit is edible and a normal dietary constituent in some cultures.

Exposure Routes and Pathways

Ingestion of unripened fruit may result in poisoning.

Mechanism of Toxicity

The mechanism of toxicity is unclear; however, it contains the toxin hypoglycins A (β-methylene cyclopropyl-L-α-aminopropionic acid) and B (a dipeptide of hypoglycin A).

Symptoms of Toxicity

Symptoms of nausea, vomiting, and hypoglycemia may be delayed following ingestion. Liver abnormalities have occurred.

Clinical Management

Fatalities and seizures have been reported but can be avoided with good supportive measures. Glucose administration for hypoglycemia and symptomatic care are effective therapies for this toxin.

Apple Seeds (*Prunus* species)

Description

A perennial flowering herb, it comes in white, pink, and purple. Other examples include apricot and peach pits and choke cherry seeds.

Uses

Cyanogenic (cyanide producing) plants of the *Prunus* species are flowering and fruit trees. Seeds and pits are commonly ingested inadvertently along with the edible fruit.

Exposure Routes and Pathways

Poisoning may occur from ingestion of crushed seeds and pits. In addition, leaves and bark may be toxic. The toxin of this species is amygdalin.

Mechanism of Toxicity

The toxin, amygdalin, releases hydrocyanic acid. Thus, toxicity is similar to cyanide, and is dose-related. Cytochrome oxidase inhibition interrupts electron transport and oxygenation at the cellular level.

Symptoms of Toxicity

Symptoms may be delayed following ingestion and include headache, dizziness, coma, seizures, and potential death.

Clinical Management

Treatment involves the use of activated charcoal in order to prevent amygdalin metabolism. In addition, there is a cyanide antidote kit available.

Castor Bean (*Ricinus communis*)

Description

A large, leafy green plant which produces capsules containing three hard, shiny, brown seeds.

Uses

The plant is used for commercial production of castor oil, a lubricant and purgative. The seeds are uses in making jewelry and rosaries.

Exposure Routes and Pathways

Human toxicity can occur if the seeds are ingested from the plants or ornaments made from the plants. If the hard outer coat remains intact, no toxicity will occur. However, if the outer coating is damaged, then ricin is released.

Mechanism of Toxicity

How does toxalbumin relate to ricin? Toxalbumins consist of two subunits (A and B) which are joined by a disulfide bond. The A subunit irreversibly binds the 60S ribosomal subunit, which blocks protein synthesis. The B subunit allows for binding and penetration across the gastrointestinal cell wall. Sufficient doses of ricin can cause cell death due to continued inhibition of protein synthesis.

Symptoms of Toxicity

Symptoms develop within 2–10 h after toxalbumin ingestions. Abdominal pain, severe vomiting, and bloody diarrhea may occur. Significant fluid losses may ensue, accompanied by tachycardia and hypotension. Systemic poisoning can be seen within 24 h, with multiple organ involvement. Hepatic and renal failure, lethargy, seizures, coma, and death may occur in severe intoxications. Allergic reactions may

occur for the seeds, or occupational exposure to the oil from this plant.

Clinical Management

Though ricin can be deadly, most exposures result in uncomfortable but limited gastroenteritis and minimal systemic toxicity. Gastrointestinal decontamination should be considered, depending on the time of ingestion. Symptomatic and supportive measures are the mainstay of treatment. There is no specific antidote for this toxin.

Jimson Weed (*Datura atura stramonium*)

Description

Jimson weed is a malodorous, fruit-bearing plant with dark green pointed leaves and tubular white flowers. It grows 3–5 ft in height. Jimson weed is native to Asia; however, it is also found throughout the United States and elsewhere. Other names for this plant and related species with the same active substance are locoseed, locoweed, devil's trumpet, Tolguacha, apple of Peru, Jamestown weed, devil's apple, thorn apple, stinkweed, hyoscyamine (leaves, roots, seeds); hyoscine (roots).

Uses

Historically, this has been recognized as a toxin and has inadvertently caused poisoning.

Exposure Routes and Pathways

Poisoning may occur through ingestion, drinking tea, or smoking the plant. Seeds have the highest concentration of toxin. Exposure may be unintentional or for experimentation purposes due to the hallucinogenic properties of the plant.

Mechanism of Toxicity

Anticholinergic poisoning occurs from belladonna alkaloids in various plants. Toxins may include atropine, hyoscyamine, and scopolamine.

Symptoms of Toxicity

Symptoms may begin within 1–4 h after ingestion. Jimson weed causes the anticholinergic toxidrome characterized by tachycardia, mydriasis, dry flushed skin, decreased bowel sounds, urinary retention, sedation, and hallucinations. Symptom resolution may vary from 1 day to 2 weeks.

Clinical Management

Treatment is supportive, though physostigmine is potentially indicated for stupor, coma, seizures, high fever, and severe agitation unresponsive to other treatment. Mild symptoms will usually resolve in a calm environment.

Monkshood (*Aconitum mapellus*)

Description

A perennial flowering herb, aconitum comes in white, pink, and purple. It is distributed throughout the Northern Hemisphere.

Uses

Aconite is used in Eastern medicine as an herb for properties of analgesia and antiinflammation. It has also been used experimentally in Western medicine to study cardiac arrhythmias.

Exposure Routes and Pathways

Poisoning may occur through ingestion and dermal exposure. The roots and flowers contain the highest concentration of alkaloid and are the most poisonous parts of the plant.

Mechanism of Toxicity

This species contains diterpene and norditerpine alkaloids, which mainly affect the cardiovascular system. The mechanism of toxicity is unclear; however, it is assumed to be due to blockade of the voltage-sensitive sodium channels.

Symptoms of Toxicity

Symptoms of toxicity are related to the cardiovascular, gastrointestinal, and neurological systems. These include bradycardia, ventricular tachycardia, nausea, and vomiting. Paresthesias of the extremities and generalized weakness have been reported. Rarely, seizures may occur in humans. This symptom is more commonly seen in animal models.

Clinical Management

Treatment is supportive, as death may occur from cardiovascular collapse. No specific antidote is available, but bradycardia and hypersalivation may be managed with atropine.

Oleander (*Nerium oleander*)

Description

Oleander is a flowering tree in the summer, which grows to a height of 25 ft, with green leaves and thick sap. Oleander is native to the Mediterranean, but found in the southern United States and elsewhere in the world. In addition to oleander, other toxic

cardiac glycosides with similar effects are lily of the valley (*Convallaria majalis*), and yellow oleander (*Thevetia peruviana*).

Uses

The oleander is cultivated as a flowering shrub in gardens. Also, *Digitalis purpurea* and *Digitalis lantana* have been used medicinally.

Exposure Routes and Pathways

Poisoning may occur through ingestion and dermal exposure. All parts of the plant contain varying amounts of cardiac glycosides. Concentrations of toxins peak during flowering season, and are found in seeds, stems, roots, and red flowers, in particular. Leaves contain oleandrin.

Mechanism of Toxicity

Toxins similar to digoxin inhibit sodium–potassium ATPase and include oleandrin, digitoxigenin, nerium folinerium, and rosagenin.

Symptoms of Toxicity

Toxicity from the cardiac glycosides includes gastrointestinal and cardiovascular symptoms. Nausea, vomiting, and irregular heartbeat may occur.

Clinical Management

Symptomatic and supportive treatment, in addition to digoxin-specific antibody fragments, can be effective for oleander toxicity.

Poison Hemlock (*Conium maculatum*)

Description

Poison hemlock is a weed that grows along roadsides. It has large fern-like leaves, and resembles some wild edible plants. Poison hemlock is found in wooded areas.

Uses

This plant was used in ancient times as a means of execution. For example, Socrates' death is attributed to Poison hemlock.

Exposure Routes and Pathways

Poisoning may occur through ingestion. Unintentional ingestion can occur from coniine when similar plants are mistaken for parsley, anise (seeds), or carrot plant. It is tuberous, similar to turnip roots.

Mechanism of Toxicity

Piperidine alkaloid toxins, such as coniine, are structurally similar to nicotine, and contained in all parts of the plant.

Symptoms of Toxicity

Initially, toxicity is manifested by nausea, vomiting, abdominal pain, tachycardia, sweating, shaking, dilated pupils, and seizures. This is followed by bradycardia, paralysis, and coma. Death can occur from respiratory failure.

Clinical Management

Gastrointestinal decontamination and aggressive treatment of symptoms is needed since no specific antidote is available and toxicity may be severe.

Common Potato or Irish Potato (*Solanum tuberosum*)

Description

Potato is an edible tuberous plant, and is toxic under certain conditions due to solanine. The toxin can be found throughout the plant, in varying concentrations. Potatoes are cultivated in many areas of the world.

Uses

The potato is edible and used in various diets.

Exposure Routes and Pathways

Ingestion of 'green' potatoes is commonly responsible for poisoning from solanine. Factors that increase the amount of toxin in the plant include exposure to light, shallow planting, excessive time in storage, and extreme temperatures.

Mechanism of Toxicity

Solanine is a glycoalkaloid that contains three sugar molecules. In animal models, solanine acts as a cardiac glycoside and inhibits cholinesterase activity.

Symptoms of Toxicity

The toxin includes gastrointestinal symptoms, with potential to impact the central nervous system. Symptoms may be prolonged, depending on the severity of the poisoning.

Clinical Management

Fluid and electrolyte replacement and supportive care is the general approach to symptoms of poisoning.

Rhododendron (Ericacricaceae family)

Description

Rhododendron is a flowering, green, shrub-like plant. Various species are found throughout Europe and the United States.

Uses

Rhododendron has been used to make tea, which can result in toxicity.

Exposure Routes and Pathways

Exposures occur after drinking tea made from the plant or sucking nectar from the flowers. Ingestion of honey contaminated by nectar from the plants may also cause toxicity.

Mechanism of Toxicity

Andromedotoxin (Grayanotoxin I) is a diterpene found in all parts of the plant. It opens sodium channels in the myocardium and increases permeability.

Symptoms of Toxicity

Cardiovascular effects, such as hypotension and both bradydysrhythmias and tachydysrhythmias may occur. In addition, gastrointestinal symptoms, perioral numbness, drowsiness and weakness are possible. Seizures and coma have also been reported.

Clinical Management

Minimal toxicity occurs in the majority of cases of rhododendron exposure. In severe exposures, decontamination and supportive care are required.

Tobacco (*Nicotiana tabacum*)

Description

Nicotiana tabacum is the principal source of nicotine. The stems and leaves of the plant are used for commercial purposes. Tobacco is now cultivated in many countries of the world.

Uses

This plant is used in the production of cigars, cigarettes, chewing tobacco, and nicotine replacement products.

Exposure Routes and Pathways

Unintentional poisoning may occur, if tobacco-containing products are ingested (e.g., by young children). Additionally, occupational exposures may cause toxicity to workers harvesting the plant. Dermal exposure can cause toxicity, even with intact skin. Wet leaves from the plant may enhance absorption.

Mechanism of Toxicity

Nicotiana tabacum contains 0.5–9% nicotine, which is the primary toxin. Nicotine binds to select acetylcholine receptors throughout the body; known as nicotine receptors. This produces initial stimulation, but inhibition later, at the receptor sites throughout the nervous system. Low doses enhance the release of catecholamines and sympathetic stimulation. Higher doses produce parasympathetic stimulation.

Symptoms of Toxicity

Initially, toxicity is manifested by nausea, vomiting, abdominal pain, seizures, tachycardia, and hypertension, followed by bradycardia, hypotension, and respiratory difficulties. Death may occur.

Clinical Management

Gastrointestinal decontamination and supportive care are the mainstay of treatment. Atropine may be used for excessive bronchial secretions. Removal of clothes that have been exposed and washing the victim are important for treatment of dermal exposure.

Water Hemlock (*Cicuta maculata*)

Description

The water hemlock is a toxic weed, with thick hollow, tuberous roots, commonly found along lakes and streams.

Uses

Water hemlock may be ingested when mistaken for the edible species, *Daucus carota* (Queen Anne's lace).

Exposure Routes and Pathways

The oral route of exposure is of primary importance with water hemlock.

Mechanism of Toxicity

The alkaloid, cicutoxin, is distributed throughout the plant, though concentrated in the roots. Cicutoxin is an unsaturated aliphatic alcohol which is postulated to exert toxicity by central cholinergic stimulation. Specifically, stimulation of both nicotinic and muscarinic receptors occur.

Symptoms of Toxicity

Initial symptoms of toxicity include nausea and vomiting. Seizures, status epilepticus, occur in severe

toxicity. Hypotension and bradycardia are followed by hypertension and tachycardia.

Clinical Management

No specific antidote is currently available. Thus, symptomatic and supportive care, especially aggressive treatment of seizures, can be life saving.

See also: Aconitum Species; Castor Bean; Hemlock, Poison; Jimsonweed; Oleander; Proteomics.

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Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Specific Poisonous Plants.

Plasticizers *See* Phthalate Ester Plasticizers.

Platinum

Shayne C Gad

- Representative Chemicals: *cis*-Dichlorodiammine platinum (cisplatin); *cis*-Platinum chloride; Potassium chloropaletinite
- Chemical Abstracts Service Registry Number: CAS 7440-06-4
- Chemical Formulas: Pt^{2+}; Pt^{4+}

Uses

Platinum and its alloys are used in jewelry, dentistry, the chemical industry, and the electrical industry. Most automobile catalytic converters contain platinum. Certain platinum compounds that have the *cis* configuration and can combine with DNA are useful therapeutic agents for many cancers that do not respond readily to conventional chemotherapy (especially testicular, ovarian, bladder, prostate, and thyroid cancers). Testicular cancer, which was once always fatal, now responds to platinum-containing drugs.

Background Information

Platinum's abundance in the earth's crust is ~0.01 ppm. Platinum has been known since ancient times and is very resistant to corrosion.

Exposure Routes and Pathways

Inhalation of industrial platinum compounds may be a problem. The general population is exposed to platinum by the dermal route, especially from jewelry. The oral route is not significant because the absorption is very poor.

Toxicokinetics

Following inhalation, lung clearance of platinum metal is very slow. Approximately 1 week following ingestion of platinum-containing water, platinum is found in the kidneys and liver. Following injection of the cancer chemotherapeutic agent, cisplatin, platinum is found mainly in the gonads. In autopsy specimens, platinum is found in adipose tissue. Serum creatinine levels correlate with *cis*-platinum doses. Most platinum is excreted in the feces.

Mechanism of Toxicity

While the metal itself is systemically of little concern, its salts are very toxic. The *cis*-platinum compounds

can react with disulfides and amino groups and form adducts with some bases in nucleic acids. Platinum compounds inhibit a few enzymes, including leucine aminopeptidase, and the hydrogenases of malate, alcohol, and lactate. Cisplatin can form crosslinks between strands of DNA.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} for platinum chloride in rats is $>3\,g\,kg^{-1}$ and the intraperitoneal LD_{50} is $>200\,mg\,kg^{-1}$. The oral and intraperitoneal LD_{50} for cisplatin in rats is considerably lower (25 and $7\,mg\,kg^{-1}$, respectively). Degenerative changes in proximal tubules including vacuolization and tubular dilation are noted within 5 days of a nephrotoxic dose of cisplatin in rats. A single exposure to cisplatin may lead to ototoxicity. Platinum is a dermal and pulmonary sensitizer.

Human

Inhalation of platinum dusts produces a pneumonitis characterized by coughing, wheezing, shortness of breath, and an asthma-like action. In some cases, cyanosis develops. Skin contact with various salts of platinum (especially the chlorides) can cause allergic dermatitis, characterized by eczematous patches. Type I hypersensitivity can be induced easily. Often the toxicity is not due to platinum itself but to its complexing with tissues. Exposure to salts can lead to cyanosis and lymphocytosis.

Chronic Toxicity (or Exposure)

Animal

Administered subcutaneously, local tumors appear. Rat (oral) TD_{Lo} is $9100\,mg\,kg^{-1}$, 26 weeks. Rats repeatedly treated with cisplatin ($2\,mg\,kg^{-1}$ twice a week for 4 weeks) exhibited neurophysiological and morphological indicators of peripheral neuropathy.

Human

Platinum salts (such as cisplatinum) produce a variety of serious side effects. It tends to deposit platinum in the corticomedullary area of the kidney and thus causes gastrointestinal upset (e.g., severe nausea and vomiting), nephrotoxicity (injuring both proximal and distal tubes), and blood changes (e.g., hypomagnesemia, leukopenia, and thrombocytopenia). Ototoxicity (e.g., tinnitus and hearing loss), peripheral neuropathy, and allergic reactions are also reported.

In Vitro Toxicity Data

Cisplatinum is an active mutagen in the Ames test and can cause sister chromatid exchanges.

Clinical Management

Symptoms of platinum inhalation usually abate soon after terminating exposure. Dermal exposure should be treated by washing the affected area immediately after exposure. To prevent skin allergic responses to platinum, it is best to control platinum dusts in the environment. The toxicity of the chemotherapeutic agent, cisplatin, can be reduced by prehydration using copious amounts of fluids.

Environmental Fate

Platinum can enter the environment through automobile emissions from the platinum-containing catalytic converter. Relatively high levels of platinum can be found along congested roadways. A number of chemotherapeutic agents contain platinum and thus their disposal can lead to environmental contamination. In industrialized regions, relatively high concentrations can be found in waterway sediments. Organic matter binds to the metal. In soil, mobility depends on pH, redox potential, and chloride concentration. Platinum will likely only mobilize under highly acidic conditions or in soil water with a high chloride content. Some platinum(IV) complexes, in the presence of platinum(II), may undergo methylation by microorganisms.

Ecotoxicology

Growth of the green alga *Euglena gracilis* was inhibited by hexachloroplatinic acid (250, 500, and $750\,\mu g\,l^{-1}$). Cisplatin inhibited growth in water hyacinth at $2.5\,mg\,l^{-1}$. The 3 week LC_{50} for hexachloroplatinic acid ($H_2[PtCl_6]$) in *Daphnia magna* was $520\,\mu g\,l^{-1}$. Reproduction was impaired at 14 and $82\,\mu g\,l^{-1}$. LC_{50} values (24, 48, and 96 h) for tetrachloroplatinic acid ($H_2[PtCl_4]$) in the Coho salmon were 15.5, 5.2, and $2.5\,mg\,l^{-1}$, respectively. Swimming behavior was affected at $0.3\,mg\,l^{-1}$.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value – time-weighted average is $1\,mg\,m^{-3}$ for elemental platinum and $0.002\,mg\,m^{-3}$ for soluble salts as platinum.

See also: Kidney; Metals.

Further Reading

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Relevant Website

http://www.inchem.org – International Programme on Chemical Safety.

Plutonium

Richard Belanger

- Representative Chemicals

  Plutonium oxide, PuO (CAS 12035-83-5)
  Plutonium dioxide, PuO_2 (CAS 12059-95-9)
  Plutonium nitride, PuN (CAS 12033-54-4)
  Plutonium tetrafluoride, PuF_4 (CAS 13709-56-3)
  Plutonium hexafluoride, PuF6 (CAS 13693-06-6)

- Chemical Abstracts Service Registry Number: CAS 7440-07-5
- Chemical/Pharmaceutical/Other Class: Metals, radioactive

Uses

The primary use for plutonium (Pu) is in nuclear power reactors, nuclear weapons, and radioisotopic thermoelectric generators (RTGs). Pu is formed as a by-product in nuclear reactors when uranium nuclei absorb neutrons. Most of this Pu is burned (fissioned) in place, but a significant fraction remains in the spent nuclear fuel. The primary plutonium isotope formed in reactors is the fissile Pu-239, which has a half-life of 24 400 years. In some nuclear programs (in Europe and Japan), Pu is recovered and blended with uranium (U) for reuse as a nuclear fuel. Since Pu and U are in oxide form, this blend is called mixed oxide or MOX fuel. Plutonium used in nuclear weapons ('weapons-grade') is metallic in form and made up primarily (>92%) of fissile Pu-239. The alpha decay of Pu-238 (half-life = 86 years) provides a heat source in RTGs, which are long-lived batteries used in some spacecraft, cardiac pacemakers, and other applications.

Background Information

The toxicity of plutonium is primarily due to its radioactive nature, and its effects have been extensively studied both in animals and in workers with occupational intakes. Shortly after its discovery in 1941, plutonium became a health concern by virtue of its property as an alpha emitter with a long half-life. The earliest attempts to predict toxicity focused on comparison to radium. Based on half-life and total alpha energy emitted, plutonium toxicity could be estimated at ~0.16 that of Ra-226. However, animal experiments quickly revealed that plutonium is much more toxic than radium.

Toxicity studies of plutonium compounds were initiated during World War II as nuclear weapons were developed. The pace was slow at first since only trace amounts of the element were made available for biological studies. In 1944, scientists at Berkeley, Rochester, and Chicago observed that plutonium deposits more unevenly in bone than radium, concentrating on areas of active bone growth. This led to the earliest safety guidelines, which were specified as maximum allowable body burden. A guideline of 1.0 μg (0.06 μCi) was generally used for the Manhattan Engineering District, while a more restrictive limit of 0.5 μg (0.03 μCi) was applied at Hanford Operations. In 1959, the International Commission on Radiological Protection (ICRP) specified a standard of 0.04 μCi, a value that stood until the system of dose limitation was revised in the late 1970s. (Current standards are specified in terms of absorbed radiation dose or radionuclide intake, as opposed to a quantity in the body.)

Exposure Routes and Pathways

The ionizing properties of Pu and other radioactive materials is one determinant of the level of hazard associated by different exposure routes. Radioactive elements are those that undergo spontaneous transformation (decay) in which energy is released either in the form of particles, such as alpha or beta particles, or waves, such as gamma or X-ray. Plutonium exists in several isomeric forms, the most important of which are Pu-238 and Pu-239. When these isotopes decay, they emit primarily alpha particles, which are densely ionizing and, therefore, damaging; however, the penetration of alpha particles into tissue is slight, so

biological damage is limited to cells in the immediate vicinity of the alpha-emitting radioactive material.

Alpha particles from plutonium cannot penetrate the epidermis, so toxicity is limited to conditions where the substance is present within the body. The primary routes of entry are inhalation, ingestion, or through wounds, cuts, or abrasions. The potential for adverse health effects caused by plutonium isotopes depends on the route of entry and subsequent deposition, redistribution, and retention, which in turn is highly influenced by the physical (e.g., particle size) and chemical forms of the isotope.

An analysis of 203 workers with internal deposits of plutonium showed that 131 were contaminated by inhalation, 48 through wounds, and eight by both routes. Most exposures to the general population involve minute quantities inhaled with ambient air or ingested in food and water. In the 1970s, a mean dietary intake of 1.6 pCi year^{-1} was estimated for New York City.

Toxicokinetics

There are several isotopes of plutonium (Pu-238 and Pu-239 being the most important), and it is the chemistry of the isotopes that determines the reactions within the environment as well their transport and reactions within the body. Ingested plutonium is primarily excreted in feces, as there is very poor absorption from the gastrointestinal tract. For inhalation, the regional deposition pattern depends primarily on particle size distribution. Within the first few days, a fraction of the deposited activity is rapidly cleared from the respiratory tract. The remaining fraction is cleared slowly, with retention half-time of months to years, depending on the chemical form (oxides, for example, tend to be cleared more slowly than nitrates). Materials absorbed from the respiratory tract are primarily deposited in bone and liver, where it is retained for many years. A very small fraction may also be deposited in testes or ovaries.

As a rule, isotopes of the same chemical form will have identical chemistry; however, Pu-238, which because of its shorter half-life is more intensely radioactive, has been shown to be more mobile in the body than the longer-lived Pu-239. Specifically, inhaled Pu-238 appears to be more rapidly cleared from the lung and transported to bone. An increased rate of radiolysis around deposited Pu-238 may account for this difference.

Mechanism of Toxicity

Plutonium is both toxic and carcinogenic. The primary mechanism responsible for both is the absorbed radiation dose delivered to cells at the sites of deposition.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute pulmonary toxicity of inhaled plutonium has been observed in experimental rodents and dogs that have inhaled large quantities of Pu-239. Changes leading to death within a year include edema, pneumonitis, and fibrosis. Bone fractures have also been observed in animals injected with large doses of Pu-239. The equivalent dose in man for these effects is more than 2.6 MBq (70 μCi). In addition, gross renal damage has been observed in animals following lethal doses of plutonium.

Human

In humans, acute pulmonary effects would only occur in extreme accident situations. It has been estimated that ~3.7 MBq (100 μCi) deposited in the lungs would be lethal to half the exposed population within 1 year.

Chronic Toxicity (or Exposure)

Animal

Chronic effects of plutonium exposure include life-shortening and cancer. These effects have been observed in numerous animal studies. The main late pulmonary effects of plutonium inhalation are pulmonary fibrosis and lung cancer. Lung cancers in animals have been reported for intakes equivalent to ~37 kBq (1 μCi) in man.

Studies in animals indicate that bone cancer is the most common form of malignancy induced by Pu-239 that has entered systemic circulation. The length of the latency period appears to depend on the amount of plutonium deposited in bone. Liver cancers have been observed in animals given Pu-239 injections, but they occur much less frequently than bone cancers.

Human

There is no evidence of life shortening or malignant disease in US workers with accidental intakes. Exposed workers in the former Soviet Union show biological effects, primarily pulmonary fibrosis and an increase in lung, bone, and liver cancers. Workers at the Mayak facility in the Russian Federation, who experienced far greater plutonium intakes than workers in other countries, have been reported to

have an excess lung cancer risk relative risk at age 60 of 0.6 per Sv of lung dose equivalent, assuming a radiation weighting factor of 20 for alpha particles. Excess bone and liver cancer mortality has also been reported in the Mayak workers with body burdens estimated to exceed 7.4 kBq (0.2 μCi), as well as among workers with unknown burdens. Although leukemia has been observed in humans exposed to relatively high levels of external radiation, it does not appear to be a significant effect of plutonium deposition in bone.

In Vitro Toxicity Data

Alpha radiation from plutonium produces cytotoxic and genotoxic effects in cultured cells. These can include cell death, chromosomal aberrations (dicentrics, translocations, and complex exchanges), and pretransformation molecular alterations such as upregulation of oncogene products coupled with inactivation of tumor suppressor genes.

Clinical Management

Clinical management can potentially reduce the effects of plutonium intake, although the effectiveness can be highly variable. Administration of the calcium salt of diethylenetriaminepentaacetic acid (DTPA) can accelerate removal of soluble forms of plutonium from body fluids and recent deposits. It is unable to remove intracellular deposits or activity buried in bone and must therefore be administered as soon as possible after an intake. In a review of 18 patients exposed to plutonium, americium, or curium, the US Food and Drug Administration concluded that administration of 1 g Ca–DTPA in 5 ml sterile aqueous solution, either by intravenous injection or as a nebulized inhalation dose, increased the rate of radioactivity elimination in urine by an average of 39-fold. Daily maintenance doses of Zn–DTPA resulted in continued elimination of radioactivity.

Bronchopulmonary lavage may also be effective for removal of inhaled plutonium, and has been recommended for occupational intakes of insoluble forms exceeding 100 times the annual limit on intake.

For wounds, any detectable plutonium in the wound or in spot urine samples should warrant considering administration of DTPA. If the activity in the wound is >5 nCi, excision of tissue should also be considered.

Environmental Fate

Although primarily a manmade substance, minute quantities of plutonium have existed and currently exist in nature. About 5000 kg of Pu-239 were dispersed into the environment by the atmospheric testing of nuclear weapons during the 1950s and 1960s, and trace amounts are present in most environmental media. Deposition (approximately three-quarters of which occurred in the northern hemisphere) reduced atmospheric levels to less than 20 kg by 1975, and as there have been few atmospheric tests conducted since that time, these levels have continued to decline. Measurable concentrations have been found in air, food, soils, and human and animal tissues. Plutonium has also reached the environment from routine and accidental releases from nuclear facilities, primarily those involved in the reprocessing of nuclear fuels.

The environmental behavior of plutonium is highly dependent on physicochemical properties of both the Pu compounds and the environmental media. As a rule, plutonium adsorbed on soil or sediment particles migrates very slowly, although the rate can be accelerated depending on Pu oxidation state and soil characteristics (mineral makeup, pH, presence of ligands). Uptake and concentration in edible plants is relatively low (concentration ratio on the order of 10^{-4} in vegetative parts).

Exposure Standards and Guidelines

Plutonium is regulated primarily as a radioactive material and the applicable standards and guidelines are set by entities involved in radiation protection. There are no US Occupational Safety and Health Administration or National Institute for Occupational Safety and Health exposure limits for plutonium.

United States of America

EPA National Primary Drinking Water Standard – 15 pCi l^{-1} (applicable standard for Pu is MCL for gross alpha particle activity, excluding uranium and radon).

NRC maximum concentration in effluent air at boundary of licensed facility (App. B to 10 CFR 20.1001-20.2401) for selected Pu isotopes:

- Pu-238 (all forms) – 2×10^{-14} μCi ml^{-1}; and
- Pu-239 (all forms) – 2×10^{-14} μCi ml^{-1}.

Annual limit on intake (occupational exposure) for inhalation of selected Pu isotopes:

- Pu-238, Class W – 7000 pCi (300 Bq);
- Pu-239, Class W – 6000 pCi (200 Bq);
- Pu-238, Class Y – 20 000 pCi (700 Bq); and
- Pu-239, Class Y – 20 000 pCi (600 Bq).

France

Plutonium in foodstuff intended for general consumption – 10 Bq kg^{-1}. Plutonium in baby foods or milk – 1 Bq kg^{-1}.

See also: Radiation Toxicology, Ionizing and Nonionizing.

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Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Plutonium.

Poinsettia

Allison A Muller

- Chemical Name: Poinsettia
- Synonyms: *Euphorbia pulcherrima*; Christmas star; Christmas flower; Painted leaf; Lobster plant; Mexican flame-leaf; Star of Bethlehem; Flower of nativity; Easter flower; Pappagallo

Exposure Routes and Pathways

Ingestion and dermal contact are possible routes of exposure.

Mechanism of Toxicity

Diterpene esters are primary dermal and gastrointestinal irritants. The amount of toxin found in the common greenhouse variety of poinsettia is minimal and very rarely causes symptoms. Irritation, whether dermal or oral, is rare. The poinsettia, despite its unfavorable reputation, appears for the most part to be innocuous.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals, particularly domestic animals, have shown a very low incidence of toxic effects. Of those that do develop symptoms, gastrointestinal irritation (nausea, vomiting, diarrhea, hypersalivation) is the most common effect seen after ingestion of poinsettia.

Human

Most human exposures occur in children with only small amounts ingested or in contact with skin. In these situations, symptoms are infrequent. Those symptoms that do occur are due to irritation of the affected area. Poinsettia ingestions may produce vomiting and diarrhea. Dermal exposures to the sap of the plant may cause irritation. Most exposures result in either no clinical effects or only minor, self-limited symptoms.

Clinical Management

For ingestion of plant material, symptomatic treatment consists of dilution with cool liquids. Ingestions rarely cause any symptoms aside from minor, self-limited gastrointestinal effects. Dermal exposures are treated with irrigation and local skin care.

See also: Gastrointestinal System.

Further Reading

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Poisoning Emergencies in Humans

Christopher P Holstege

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Introduction

Poisoning emergencies are a common occurrence. In 2002, The *Toxic Exposure Surveillance System* of the American Association of Poison Control Centers reported 2 380 028 toxic exposures and 1153 resultant fatalities. Of these total exposures, 548 093 (22.2%) were managed in a healthcare facility and 72 877 were admitted to a critical care unit (3.1%). The mortality rate associated with these overdose patients was less than 1%. Thorough evaluation, adequate supportive care, and the use of a few specific antidotes have resulted in lowered morbidity and mortality if the poisoned patient arrives at the hospital in time for the healthcare team to intervene. In select cases, decreasing further toxin absorption by various decontamination procedures may be of benefit.

Poisonings are among the most preventable public health problems. The majority of exposures are accidental (86%) and occur in children under 6 years of age. However, poisoning exposures are not limited to children and occur during every decade of life. Children aged 18–36 months are at the greatest risk due to excessive hand-to-mouth behavior and their innate curiosity, which results in extensive exploration of their environment. The carelessness by caretakers and the lack of adult awareness about what constitutes a poison also contributes to the risk of childhood exposure. Childhood exposures are largely preventable by recognizing the toxic potential of medications, herbals, household products, cosmetics, and plants and by keeping these agents out of the reach of children. Homes should clearly keep readily available the number of the local poison center. In the United States, all citizens can dial the toll free number 1-800-222-1222 and reach their local poison center. The American Association of Poison Control Center keeps an updated list of poison centers that can be located at their website.

Across all age groups, medications are involved in the majority of reported poisonings. Cosmetic products are the leading cause of pediatric poisoning exposures, followed closely by cleaning substances, analgesics, topical agents, and plants. These agents result in the majority of exposures because they are commonly found in households where children reside. Poisonings are associated with a relatively low mortality rate – only 0.048% of poisoned victims have fatal outcomes. Adults account for 98% of these fatalities and children account for only 2%. Most adult poisoning-related fatalities involve analgesics and psychiatric agents.

Management

All patients presenting with potential toxicity following exposure to various agents should be thoroughly assessed. The patient's airway should be patent and adequate ventilation assured. If necessary, endotracheal tube intubation should be performed. Too often physicians are lulled into a false sense of security when a patient's oxygen saturations are adequate on high flow oxygen. If the patient has either inadequate ventilation or a poor gag reflex, then the patient may be at risk of subsequent CO_2 narcosis with worsening acidosis or pulmonary aspiration. The initial treatment of hypotension consists of intravenous fluids. There should be close monitoring of the patient's pulmonary parameters to ensure that pulmonary edema does not develop as fluids are infused. The healthcare providers should consider placing the potentially poisoned patient on continuous cardiac monitoring with pulse oximetry. Frequent neurological checks should be considered. In all patients with altered mental status, the patient's glucose should be checked. These patients should receive a large bore peripheral intravenous line and all critically ill patients should have a second line placed either peripherally or centrally.

Decontaminating the Poisoned Patient

Approximately 80% of all poisonings occur by ingestion and subsequently the most common type of decontamination performed is gastrointestinal decontamination using a variety of techniques including emesis, gastric lavage, activated charcoal, cathartics, and whole bowel irrigation (WBI). Poisonings may also occur by dermal and ocular routes, which necessitate external decontamination. Significant controversy exists concerning the need for routine gastric emptying in the poisoned patient. Current available evidence dissuades one from the routine use of gastric decontamination. Gastric decontamination may be considered in select cases and specific scenarios. Before performing gastrointestinal decontamination techniques, the clinician responsible for the care of the poisoned patient must clearly understand that these procedures are not without hazards, and any decision on their use must

consider whether the benefit of decontamination outweighs any potential harm.

Dermal Decontamination

Patients presenting to healthcare facilities with dermal contamination pose a potential risk to healthcare personnel. Contaminated patients should not gain entrance into the healthcare facility prior to decontamination. Personnel involved in the dermal decontamination may need to don personal protective equipment. Most chemical exposures do not pose a risk of secondary exposure. For exposures that occur in the workplace, Material Safety Data Sheets can be obtained and either the local poison center or the Agency for Toxic Substances and Disease Registry can be contacted to obtain advice on what level of protection is appropriate. Contaminated clothing and valuables should be placed in an impervious bag to avoid potential of gassing.

Dermal decontamination can be performed by using soap and copious warm water irrigation. Starting from head to toe, irrigate the exposed skin and hair for 10–15 min and scrub with a soft surgical sponge, taking care not to abrade the skin. Irrigate wounds for an additional 5–10 min with water or saline. Clean underneath the nails with a brush. Stiff brushes and abrasives should be avoided as they may enhance dermal absorption of the toxin and can produce skin lesions that may be mistaken for chemical injuries. Sponges and disposable towels are effective alternatives.

Ocular Decontamination

Ocular irrigation should be performed as rapidly as possible by instillation of a gentle stream of lukewarm tap water into the affected eye(s). The skin contiguous to the eye should also be irrigated. In minor ocular toxicity cases, this procedure can be conducted in the home. If irritation persists following home irrigation, referral to an emergency department may be necessary. In the emergency department, the patient should undergo ocular irrigation with sterile normal saline for a period of at least 1 h. Exposures to some corrosives may necessitate prolonged ocular irrigation. Irrigation of the eyes should be directed away from the medial canthus to avoid forcing contaminants into the lacrimal duct. Longer irrigation times may be needed with specific substances and the endpoint of irrigation should be normalization of the eye's pH.

Gastrointestinal Decontamination

Emesis, gastric lavage, activated charcoal, cathartics, and WBI are the most common means of gastrointestinal decontamination. With emerging evidence, gastric lavage and syrup of ipecac-induced emesis are rarely being utilized to decontaminate the poisoned patient. At this time, the documented risks associated with these procedures should be carefully weighed in light of the rare indications. Activated charcoal as the sole means of gastric decontamination is increasing in popularity, but its efficacy has specific limitations. The major issue currently facing the clinician is the choice of gastrointestinal decontamination in the significantly poisoned patient. The choice of decontamination method for these patients must be individualized using both evidence-based medicine and clinical acumen. No patient should undergo any of the available procedures unless it is anticipated that decontamination will provide clinical benefit.

Emesis

Numerous emetics have been advocated in the past for the treatment of the poisoned patient. Past emetics have included apomorphine, egg whites, saltwater, copper sulfate, and household dish-washing liquid. However, the use of these agents is fraught with ineffectiveness and potential harm to the patient. The only acceptable emetic that may be considered is syrup of ipecac.

Syrup of ipecac is available as a nonprescription product in many countries. It is derived from the dried rhizome and roots of the *Cephaelis ipecacuanha* or *Cephaelis acuminata* plant. These plants contain the potent emetic alkaloids emetine and cephaeline, which induce vomiting by both direct local gastrointestinal effects and central nervous system actions. Emesis following syrup of ipecac ingestion typically occurs within 20 min of ingestion and persists for 30–120 min.

There have been numerous animal and human volunteer studies examining both the efficacy of syrup of ipecac to expel specific ingested agents from the stomach and its ability to decrease serum drug levels. In these studies, the amount of marker removed by syrup of ipecac was highly variable and the efficacy at expelling experimental markers decreased as the administration time post-ingestion increased. Syrup of ipecac is of very limited benefit if more than 60–90 min have elapsed since the time of ingestion. No studies have demonstrated that syrup of ipecac improves patient outcome. In fact, recent studies suggest there is no reduction in resource utilization or improvement in patient outcome from the use of syrup of ipecac at home. In 2003, the American Academy of Pediatrics recommended that ipecac should no longer be used routinely as a home treatment strategy and that existing ipecac in the home

should be disposed of safely. Also in 2003, the US Federal Drug Administration (FDA) Nonprescription Drugs Advisory Committee met to discuss whether there was sufficient evidence of the benefits of ipecac syrup to outweigh the potential for misuse, abuse, and adverse effects associated with it as an over-the-counter (OTC) drug. At the conclusion of the meeting, the Committee recommended by a six-to-four vote that the FDA rescind ipecac's OTC status. The position statement written by the American Academy of Clinical Toxicologists and the European Association of Poison Centers and Clinical Toxicologists declared that the routine administration of ipecac should be abandoned.

In the rare cases where syrup of ipecac is administered to a patient, it should only be given to an alert, conscious patient who has ingested a potentially toxic amount of a poison no more than 60 min prior to administration. The administration of syrup of ipecac is contraindicated in any person who demonstrates compromised airway protective reflexes or has the potential to lose such protective reflexes. It should be avoided in persons who have ingested substances that could result in coma, seizures, cardiovascular collapse, or paralysis. Syrup of ipecac is also contraindicated in persons who have ingested corrosive substances (acids or alkalis), hydrocarbons, and foreign bodies that could potentially result in airway obstruction. Caution should be exercised in using syrup of ipecac in patients who possess medical conditions that could be further compromised by the induction of emesis, such as patients with bleeding diatheses. The most commonly reported complications of ipecac administration include diarrhea, lethargy, and prolonged vomiting. Other reported complications include pulmonary aspiration of gastric contents, bradycardia, cerebral hemorrhage, gastric rupture, gastric diaphragmatic herniation, Mallory–Weiss tear, and pneumomediastinum. The use of ipecac may both delay the administration and diminish the effectiveness of other methods of gastrointestinal decontamination.

Gastric Lavage

The efficiency of gastric lavage to remove a marker significantly decreases with increasing time following ingestion. This is due to the fact that the greater the time after ingestion, the more time there is for the marker to be absorbed and for the marker to pass out of the stomach. It is rare that gastric lavage can be performed within the first hour after toxic ingestion. Not only does it take time for these patients to present to the emergency department, but it also takes time for evaluation, stabilization, and for the gastric lavage to take place. Based on the available literature, gastric lavage should not be routinely employed in the management of poisoned patients. Oral charcoal alone is considered superior to gastric lavage if a drug is adsorbed by charcoal.

The performance of gastric lavage is contraindicated in any person who demonstrates compromised airway protective reflexes unless they are intubated. Gastric lavage is also contraindicated in persons who have ingested corrosive substances (acids or alkalis), hydrocarbons (unless containing highly toxic substances such as paraquat, pesticides, heavy metals, halogenated and aromatic compounds), have known esophageal strictures, and a history of gastric bypass surgery. Caution should be exercised in performing gastric lavage in patients who possess medical conditions that could be compromised by performing this procedure, such as patients with bleeding diatheses, and in combative patients.

Numerous complications have been reported in association with gastric lavage. Depending on the route selected for tube insertion, damage to the nasal mucosa, turbinates, pharynx, esophagus, and the stomach have all been reported. After tube insertion, it is imperative to confirm correct placement. Radiographic confirmation of tube placement should especially be considered in young children and intubated patients. Instillation of lavage fluid and charcoal into the lungs through tubes inadvertently misplaced within the airways has been reported. The large amount of fluid administered during lavage has been reported to cause patient fluid and electrolyte disturbances. These disturbances have been seen with both the use of hypertonic and hypotonic lavage fluids in the pediatric population. Hypothermia is a possible complication if the lavage fluid is not pre-warmed. Pulmonary aspiration of gastric contents or lavage fluid is the primary potential risk during gastric lavage, especially in patients with compromised airway protective reflexes.

Activated Charcoal

Activated charcoal acts both by adsorbing a wide range of toxins present in the gastrointestinal tract and by enhancing toxin elimination, if systemic absorption has already occurred. It enhances elimination by creating a concentration gradient between the contents of the bowel and the circulation, but it also has the potential of interrupting enterohepatic circulation if the particular toxin is secreted in the bile and enters the gastrointestinal tract prior to reabsorption. Oral activated charcoal is given as a single dose or in multiple doses.

Single dose activated charcoal is indicated if the clinician estimates that a clinically significant

fraction of the ingested substance remains in the gastrointestinal tract, the toxin is adsorbed by charcoal, and further absorption may result in clinical deterioration. It may also be administered by multiple dosing if the clinician anticipates that the charcoal will result in increased clearance of an already absorbed drug. In 1997, the American Academy of Clinical Toxicology released a position statement recommending that activated charcoal should not be routinely administered but should be reserved for cases in which serious toxicity is anticipated. It is most effective within the first 60 min after oral overdose and decreases in effectiveness over time.

The administration of charcoal is contraindicated in persons who demonstrate compromised airway protective reflexes, unless they are intubated. It is contraindicated in persons who have ingested corrosive substances (acids or alkalis). Charcoal not only provides no benefit in a corrosive ingestion, but its administration could precipitate vomiting, obscure endoscopic visualization, and lead to complications if a perforation develops and charcoal enters the mediastinum, peritoneum, or pleural space. Charcoal should be avoided in cases of pure aliphatic petroleum distillate ingestion. Caution should be exercised in using charcoal in patients who possess medical conditions that could be further compromised by charcoal ingestion, such as those with gastrointestinal perforation or bleeding. Since it is often impossible to determine the exact nature of an ingestion, a liberal use policy is advocated for potential mixed overdoses.

Charcoal is generally very safe and few adverse effects from the use of single dose activated charcoal have been reported despite its widespread use. There are no reports of gastrointestinal obstruction associated with single dose activated charcoal. The most common complications of charcoal administration include constipation, diarrhea, and vomiting. Pulmonary aspiration of activated charcoal is a dreaded complication that can result in pneumonitis, obstruction of the respiratory tree, and bronchiolitis obliterans. Aspiration of large amounts of charcoal can be fatal.

The use of multidose activated charcoal (MDAC) may be indicated in select cases. Its use has been advocated to prevent continued absorption of a drug that may still be present within the gastrointestinal tract and to increase the clearance of a drug that has already been absorbed. MDAC prevents continued absorption by binding a drug that may be either present throughout the gastrointestinal tract or one that exists as an extended-release or enteric-coated preparations. MDAC enhances elimination of a drug by interrupting enterobiliary recirculation or augmenting enterocapillary exsorption. By interrupting enterobiliary recirculation, charcoal binds to an active drug that is secreted by the biliary system, subsequently preventing reabsorption. By augmentation of enterocapillary exsorption, charcoal produces sink conditions that drive diffusion of the drug from the capillaries into the entraluminal space from where it is subsequently eliminated. This process is called 'intestinal dialysis'. MDAC is contraindicated if there is evidence of bowel obstruction. An ileus is a relative contraindication. The administration of MDAC is contraindicated in any patient who does not have an intact or protected airway. MDAC should be avoided in patients who have repetitive emesis, especially when associated with decreased mental status or a decreased gag reflex. The concurrent use of cathartics with MDAC remains unproven and is not recommended. The first dose of activated charcoal should be $1\,g\,kg^{-1}$ (maximum of 100 g). If a cathartic is used, it should be administered only with the first dose of charcoal to decrease the risk of cathartic-induced electrolyte abnormalities that can potentially develop, especially in children. The initial dose of charcoal is followed by $0.5\,g\,kg^{-1}$ (up to 50 g) of activated charcoal every 4 h. If repeat examination reveals an absence of bowel sounds or reveals a distended abdomen, MDAC should be terminated and the physician should consider placement of a nasogastric tube on low intermittent suction. The use of antiemetics may help decrease the incidence of vomiting associated with MDAC. Charcoal therapy should be continued until there is clinical improvement and plasma drug levels have fallen to acceptable levels. There have been reports of gastrointestinal obstruction and perforation from MDAC therapy, especially in conjunction with the ingestion of drugs with anticholinergic properties.

Cathartics

The use of cathartics is intended to decrease the absorption of substances by accelerating the expulsion of the poison from the gastrointestinal tract. However, most data suggest negligible clinical benefit from cathartic use. There is little evidence that a single dose of aqueous activated charcoal is significantly constipating; however, cathartics are often given for this potential problem. The routine administration of a cathartic in combination with activated charcoal is not endorsed by the American Academy of Clinical Toxicology or the European Association of Poison Centres and Clinical Toxicologists. In addition, the administration of a cathartic alone has no role in the management of the poisoned patient.

Cathartics are contraindicated if there is volume depletion, hypotension, significant electrolyte imbalance, corrosive ingestion, ileus, recent bowel surgery,

and intestinal obstruction or perforation. The administration of cathartics is also contraindicated with patients who do not have an intact or protected airway. They should be avoided in patients who have repetitive emesis, especially when associated with decreased mental status or a decreased gag reflex. Cathartics should be used cautiously in young children and the elderly because of the propensity of laxatives to cause fluid and electrolyte imbalance.

There are two types of osmotic cathartics: saccharide cathartics (sorbitol) and saline cathartics (magnesium citrate, magnesium sulfate, sodium sulfate). Many charcoal formulations come premixed with sorbitol, but there is considerable variation in the sorbitol content. Multiple doses of cathartics should be avoided. The administration of sorbitol has been associated with vomiting, abdominal cramps, nausea, diaphoresis, and transient hypotension. Multiple doses of sorbitol have been associated with volume depletion. Multiple doses of magnesium-containing cathartics have been associated with severe hypermagnesemia. Children are particularly susceptible to the adverse affects of cathartics, and therefore cathartics should be used with caution, or totally avoided, in children.

Whole Bowel Irrigation

Whole bowel irrigation (WBI) has emerged as the newest technique in gastrointestinal decontamination. It involves the enteral administration of an osmotically balanced polyethylene glycol-electrolyte solution (PEG-ES) in sufficient quantity and rate to physically flush ingested substances through the gastrointestinal tract, purging the toxin before absorption can occur. PEG-ES is isosmotic, is not systemically absorbed, and will not cause electrolyte or fluid shifts. Available data suggest that the large volumes of this solution needed to mechanically propel pills, drug packets, or other substances through the gastrointestinal tract are safe, including in pregnancy and in young children. WBI may be considered for ingestions of exceedingly large quantities of potentially toxic substances, ingestions of toxins that are poorly adsorbed to activated charcoal, ingestions of delayed-release formulations, late presentation after ingestion of a toxin, pharmacobezoars, and body stuffers or packers. The most common indication for WBI in the Emergency Department (ED) is for the treatment of toxic sustained-release medications (such as calcium channel blockers, theophylline, lithium) and iron tablets. WBI is contraindicated in patients with gastrointestinal obstruction, perforation, ileus, and corrosive ingestion. It should also be avoided in patients with hemodynamic instability or an unprotected airway. WBI should be avoided with patients who have repetitive emesis, especially when associated with decreased mental status or a decreased gag reflex. WBI should be used cautiously in debilitated patients.

Cooperative patients with intact airway protective reflexes may drink the solution. However, the large volume and taste often limit even the most motivated patient's ability to comply. If the patient is unable or unwilling to drink this solution, it should be administered through a small-bore nasogastric tube after placement is confirmed. Unconscious patients with protected airways may receive WBI. Prewarming the irrigant to a temperature of $\sim 37^{\circ}C$ avoids the potential complication of hypothermia. The endpoint of WBI is the arrival of clear rectal effluent and/or resolution of toxic effect.

There have been few reported complications from WBI therapy, especially pertaining to acute poisonings. Nausea, vomiting, abdominal cramps, and bloating have been described. Nausea and vomiting may make administration of WBI difficult. Antiemetics and a 15–30 min break followed by a slower rate may allow readministration. As discussed with the other methods of decontamination, attention should be directed to the airway and the potential for aspiration. Administration of a large amount of chilled or room temperature WBI fluid to pediatric patients could potentially cause hypothermia. Warmed fluid should be considered in these patients.

Pharmacologic Antagonists

The number of pharmacologic antagonists or *antidotes* is quite limited. There are few agents that will rapidly reverse toxic effects and restore a patient to a previously healthy baseline state. Administering some pharmacologic antagonists may actually worsen patient outcome compared to merely employing basic supportive care. As a result, antidotes should be used cautiously and with clearly understood indications and contraindications.

Atropine

Atropine is the initial drug of choice in symptomatic patients poisoned with organophosphates or carbamates. Atropine acts as a muscarinic receptor antagonist and blocks neuroeffector sites on smooth muscle, cardiac muscle, secretory gland cells, peripheral ganglia, and in the central nervous system. Atropine is therefore useful in alleviating bronchoconstriction and bronchorrhea, relieving tenesmus, abdominal cramps, nausea and vomiting, resolving

bradydysrythmias, and halting seizure activity. Atropine can be administered by either the intravenous, intramuscular, or endotracheal route. The dose varies with the type of exposure, but typically even the worst cases require less than 20 mg in the first 24 h. There are a few reports of severe organophosphate pesticide poisonings requiring hundreds of milligrams of atropine each day. For the mildly and moderately symptomatic patient, 2.0 mg kg^{-1} for adults and 0.02 mg kg^{-1} for children (minimum of 0.1 mg) is administered every 5 min. In the severely poisoned patient, dosages may need to be increased and given more rapidly. Tachycardia is not a contraindication to atropine administration in these patients. Drying of the respiratory secretions and resolution of bronchoconstriction are the therapeutic end-points used to determine the appropriate dose of atropine. Atropine has no effect on the nicotinic receptors and therefore has no effect on autonomic ganglia and neuromuscular junction. Therefore, muscle weakness, fasciculations, tremors, and paralysis are not an indication for further atropine dosing. Atropine does have a partial effect on the central nervous system and may be helpful in resolving seizures.

Deferoxamine

Deferoxamine is an effective chelator of iron. Deferoxamine chelates iron and converts it to a water-soluble complex, ferrioxamine, which is eliminated readily via the urine. Indications for deferoxamine infusion include significant clinical signs of iron toxicity, metabolic acidosis, shock, serum iron levels $> 500\ \mu g\ dl^{-1}$, and/or an X-ray positive for multiple pills. Deferoxamine should be infused intravenously at a starting rate of 15 mg $kg^{-1}\ h^{-1}$, not to exceed 1 g h^{-1}, over a total of 6 h and then reevaluated. Deferoxamine-induced hypotension may occur at fast rates, and adequate hydration should be assured before infusion initiation. As iron is chelated and excreted, urine may develop a characteristic rusty-red ('vin rose') appearance.

Crotalidae Antivenin

Currently in the United States, there are two available Crotalidae antivenoms: polyvalent IgG (Wyeth-Ayerst Laboratories) and polyvalent Fab immunoglobulin fragments (CroFab-® by Protherics Inc.). Use of antivenin in the appropriate doses can control local swelling and serious systemic effects (e.g., neurologic effects and coagulopathies) that occur in patients who have been envenomated. However, the antivenin should not be used prophylactically since a significant number of snake bites are dry bites. There are numerous dosage regimens that vary with the degree of systemic toxicity and regional treatment preferences. Consultation with a poison center or a clinical toxicologist is advised for the most contemporary treatment recommendations.

There have been numerous reports of immediate hypersensitivity reactions associated with the use of crotalidae antivenom polyvalent IgG (Wyeth-Ayerst Laboratories). Incidence rates for immediate hypersensitivity reactions associated with the use of this product range from 23% to 56%. This high reaction rate may be in part due to the large amount of non-venom neutralizing proteins within this partially purified horse antivenom. In addition, this product contains the Fc portion of the antibodies that may result in cross-linking on cell surface receptors and lead to mast cell and basophil degranulation. CroFab-® is reported to have a lower risk of immediate hypersensitivity reactions. The product contains reduced amounts of the immunogenic Fc portion of the antibody.

Digoxin Immune Fab

A milestone in the treatment of cardiac glycoside poisoning was the development of drug-specific antibodies. Digoxin-specific Fab fragments (Digibind or DigiTab) are antibody fragments produced by enzymatic cleavage of sheep immunoglobulin (IgG) antibodies to digoxin. Fab fragments can reverse digitalis-induced dysrhythmias, conduction disturbances, myocardial depression, and hyperkalemia in severely poisoned patients. Most patients have an initial response to cardiac glycoside toxic dysrhythmias within 30 min of Fab administration and those who responded had complete resolution by 4 h. Animal studies and case reports have demonstrated the efficacy of Fab fragments to the cardiac glycoside contained in plants. Adverse reactions to Fab administration have been few and include rare but mild hypersensitivity reactions, precipitous drops in serum potassium, and supraventricular tachydysrhythmias previously controlled by digoxin.

Fab fragment therapy should be administered for the following indications: (1) potassium $> 5.0\ mEq\ l^{-1}$ following acute ingestion, (2) serum digoxin concentration $> 10\ ng\ ml^{-1}$, (3) patients with potentially life-threatening dysrhythmias. Often, chronically poisoned patients can be managed by discontinuing digoxin and close monitoring. However, the threshold for treatment with Fab should be lower in chronically poisoned patients with signs of cardiac toxicity or those who have chronic pulmonary disease, hypokalemia, hypothyroidism, renal insufficiency, or underlying cardiac disease. If patients are managed conservatively the Fab dose to

be administered should be calculated and the Fab fragments made available at the bedside while the patient is monitored for worsening toxicity.

Although serum digoxin levels should not be the sole factor in determining the need to administer Fab, dosage calculations for Fab are based on the serum digoxin level or estimated body load of digoxin. It is assumed that equimolar doses of antibody fragments are required to achieve neutralization. Thirty-eight milligrams of Fab (one vial) will bind 0.5 mg of digoxin. A severely toxic patient in whom the quantity ingested acutely is unknown should be given 5–10 vials at a time and the clinical response observed. If cardiac arrest is imminent or has occurred, the dose can be given as a bolus. Otherwise, it should be infused over 30 min. In contrast, patients with chronic therapeutic overdose often have only mildly elevated digoxin levels and respond to one to two vials of Fab. The recommended dose for a given patient can be determined using the tables in the package insert or by contacting a regional poison center or toxicology consultant.

Free digoxin levels are decreased to zero within 1 min of Fab fragment administration, but total serum digoxin levels are markedly increased. Since most assay methods measure both bound and free digoxin (total), very high digoxin levels are seen after Fab fragment therapy, but they have no correlation with toxicity. Serum levels may be unreliable for several days after Fab treatment. The digoxin–Fab complex is excreted in the urine and in patients with renal failure; elimination of the digoxin–Fab complex is prolonged and free digoxin levels gradually increase over hours after Fab administration. Rebound cardiac glycoside toxicity is rare but has been reported. Hemodialysis does not enhance elimination of the digoxin–Fab complex.

Flumazenil

Benzodiazepines are involved in many intentional overdoses. While these overdoses are rarely fatal when a benzodiazepine is the sole ingestant, they often complicate overdoses with other central nervous system depressants (e.g., ethanol and sedatives) due to their synergistic activity. Flumazenil finds its greatest utility in the reversal of benzodiazepine-induced sedation from minor surgical procedures. The initial flumazenil dose is 0.2 mg and should be administered intravenously over 30 s. If no response occurs after an additional 30 s, a second dose is recommended. Additional incremental doses of 0.5 mg may be administered at 1 min intervals until the desired response is noted or until a total of 3 mg has been administered. Flumazenil should not be administered as a nonspecific coma-reversal drug and should be used with extreme caution after intentional benzodiazepine overdose since it has the potential to precipitate withdrawal in benzodiazepine-dependent individuals and/or induce seizures in those at risk.

Fomepizole

Fomepizole (4-methylpyrazole) is an alcohol dehydrogenase inhibitor. It is administered in cases of suspected or confirmed ingestion and intoxication with ethylene glycol or methanol. Fomepizole should be administered intravenously as a loading dose of $15\,mg\,kg^{-1}$, followed by doses of $10\,mg\,kg^{-1}$ every 12 h for four doses, then $15\,mg\,kg^{-1}$ every 12 h thereafter; all doses should be administered as a slow intravenous infusion over 30 min. During hemodialysis, the frequency of dosing should be increased to every 4 h. Therapy should be continued until ethylene glycol or methanol concentrations are less than $20\,mg\,dl^{-1}$ and the patient is asymptomatic.

Hydroxocobalamin

Hydroxocobalamin (vitamin B_{12a}), currently investigational in the United States, is a safe and effective alternative that is currently being used in Europe for the treatment of cyanide toxicity. It acts as a chelating agent for cyanide. The reaction of hydroxocobalamin with cyanide results in the displacement of a hydroxyl group by a cyano group to form cyanocobalamin (vitamin B_{12}), which is then excreted in the urine. One molecule of hydroxocobalamin binds one molecule of cyanide. Hydroxocobalamin is given intravenously in a 5% dextrose solution. The usual adult dose is 4 g, which may be increased in cases of massive cyanide poisoning. The most common side effect is an orange-red discoloration of the skin, mucous membranes, and urine, which lasts for $\sim$12 h.

N-Acetylcysteine

Acetaminophen overdosage, whether accidental or intentional, is the most common type of poisoning event reported to American poison centers. Most acetaminophen overdoses do not produce adverse effects because most of these are minor exposures in children. However, significant overdoses may need to be treated with *N*-acetylcysteine (NAC; Mucomyst) if the patient has a toxic serum acetaminophen concentration or is in hepatic failure. NAC increases glutathione levels and serve as a glutathione surrogate. An acetaminophen overdose may deplete glutathione, permitting the toxic metabolite to destroy hepatocytes. NAC is most effective if administered within 8 h of the acetaminophen ingestion; however, it is still effective

days after the ingestion when patients are already in hepatic failure and acetaminophen levels are no longer detectable.

NAC is approved for both oral and intravenous administration:

- *Oral*: 140 mg kg^{-1} loading dose followed by 70 mg kg^{-1} every 4 h for 17 doses.
- *Intravenous*: 150 mg kg^{-1} loading dose followed by 50 mg kg^{-1} over 4 h followed by 100 mg kg^{-1} infused over 16 h.

Parenteral administration of NAC eliminates compliance problems associated with oral therapy (very bad taste and odor due to the sulfhydryl groups) and circumvents the problems associated with acetaminophen-induced vomiting.

Naloxone

Opioid poisoning from the abuse of morphine derivatives or synthetic narcotic agents may be reversed with the opioid antagonist naloxone (Narcan). Naloxone is commonly used in comatose patients as a therapeutic and diagnostic agent. The standard dosage regimen is to administer from 0.4 to 2.0 mg slowly, preferably intravenously. Intramuscular administration is an alternative parenteral route, but if the patient is hypotensive, naloxone may not be absorbed rapidly from the intramuscular injection site. The intravenous dose should be readministered at 5 min intervals until the desired endpoint is achieved – restoration of respiratory function and an improved level of consciousness. If the intravenous route of administration is not viable, alternative routes in addition to intramuscular injection are administration via the endotracheal tube in intubated patients as well as intralingual and sublingual injection. Intraosseous administration may be an alternative route in pediatric patients.

A patient may not respond to naloxone administration for a variety of reasons: insufficient dose of naloxone, the absence of an opioid exposure, a mixed overdose with other central nervous and respiratory system depressants, or medical or traumatic reasons.

Physostigmine

Once touted as the medication of choice to treat lethal tricyclic antidepressant overdoses, physostigmine (antilirium^{TM}) has very limited uses today in overdose management. Physostigmine is a cholinesterase inhibitor and finds its primary application in the treatment of severe anticholinergic poisoning. When indicated, physostigmine is administered preferably in small incremental doses of 2 mg mixed in 10 cc of saline by slow intravenous infusion over 10 min. Rapid injection or the administration of large doses may produce a cholinergic crisis or seizure activity.

Pralidoxime Chloride

Pralidoxime chloride (2-PAMCL, Protopam^{TM} Chloride) reactivates acetylcholinesterase (AChE) by exerting a nucleophilic attack on the phosphorus resulting in an oxime–phosphate bond that splits from the AChE leaving the regenerated enzyme. This reactivation is clinically most apparent at skeletal neuromuscular junctions, with less activity at muscarinic sites. Pralidoxime must therefore be administered concurrently with adequate atropine doses. In addition, the process of aging will prevent pralidoxime from regenerating the AChE active site and, as a result, is ineffective after aging has occurred. Therefore, the sooner pralidoxime is administered, the greater the clinical effect. The recommended dose of pralidoxime is 1.0 gm for adults or 15–25 mg kg^{-1} for children by the intravenous route. Slow administration over 15–30 min has been advocated to minimize side effects. These side effects include hypertension, headache, blurred vision, epigastric discomfort, nausea, and vomiting. In multiple animal models, the pralidoxime serum concentration to achieve therapeutic efficacy was reported to be 4 mg l^{-1}. The above dose will attain these levels, but pralidoxime is rapidly excreted and the concentration falls below 4 mg l^{-1} within 2 h. Subsequently, repeat pralidoxime should be administered at hourly intervals if progressive worsening or serious signs of toxicity persist. In order to achieve a steady-state blood level of pralidoxime following loading, it has been recently recommended that a continuous intravenous infusion be administered. Continuous intravenous infusion for insecticide organophosphate poisoning has proven to be safe and effective. As pralidoxime is rapidly excreted in the urine, adequate hydration should be maintained during therapy. Theoretically, dosing should be lowered for patients with renal failure. If medical personnel are unable to initially obtain intravenous access, a solution for intramuscular use can be made by mixing the contents of a 1 g vial with 3 ml of sterile saline. Intramuscular administration to a patient with an adequate blood pressure will produce a therapeutic plasma concentration of 4 mg l^{-1} within 10 min.

Pyridoxine

Isoniazid, hydrazine, and the *Gyrometria* species of mushrooms can decrease the brain concentrations of gamma-aminobutyric acid by inhibiting pyridoxal-5-phosphate activity, resulting in the development of severe seizure activity. The administration of

pyridoxine (vitamin B_6) can prevent or actively treat the central nervous system toxicity associated with isoniazid poisoning. Pyridoxine is administered on a gram-for-gram basis with isoniazid (i.e., the amount of pyridoxine should equal the amount of isoniazid). If the ingested amount of the agent above is unknown, the dose of pyridoxine should be 5 g administered intravenously. This dose can be repeated.

See also: Anticholinergics; Atropine; Charcoal; Deferoxamine; Gastrointestinal System; Lithium; Pesticides; Polyethylene Glycol; Pyridoxine.

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Relevant Website

http://www.aapcc.org – American Association of Poison Control Centers.

Pokeweed

Ann P Slattery

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- Synonyms: *Phytolacca americana*; *P. pecandra*; *P. rigida*; American nightshade; Cancerroot; Crowberry; Indian polk; Inkberry; Pigeonberry; Pokeberry; Red ink plant; Red weed; Red wood; Scoke

Uses

Pokeweed is a tall perennial shrub growing up to 12 ft. This shrub can be found in damp fields, along fences, and wooded areas of southeastern Canada, eastern United States, as well as California and Hawaii. The stalks are reddish. There are small, greenish-white flowers and berries in opposite clusters. The berry is dark purple, almost black in color, and matures late summer to autumn. Pokeweed has been used in tea and as a herbal medicine. In folk medicine, pokeweed as a tincture has been used for arthritis and chronic rheumatism. Taken by mouth pokeweed was used as a purgative and as an emetic. The young leaves, if boiled and drained twice, are supposedly edible.

Exposure Routes and Pathways

Exposure to pokeweed is by ingestion and dermal contact.

Mechanism of Toxicity

The toxic activity of the plant is unclear. Pokeweed mitogen (PWM) noted in the plant fluids may initiate changes in the immune system that alter T- and B-lymphocytes. The gastrointestinal irritant properties of phytolaccinic acid and related triterpenoid glycosides (saponins) cause diarrhea and severe vomiting.

Acute and Short-Term Toxicity (or Exposure)

Human

Pokeweed contains phytolaccatoxin and related triterpenes. All plant parts are poisonous, especially the roots. Uncooked berries have been known to poison children. Toxic exposures have occurred from eating the uncooked leaves in salads or when the root is mistaken for horseradish, parsnip, or ginseng. Effects appear 30 min to 6 h after exposure. Symptoms include nausea, abdominal cramps, profuse sweating, and foamy diarrhea. Other effects include oral burning, a bitter taste in the mouth, dyspnea, weakness, tremors, and seizures. One case of Mobitz Type I heart block has been reported after ingesting pokeweed, but is believed to be secondary to parasympathetic effects from prolonged vomiting. Symptoms may last up to 48 h. As few as 10 berries can result in toxic effects. Dermal exposures result in irritation, pain, and the sensation of heat.

Clinical Management

Symptoms usually resolve within 24 h. In significant exposures, treatment should include gastric lavage (depending on time since exposure) followed by activated charcoal. Symptomatic and supportive care should include rehydration and correction of electrolyte imbalance. Promethazine may decrease gastrointestinal symptoms. There is no antidote for exposure to pokeweed.

See also: Charcoal; Gastrointestinal System.

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Pollutant Release and Transfer Registers (PRTRs)

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Background

Over the past decade, the public's right to know has moved to the forefront of environmental policy-making and action. Principle 10 of the Rio Declaration, articulated at the 1992 UN Conference on Environment and Development (UNCED), popularly known as the 'Earth Summit', the Organisation for Economic Cooperation and Development (OECD) Council Recommendation (1996), and the Aarhus Convention (1998) all emphasize the importance of providing public access to environmental information. In most OECD countries, the involvement of the public in environmental decision-making is regarded as an important component of sustainable development. A key tool that governments are using to provide data to the public about the releases and transfers of potentially hazardous pollutants is the Pollutant Release and Transfer Register (PRTR).

What is a PRTR?

A PRTR is an environmental database or inventory of potentially harmful chemicals and/or pollutants released to air, water, and soil, and transferred off-site for treatment. According to the OECD Council Recommendation (C(96)41/FINAL), as amended by (C(2003)87), and the PRTR Protocol under the Aarhus Convention, the core elements of a PRTR system are: (1) a listing of chemicals, groups of chemicals, and other relevant pollutants that are released to the environment or transferred off-site; (2) integrated multimedia reporting of releases and transfers (to air, water, and land); (3) reporting by source, covering point sources and nonpoint sources, where appropriate; (4) periodic reporting (preferably annually); and (5) making data available to the public. A PRTR brings together in one place information about what pollutants are being released, where, how much, and by whom.

Each country has its own set of requirements for reporting. However, releases from nonpoint sources, although contributing a large share to any industrialized country's pollution burden, are so far included only in a limited number of PRTR systems. These nonpoint, or diffuse, sources include area sources (e.g., residential wood combustion, dry cleaners), mobile sources (e.g., automobiles, aircrafts, trains), biogenic sources (e.g., vegetation and microbial activity), and geogenic sources (e.g., soil erosion and volcanoes).

PRTR History

The OECD Council Recommendation (1996) and the subsequent Guidance Manual (1996) provided a catalyst for the development of PRTRs across the OECD countries and elsewhere. Since 1996, the number of OECD countries with operating PRTR systems has more than doubled. By 2004 at least 14 OECD countries had an operational PRTR in place (Australia, Canada, Denmark, Hungary, Ireland, Japan, Korea, Mexico, the Netherlands, Norway, Slovak Republic, Sweden, United Kingdom, and the United States). Many more countries, within the OECD and beyond, have already taken concrete steps toward the establishment of a PRTR.

Since the 'Earth Summit', there has been a more general call for information exchange on toxic chemicals and chemical risks. The Aarhus Convention created a framework and process for the potential integration of current national PRTRs, cleaner production activities, and improvement of the 'right to know' processes in general. A Working Group on PRTRs under the Convention was established in 2000 and charged with the task of preparing a legally binding instrument, a Protocol on PRTRs. The Protocol would be open to all States,

*The opinions expressed in this paper are those of the authors and do not necessarily represent the views of the OECD or of the member governments.

whether or not Parties to the Convention. The Protocol was formally adopted and signed at the Fifth Ministerial Conference, 'Environment for Europe', in Kiev, Ukraine, May 21, 2003. More than 30 States took part in the negotiations and 36 countries and the European Community signed the Protocol. Also, a new Working Group on PRTRs was established at the Kiev meeting, and its first meeting took place in February 2004 in Geneva.

Some PRTRs have been up and running for many years and even predate the Earth Summit, others have been initiated more recently, and yet others are still in the planning stages. This overview takes a closer look at some of the more fully developed PRTRs, while making reference also to those in earlier phases of development.

North America

United States' Toxics Release Inventory (TRI)

In 1984, a deadly cloud of methyl isocyanate killed thousands of people in Bhopal, India. Shortly thereafter, there was a serious chemical release at a sister plant in West Virginia. These incidents underscored demands by industrial workers and communities in several states for information on hazardous materials. Public interest and environmental organizations around the United States accelerated demands for information on toxic chemicals being released to the environment. Against this backdrop, the Emergency Planning and Community Right-to-Know Act (EPCRA) was enacted in 1986.

EPCRA's primary purpose is to inform citizens of chemical hazards in their communities. Sections 311 and 312 of EPCRA require businesses to report the locations and quantities of chemicals stored on-site to State and local governments in order to help communities prepare to respond to chemical spills and similar emergencies. EPCRA Section 313 requires the US Environmental Protection Agency (EPA) and the States to annually collect data on releases and transfers of certain toxic chemicals from industrial facilities, and make the data available to the public in the Toxics Release Inventory (TRI).

Reporting year 2002 is the 16th year that TRI data has been collected (2002 data were released by the EPA in June 2004). The amount and nature of data have changed over the years. Its initial list of chemicals has more than doubled to some 650. Passage of the Pollution Prevention Act of 1990 broadened the information TRI collects to include off-site transfers to recycling and energy recovery as well as on-site management of toxic chemicals. Beginning with reporting year 2000, thresholds were lowered for persistent, bioaccumulative toxic (PBT) chemicals. The SIC codes (Standard Industrial Classification System) covered have been expanded to include other industry sectors not initially covered, such as metal and coal mining, electrical utilities that combust coal and oil, and hazardous waste treatment facilities, as well as federal facilities.

EPA offers TRI data back through 1988 via its TRI Explorer search engine. TRI data are also offered from 1995 onwards on the National Library of Medicine's (NLM) TOXNET system. Being part of TOXNET allows easy linkages between TRI and other NLM files, such as the Hazardous Substances Data Bank and TOXLINE, containing health and environmental effects information. NLM has also implemented a TRI mapping feature called TOX-MAP that allows for geographic visualization of TRI data. An interesting value-added version of TRI is Scorecard, a project of Environmental Defense. Scorecard uses the most current TRI data and integrates it with information from other databases, including EPA's National Emissions Trends (NET) database and the Canadian National Pollutant Release Inventory (see below). Scorecard actually covers ~7000 chemicals, including the TRI set. Their full complement includes high production, toxicity, or exposure US chemicals that are part of federal or California regulatory programs. Scorecard, however, only offers TRI data from the most recent reporting year.

Canada's National Pollutant Release Inventory (NPRI)

Canada's NPRI is an outgrowth of the government's Green Plan initiative and currently falls under the renewed Canadian Environmental Protection Act (CEPA). The year 2002 was the tenth reporting year for NPRI. The 1999 renewal of the Canadian Environmental Protection Act (CEPA) included provisions that require mandatory NPRI reporting and the annual publication of a summary report. Many of the reporting requirements and thresholds are similar to the United States' TRI. Neither system requires reporting on greenhouse gases. However, the NPRI includes releases from diffuse sources, while TRI does not include this requirement. The online database search screen permits entry of terms related to the facility, chemical name or CAS registry number, province/territory/city/postal code, and SIC code (Canadian or American).

NPRI covers some 260 chemicals (in 2001). However, only 204 substances were the same in NPRI and TRI in 2001. NPRI provides information on on-site releases and off-site transfers for final disposal and other treatment. Reporting on off-site transfers to recycling and energy recovery was made

mandatory in 1998. Also, reporting on pollution prevention activities has been mandatory since 1997. However, no quantitative estimates on the achieved pollution reduction are required.

Mexico's Registro de Emisiones y Transferencia de Contaminantes

Mexico has made great strides recently in the development of its PRTR program. Voluntary reporting began with the *Registro de Emisiones y Transferencia de Contaminantes* (RETC) program. In December 2001, legislation was passed providing for a mandatory, pulicly accessible PRTR. President Fox has now signed Mexico's mandatory reporting rule and it was formally published in Mexico's *Diario Oficial* on 3 June 2004. This puts Mexico and its North American partners at the forefront of international cooperation in promoting publicly accessible pollutant release and transfer registers. Much work still remains, however, as Mexico must now formally designate the substances to be reported. This will be based upon a list of 104 chemicals under the former voluntary reporting rule.

North American Pollutant Release and Transfer Register (Commission for Environmental Cooperation (CEC)

North America is well positioned to serve as a global leader in the development and use of PRTRs nationally and regionally. Each of the three North American countries, as discussed above, has a national PRTR program. First reporting years for the United States, Canada, and Mexico were 1987, 1993, and 1997, respectively.

The CEC's North American Pollutant Release and Transfer Register project tracks and publishes information on the amounts, sources, and handling of toxic chemicals from industrial activities in North America, including analyses of trends in pollutant releases and transfers since the early days of NAFTA. Each year the CEC publishes the '*Taking Stock*' report and website, which provides a unique regional picture of pollutant data in North America, based on available data from the national PRTR systems.

In May 2002, the CEC published *Taking Stock 1999*, the sixth in series. The report featured the first-ever 5 year look at trends in pollutant releases and transfers in North America. To date, *Taking Stock* includes data from Canada and the United States only. Comparable data from Mexico are not yet available. Since the start of the CEC PRTR project, there has been roughly a 50% increase in the amount of data that are comparable between the Canadian and US PRTRs. The most recent '*Taking Stock 2001*' was released in June 2004, focusing on the releases of toxic substances to the air and trends of releases and tansfers in 1995–2001.

Europe

The Netherlands

The Dutch PRTR is a bit different from other PRTRs and comprises the inventory, analysis, localization, and presentation of emission data of both industrial and nonindustrial sources in the Netherlands. The PRTR is used as the national instrument to monitor the emissions from all sources to air, water, soil, and off-site transfers as waste. In total, some 800 substances are included in the Dutch PRTR. Data cover industry, public utilities, traffic, households, agriculture, and natural sources, and it is to some extent open to public. The emission data are partly updated every year and some 170 most important substances are covered in a report that is published annually in close cooperation with all actors in the field.

Sweden

The Swedish PRTR system (KUR) contains annual information on emissions of a number of chemical substances and groups of substances by large facilities. The creation of a register is one step in the EPA's program to improve public access to information on national emissions and also to comply with international agreements entered into by Sweden. The figures in the PRTR are taken from annual facility reports and are mainly used by the supervisory authorities. Only IPPC (EC Directive on Integrated Pollution Prevention and Control) facilities report emissions of chemical substances and groups of substances. IPPC facilities are large facilities with a capacity above certain thresholds. Small and medium size enterprises (SMEs) are therefore not included in the register, neither are emissions from diffuse sources (e.g., the use of pesticides in agriculture). However, releases from products are to some extent covered in the KUR. The number of facilities currently listed in the register is 1050. All figures are reported as total emissions per year and are not related to production volumes. This implies that available emission figures cannot be the basis for a judgment of the environmental impact of a facility; neither can they be compared with other facilities operating under different conditions. The PRTR register contains 70 substances/groups of substances in total. The selection is based on the requirements of the EPER-reporting (European Pollutant Emission Register) and those substances prioritized by OSPAR (The Convention for the Protection of the Marine Environment

of the North-East Atlantic) in 2000 as substances of concern.

United Kingdom

The United Kingdom's Pollution Inventory (formerly Chemical Release Inventory) contains details on large industrial sites as designated in the 1990 Environmental Protection Act. Local authorities regulate smaller sites. Data collection started in 1991. Presently reporting covers some 180 chemicals, including greenhouse gases and releases from diffuse sources. Inclusion of emission data from landfill sites and waste transfer stations is a new feature for 2002 data. It is currently optional for emitters of radioactive substances to report to the pollution inventory. Over the next few years reporting to the inventory of activities involving radioactive substances is likely to become compulsory. The food and drink, surface coating, and intensive agriculture industries will also begin to provide emissions data to the inventory over the next few years. The pollution inventory has been adapted to meet the reporting requirements of the European Pollutant Emission Register.

European Pollutant Emission Register

In July 2000, the European Commission adopted a decision on the implementation of a European Pollutant Emission Register (EPER) according to Article 15 of Council Directive 96/61/EC concerning Integrated Pollution Prevention and Control (IPPC). The general purpose of the IPPC Directive is to reduce pollution by industry and to control emissions from larger facilities. National governments of all EU Member States are required to maintain inventories of emission data from specified industrial sources and to report emissions from individual facilities to the European Commission. The reported data will be made accessible in a public register (EPER), which is intended to provide environmental information on major industrial activities. EU Member States were required to submit their first report in June 2003 covering emissions in 2001. The next report will be delivered in June 2006 and will cover emissions in 2004. The present EPER can be considered as a first step toward the development of a fully integrated PRTR for Europe according to the requirements of the PRTR Protocol under the Aarhus Convention.

The objectives of the EPER are: (1) collection of comparable emission data from ~20 000 individual polluting industrial sources and activities as specified in the IPPC Directive; (2) storage of the reported data in a database or register (EPER), which is publicly accessible; the register relates to emissions to air and water for 50 major pollutants; and (3) dissemination of the registered data to the public by written reports and the Internet.

Every 3 years, the European Commission will publish a report on the inventoried emissions and their individual sources. For the first time, it will be possible for the public to compare emissions from individual facilities, industrial sectors, or countries. Governments will use the EPER to monitor progress of achievements by industry in meeting environmental targets in national or international agreements or protocols.

Other Countries

Australia's National Pollutant Inventory (NPI)

Australia's NPI is an Internet database designed to provide the community, industry, and government with information on the types and amounts of certain substances emitted to the environment. In total, 90 substances are reported to the NPI. A limited reporting started in 1998–99, but the coverage of all present 90 substances commenced in 2001–02. Greenhouse gases, ozone depleting substances, and transfers of waste/chemicals are not reported to the NPI. However, releases from diffuse sources will be included on the database.

Japan

Japan published in March 2003 its first PRTR report for 2001 data on 354 chemicals based on a legislative framework. The report includes release and transfer data submitted by industry for 35 000 facilities and estimated release data for diffuse sources. Information is available in English, also for 2002.

PRTRs under Development

The foregoing discussion has, for the most part, looked at fully developed PRTRs within a fairly strict context as defined by the OECD and various international conventions. It should be noted that a larger array of countries have in place pollution inventories or a PRTR under development or consideration. Among these are all European Union Member States, Brazil, Bulgaria, Chile, Croatia, Cuba, Egypt, Kazakhstan, Moldova, Romania, Russia, South Africa, Switzerland, and Thailand. These countries, and many others, may or may not ultimately adhere to the requirements set up for a full-scale PRTR, but their efforts to report to the public on pollution are clearly in the right direction.

See also: Environmental Protection Agency, US; National Library of Medicine/TEHIP; Organisation for Economic Cooperation and Development.

Relevant Websites

http://www.environment-agency.gov.uk – Environment Agency Pollution Inventory, UK.
http://europa.eu.int – European Pollutant Emission Register.
http://www.emissieregistratie.nl – Milieumonitor, The Netherlands.
http://www.npi.gov.au – National Pollutant Inventory, Australia.
http://www.ec.gc.ca – National Pollutant Release Inventory, Canada (NPRI).
http://www.cec.org – North American Pollutant Release and Transfer Register.
http://www.oecd.org – OECD Pollutant Release and Transfer Registers.
http://www.prtr.nite.go.jp – PRTR, Japan.
http://www.naturvardsverket.se – Sweden's PRTR (KUR).
http://www.epa.gov – Toxics Release Inventory, US (TRI).
http://www.unece.org – United Nations Economic Commission for Europe (UNECE), the Protocol on Pollutant Release and Transfer Registers to the Convention on Access to Information, Public Participation in Decision-making and Access to Justice in Environmental Matters (Aarhus Convention).

Pollution Prevention Act, US

Shayne C Gad

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- Agency: US Environmental Protection Agency
- Year Passed: 1990
- Groups Regulated: Industrial manufacturers

Synopsis of Law

Rather than continue to spend millions of dollars annually to control the millions of tons of pollution each year, Congress decided to encourage industry to reduce source pollution through cost-effective changes in production, operation, and use of raw materials. These actions prevent pollution, and can also reduce the amount of raw materials used, limit liabilities of compliant industries, and reduce risks to workers as well as to the environment.

Prior to passage of the Pollution Prevention Act (PPA), control efforts within industry were reactive, focusing on treatment and disposal of waste, with pollution prevention also referred to as 'P2'. The act proposed a front-end approach to pollution control, reducing the amount of materials entering the production process. It also suggested technical support to business in order to put source reduction into practice. The policy states the following:

The Congress hereby declares it is to be the national policy of the United States that pollution should be prevented or reduced at the source whenever feasible; pollution that cannot be prevented should be recycled in an environmentally safe manner, whenever feasible; and disposal or other release into the environment should be employed only as a last resort and should be conducted in an environmentally safe manner.

Under the PPA, the Environmental Protection Agency (EPA) established an office responsible for creating standards to measure source reduction, ensuring that EPA policy is consistent with this initiative, and providing the public with such information. The act also established a Source Reduction Clearinghouse to promote industry efforts by providing information and workshops, helping set measurable goals, and establishing incentive and reward systems for efforts or innovations. Incentive systems included matching grants to states to establish their own source reduction programs.

The PPA also included specific source reduction actions in conjunction with the businesses required to file an annual toxic chemical release form under the Superfund Amendments Reauthorization Act. The additional toxic chemical source reduction and recycling report documents the amount of the chemical entering the waste stream, the amount that is recycled, and efforts to reduce source use. In turn, EPA is required to provide a detailed evaluation report of the source reduction program to Congress every 2 years.

See also: Clean Air Act (CAA), US; Clean Water Act (CWA), US; Comprehensive Environmental Response, Compensation, and Liability Act, US; National Environmental Policy Act, US; Toxic Substances Control Act, US.

Relevant Website

http://www.epa.gov – US Environmental Protection Agency (EPA), Pollution Prevention. See also: Pollution Prevention Act of 1990.

Pollution, Air

Terry Gordon

Types and Sources of Air Pollutants

Both man-made and natural sources contribute to the particles and gases that pollute our ambient environment. Episodic natural events, such as fires, wind erosion, and volcanic eruptions, produce considerable amounts of particulate matter and gases including mineral ash, pyrolysis products of combustion, carbon monoxide, and carbon dioxide. Although these 'natural' particles and gases can have significant global effects such as short- and long-term alterations in weather conditions, little is known about the health effects resulting from inhalation of these materials. Obviously, little can be done to alter the contribution of pollutants from natural sources such as volcanic eruptions. A great deal more information is known regarding the generation, underlying chemistry, and adverse health effects of air pollutants from man-made (anthropogenic) sources. This continued interest in understanding the generation and health effects of man-made air pollutants stems largely from the fact that measures can be taken to control these pollutants and thus modify adverse health effects. Such control measures are often promulgated and regulated at the governmental level and are based on comparisons of the tangible (e.g., increased morbidity and mortality and financial) and intangible (e.g., quality of life) costs to society and the environment versus the cost of control measures.

Both stationary and mobile sources contribute to the particulate matter and gases that make up polluted urban and rural environments. Fossil fuel-powered electricity plants, heat generators, and waste incineration sites represent the major stationary point sources of pollution. Industrial processing plants such as smelters also produce a wide range of particulate matter and gases. Because these pollution sources are stationary, significant differences in both the quantity and the makeup of regional air pollution can occur. For example, sulfur is present in fossil fuels (primarily in coal) used for heat production in the northeastern United States. The resulting acid components of ambient particles in the northeastern United States are thus primarily sulfates, whereas the acidic fraction of airborne particles in California is primarily nitrates.

A major portion of ambient air pollution results from gasoline- and diesel-powered automobiles and trucks. The mobile and ubiquitous nature of motor vehicles makes their pollution products widespread. While the presence of lead in automobile exhaust has been virtually eliminated, the contribution of motor vehicles to ambient concentrations of nitrogen oxides, hydrocarbons, and carbon monoxide in urban atmospheres is great. Indeed, transportation sources are responsible for 45% of the nitrogen oxides and 80% of the carbon monoxide emissions in the United States. Sunlight can drive a series of chemical reactions involving nitrogen oxides and hydrocarbons (a process known as photochemical oxidation), which result in secondary pollutants such as ozone. Though a secondary pollutant, ambient ground-level ozone is a major health concern for both urban and rural dwellers and produces agricultural crop losses and tree damage approaching several billions of dollars each year.

Regardless of the source of primary and secondary air pollutants, meteorological conditions play a significant role in the formation and transport of gases and particulate matter. One well-documented example occurs when sulfur dioxide released from industrial point sources in the northeastern United States forms acidic particles. These acidic precursors undergo long-range transport resulting in adverse effects due to acid rain in southern Canada. Thus, movement of masses of air can reduce ambient levels of pollutants in one region at the expense of air quality in another region. Meteorologic conditions also influence the creation of photochemical smog. Inversions occur when cooler air is trapped beneath a blanket of warm air, resulting in stagnant weather patterns. In southern California, the combination of inversions, sunlight, and motor vehicle emissions drives the photochemical reaction of trapped precursors and results in high ozone concentrations.

Regulation of Air Pollution

Reductions in ambient concentrations of some but certainly not all air pollutants have taken place over the past decade. In the United States, the US Environmental Protection Agency (EPA) is the primary agency responsible for promulgating and regulating air pollution standards. National ambient air quality standards (NAAQS) have been established for six classes of outdoor pollutants: lead, carbon monoxide, ozone, nitrogen dioxide, sulfur dioxide, and particulate matter. These standards (**Table 1**) are periodically reviewed and updated based on currently available data regarding adverse health effects. Bases on new evidence, the standards for ozone and

Table 1 National ambient air quality standards (NAAQS)

Pollutant	*Primary standard*	*Type of average*	*Nonattainment population*[a]
Lead	$1.5\,\mu g\,m^{-3}$	Quarterly average	0.01
Carbon monoxide	35 ppm ($40\,mg\,m^{-3}$)	1 hour	19.0
	9 ppm ($10\,mg\,m^{-3}$)	8 hour	
Ozone	0.08 ppm	8 hour	>100
	0.12 ppm	1 hour	>100
Nitrogen dioxide	0.053 ppm ($100\,\mu g\,m^{-3}$)	1 year	0
Sulfur dioxide	0.14 ppm ($365\,\mu g\,m^{-3}$)	24 hour	2.7
	0.03 ppm ($80\,\mu g\,m^{-3}$)	1 year	
Particulate matter (PM)			
PM 10	$150\,\mu g\,m^{-3}$	24 hour	29.2
	$50\,\mu g\,m^{-3}$	1 year	
PM 2.5	$65\,\mu g\,m^{-3}$	24 hour	data not available
	$15\,\mu g\,m^{-3}$	1 year	data not available

[a] Millions of persons living in counties with air quality levels not meeting NAAQS in 1992.

particulate matter were recently changed. The ozone standard was amended to include an 8 h average to reflect results of clinical studies that demonstrated cellular and biochemical pulmonary changes in exercising human subjects exposed to 0.10 ppm ozone for several hours. The particulate matter standard was also changed to include a standard for fine particles. Those metropolitan sites in which a particular pollution level exceeds the NAAQS are designated as nonattainment areas. Currently, more than half of the population in the United States lives in a nonattainment area for at least one regulated air pollutants (**Table 1**). The Air Quality Index, a more general ranking of air quality, is used for the daily reporting of air quality to the layperson via newspapers and telecasts in most major cities (**Table 2**). It is noteworthy that five pollutants account for ~98% of the total mass of air pollution and that gaseous emissions make up the majority of air pollutants (**Table 3**). These figures do not take into account fugitive dust emissions that are not inhalable (i.e., coarse dust which is generated by wind erosion, farming, construction, and mining and quickly settles out due to its large size). Despite extensive legislation (three Clean Air Acts within the past 30 years) to set primary and secondary standards for the protection of human health and the environment, respectively, considerable numbers of air toxics are currently unregulated.

Control and reduction of ambient air pollutants in the United States has met with varying degrees of success. Unleaded gasoline now accounts for 99% of all gasoline sales. This change has virtually eliminated mobile sources as emitters of lead and reduced ambient lead levels by more than 75%. Likewise, stationary point sources of lead emissions, primarily industrial smelters, have dropped by more than 90% over the past three decades, although significant problems exist with individual smelters. Over the past decade, programs in reducing all Criteria pollutants have been successful. The reduction program for nitrogen dioxide has been partially successful only recently. A slight decrease in total nitrogen dioxide emissions occurred during a time period in which total motor vehicle miles in the United States increased substantially. A lack of major changes in ambient levels of nitrogen oxides and volatile organic compounds has resulted in only marginal success in reducing ambient levels of the secondary pollutant ozone. The long-term trend for ozone concentrations

Table 2 Air Quality index values

Index value color	*Descriptor*[a]	*Level of health concern*
301–500	Maroon	Hazardous
201–300	Purple	Very unhealthy
151–200	Red	Unhealthy
101–150	Orange	Unhealthy for sensitive groups
51–100	Yellow	Moderate
0–50	Green	Good

[a] General health descriptor used in the lay press and media.

Table 3 Emission estimates for the United States (2003)

Pollutant	*Total emissions*[a]	*10 Year trend*[b]
Carbon monoxide	93.7	21% decrease
Nitrogen dioxide	20.5	12% decrease
Sulfur dioxide	15.8	31% decrease
Volatile organics	15.4	25% decrease
Particulate matter (PM)		
PM 10	2.3	22% decrease
PM 2.5	1.8	17% decrease
Lead	0.003	5% decrease

[a] Millions of tons/year for 2003.
[b] Percentage change in estimated emissions between 1993 and 2003.

is downward, although meteorologic conditions appear to modify peak ozone levels monitored throughout the United States (high ozone levels have been measured during summers with hot, dry conditions and low levels measured during cool summers). In summary, legislative efforts have been successful in reducing ambient air pollution over the past three decades. Reduction of emissions from mobile sources such as motor vehicles has met with the greatest success, while reducing emissions from stationary point sources has often proven difficult as a result of conflicting interests of business, state and federal regulations, and enforcement agencies. While progress has been made in reducing ambient oxidant pollutants, it should be noted that a significant problem still exists and in the United States more than 100 million people lived in countries that exceeded the ozone standard in 2003.

Health Effects of Air Pollution

There is mounting evidence that a number of air pollutants play a causal role in adverse health effects and that copollutants such as acid aerosols, ozone, and nitrogen oxides can have synergistic effects with each other. The major challenges for environmental health scientists are to identify the acute and long-term adverse health effects of ambient air pollution, pinpoint the relevant concentrations at which these effects occur, and determine sensitive subpopulations. This latter point is important in developing risk assessment paradigms as current federal legislation in the United States acknowledges the importance of protecting the health and welfare of all individuals.

In general, a great deal more is known about the acute effects of ambient air pollutants than is known about the chronic effects. The following discussion will outline the findings of epidemiologic, controlled clinical and animal studies that have examined the adverse health effects of outdoor air pollutants. More detailed information can be found in the Further Reading section.

Ozone

Exposure to ozone in the ambient air is a major health concern in urban and rural communities throughout the United States. Current strategies to control the exposure of the general population to this highly reactive gas have been only marginally successful. Indeed, tens of millions of people reside in communities in which the 1 h and 8 h ozone NAAQS has been exceeded.

Substantial evidence from epidemiological and controlled clinical studies suggests that acute ozone exposure at current ambient levels is associated with adverse respiratory effects in human subjects. The functional and symptomatic response of human subjects to inhaled ozone, however, appears to be highly variable. After performing moderate exercise during a single 6.6 h exposure to 0.12 ppm ozone, the change in forced expiratory volume in 1 s (FEV1) ranged from no decrement to -39% in healthy adult volunteers. The decrement in FEV1 and the increase in respiratory symptoms were dose dependent, with some volunteers responding to as little as 0.08 ppm ozone. Significant increases in the airway responsiveness to inhaled methacholine have also been observed after exposure to near-ambient ozone concentrations in laboratory studies. These functional effects are accompanied by an inflammatory response that occurs shortly after exposure and persists for at least 1 day. An influx of neutrophils and an increase in a number of mediators, including eicosanoids, neutrophil elastase, and cytokines, were measured in bronchoalveolar lavage fluid recovered from subjects exposed to near-ambient concentrations of ozone.

The adverse functional effects observed in controlled clinical studies are similar to those reported during exposure to ambient air. Decrements in lung function have been noted in a series of camp studies in which children were exposed to ambient ozone during normal outdoor play activity. Compared to controlled chamber studies, greater decrements in lung function were observed in the camp studies when the data were normalized for ozone concentration. A number of factors may explain the greater response in the camp study, but the most likely reason is the simultaneous exposure to ambient copollutants such as acid aerosols. Epidemiologic studies have found strong correlations between respiratory symptoms, such as cough, throat irritation, and chest discomfort, and ambient ozone levels. Exacerbation of asthma, increases in hospital admissions for respiratory infections, and excess mortality have also been reported to be associated with oxidant air pollution episodes. Thus, a number of epidemiologic, field, and clinical studies provide evidence that adverse respiratory effects occur after acute exposure to ozone at or below the current NAAQS. Animal studies have corroborated these findings, although test animals in general appear to be less sensitive than human subjects to ozone.

Despite ample evidence for an acute response to ozone in human subjects, relatively little is known about the cumulative effects of acute injury and possible progression to adverse chronic lung

dysfunction. Many studies have found that the functional decrements and symptoms observed after a single exposure to ozone lessen or are absent upon repeated exposure. The phenomenon of tolerance to the acute effects of ozone was described decades ago in animal studies. Clinical studies examining ozone-induced tolerance have clearly demonstrated that functional and inflammatory changes that are typically observed after the first day of exposure are attenuated by the second or third day of exposure for both normal and asthmatic subjects. Interestingly, the development of tolerance after repeated ozone exposure appears to occur for some functional parameters but not for others. For example, it has been observed in healthy adults that despite the rapid development of tolerance to decrements in FEV1 following repeated ozone exposure, ozone-induced increases in airway responsiveness to methylcholine were sustained throughout the five daily exposures. In addition, increases in markers of inflammation, such as an influx of neutrophils, are attenuated after five daily exposures. Markers of cell injury, however, do not appear to adapt as readily to repeated ozone exposure. These latter findings and similar results observed in animal studies suggest that although the respiratory tract is able to adapt to a major portion of the acute effects of ozone, long-term consequences may occur.

The few population-based and animal toxicology studies examining the chronic pulmonary effects of ozone suggest that ozone may be associated with long-term reductions in lung function and pathological changes. Animal studies using concentrations above the current NAAQS reveal that the centriacinar region of the airways and the nasal cavity are the most sensitive to pathological changes induced by chronic ozone. Epidemiologic studies have demonstrated that chronic exposure to ozone is associated with decrements in lung function and increases in the incidence and severity of asthma. The ability of these epidemiologic studies to establish cause and effect is hampered by confounding factors such as copollutants. Thus, the question whether chronic adverse health effects are clearly associated with ambient ozone exposure has not been answered at this time.

Sulfur Oxides

Significant and, on occasion, disastrous adverse health effects have accompanied acute air pollution episodes involving reducing-type pollutants. In the middle of this century, meteorologic inversion conditions resulted in high levels of particulate matter and sulfur dioxide in the Meuse Valley in Belgium, Donora in Pennsylvania, and London. Excess mortality accompanied each of these pollution episodes and has been attributed to the smoke and sulfur dioxide generated by fossil fuel combustion. A number of recent epidemiologic, clinical, and animal studies have confirmed that both particulate matter and sulfur oxides produce adverse health effects. These adverse effects have been observed during pollution episodes in which the gas and particle concentrations do not approach the magnitude of the three incidences mentioned previously. Delineating the relative contribution of particulate matter and sulfur oxides to these adverse effects is difficult because of the chemicophysical association of sulfur oxides and particles. This section is limited to the current state of knowledge on sulfur oxides and acid aerosol-related health effects. The following section will discuss particulate matter-related effects.

Sulfur dioxide is generated during the combustion of fossil fuels (primarily coal) containing traces of sulfur. Controlled laboratory studies using human subjects and test animals have demonstrated that sulfur dioxide can produce functional and pathological changes. These changes include increases in airway resistance and in mucus production. In general, the concentrations of sulfur dioxide necessary to produce these changes are greater than those encountered in the ambient environment. A notable exception is the bronchoconstrictive effect of sulfur dioxide on atopic and asthmatic subjects. Inhalation of 0.4 or 0.5 ppm sulfur dioxide in combination with moderate exercise causes substantial bronchoconstriction, shortness of breath, and cough in these sensitive individuals. Similar changes occur in normal (nonatopic) individuals only after exposure to at least a magnitude greater concentration of sulfur dioxide.

Despite the clear evidence of a subpopulation of individuals sensitive to near-ambient peak levels of sulfur dioxide, the two-decade-old NAAQS for sulfur dioxide has not been changed nor has a short-term peak standard been instituted. A considerably greater amount of attention has been placed on the contribution of airborne particulates, particularly those associated with sulfur oxides, to adverse health effects. The carbon-, mineral-, and heavy metal-based particles produced during fossil fuel combustion and smelting promote the conversion of sulfur dioxide to sulfuric acid. Recognition of sulfur dioxide–particle interactions comes as a result of findings garnered from a number of animal studies and the characterization of sulfuric acid, ammonium sulfate, and ammonium bisulfate associated with atmospheric particles. The importance of the coexistence of sulfur oxides and particulate matter is reflected in the

difficulty of epidemiology studies to separate the contribution of each pollutant to adverse health effects.

Epidemiological evidence from both Europe and North America suggests that acid aerosols formed by gas–particle interactions in the atmosphere play a major role in the adverse health effects seen during severe and moderate pollution episodes. The increases in mortality observed in London from 1958 to 1972 were more closely associated with acid aerosol concentrations than other pollutants such as smoke and sulfur dioxide. In the United States and Canada, cross-sectional analyses have demonstrated that ambient sulfate concentrations are better than indices of particulate concentrations as a predictor of excess mortality and hospital admissions due to air pollution. A prospective cohort study, known as the Six Cities Study, has found that increased mortality from cardiopulmonary deaths and lung cancer were strongly associated with sulfate and particulate concentrations. This same study has demonstrated that the incidence of bronchitis in children is correlated with ambient levels of acid aerosols. Similarly, in northern Europe, an acidic pollution episode in 1985 has been linked with significant excesses in respiratory mortality and morbidity and with persistent decrements in pulmonary function in children. In summary, a large body of evidence suggests that acid aerosols play a significant role in the adverse health effects attributed to air pollution.

Epidemiology studies are limited in their ability to establish direct cause and effect relationships. Many confounding factors such as smoking, occupational exposure, and copollutants such as ozone may contribute to observed effects and, for this reason, investigators have exposed human volunteers and animals to acid aerosols under controlled conditions.

Animal studies have demonstrated that exposure to near-ambient concentrations of sulfuric acid produces both conducting airway and alveolar changes, including increased airway resistance, airway hyperresponsiveness, and alterations in clearance mechanisms and macrophage function. Controlled human exposures to acid aerosols, however, have demonstrated few pulmonary effects at concentrations below 500–1000 $\mu g\,m^{-3}$. The adverse effects reported to occur after acute exposures to sulfuric acid aerosols have largely been observed in atopic subjects, are small in magnitude, and are readily reversible. Therefore, a research need has developed to explain the difference between the results of epidemiological studies and the paucity of data demonstrating adverse health effects in controlled human studies. One possible cause of this discrepancy is the type of acid aerosols used in the laboratory studies. Although pure sulfuric acid droplets are used almost exclusively in controlled exposures, ambient acid aerosols are chemically complex and are proposed to be composed of a core consisting of carbon, minerals, or heavy metals surrounded by acidic (sulfuric or nitric acid) surface material. Thus, knowing which chemical species is responsible for acid aerosol-induced adverse health effects is fundamental in developing proper control strategies for reducing air pollutants at their source.

Particulate Matter

Particulate emissions are by-products of fuel combustion, industrial processes, and motor vehicles and are believed to have a significant potential for causing adverse health effects. Carbonaceous material present in atmospheric aerosols is a combination of elemental carbon and organic and inorganic compounds. Particulate matter may also consist of fly ash, minerals, or road dust and contain traces of a number of heavy metals. Population-based studies have consistently found that the association between adverse respiratory effects and particulate concentrations occurs in a number of regions throughout the United States. This association is strongest for PM_{10} and $PM_{2.5}$ indices (particulate matter less than 10 and 2.5 μm in diameter, respectively). The observed adverse effects include increases in total mortality, mortality due to respiratory and cardiovascular causes, chronic bronchitis, and hospital visits and admissions for asthma. Elderly or unhealthy individuals and infants appear to comprise subpopulations that are most sensitive to the adverse health effects of PM.

Because the chemical makeup of particles varies greatly from region to region, the identification of the factor(s) responsible for the adverse health effects associated with PM is merely conjecture at this time. Few controlled human studies have used realistic particles and thus have contributed little to our understanding of particle-induced injury. Animal studies have been somewhat more productive and have demonstrated that particle-induced lung injury may be dependent on particle size, the presence of transition metals, and surface acid content. Effects of exposure to carbonaceous particles have been reported in studies investigating the toxicological significance of automotive diesel engine exhaust and fly ash. Long-term exposures to automotive diesel engine exhaust were found to cause focal fibrotic and proliferative lung disease accompanied by a progressive accumulation of soot in the lungs and impaired alveolar clearance. Exposure of rats to high concentrations of diesel exhaust was also associated

with an increase in lung cancer. Only minimal lung injury and irritant potency have been noted after repeated exposure of test animals to resuspended fly ash. Animal studies using freshly formed fly ash suggest that physical, chemical, and especially surface characteristics of the fly ash change substantially during the collection, storage, and resuspension processes. More recent studies have used concentrated ambient PM to examine the biological plausibility and mechanisms underlying the adverse health effects of PM. These studies include both acute animal and human controlled exposure studies using individual components (e.g., ultrafine carbon particles) as well as concentrated ambient PM. Additionally, repeated exposure studies of concentrated ambient PM in test animals and *in vitro* studies have been performed to delineate the components of PM, which may contribute to the respiratory and cardiac effects observed in epidemiology studies. As yet, animal toxicology and controlled clinical studies have not yet provided clear answers to questions regarding the factor(s) responsible for the adverse health effects temporally associated with PM pollution episodes.

Nitrogen Oxides

Nitrogen oxide is produced in high-temperature combustion processes and is rapidly converted to nitrogen dioxide. Nitrogen dioxide is an irritant gas that produces oxidant lung injury similar to that produced by ozone. Nitrogen dioxide is far less potent than ozone and few functional or pathological changes have been observed in animals exposed to <0.5 ppm nitrogen dioxide. Pathological changes occur primarily in the terminal bronchioles and the alveolar region, although changes in mucociliary clearance have been observed during chronic exposures. Both long- and short-term exposures to nitrogen dioxide can increase the susceptibility of animals to respiratory infection. Studied in a number of animal species, this effect includes increased mortality, decreased survival time, and impaired clearance of instilled pathogens. These findings reflect those obtained in epidemiologic studies that have found an increased incidence of respiratory infections in homes with gas appliances.

Nitrogen oxides other than nitrogen dioxide have been studied for possible adverse health effects. Chemical analysis of ambient aerosols collected in southern California has revealed that nitrates exhibit particularly high values compared to other parts of the United States. These aerosols are generally acidic in nature and are composed of nitric acid and nitrate salts that are formed through photochemical reactions with nitrogen dioxide and other oxides of nitrogen. These forms of nitrogen oxides contribute to acid aerosol formation in the ambient air and result from particle surface–gas interactions similar to those which have been described for sulfuric acid generation. Unique to the conditions of the coastal regions of California, acid fog forms from the interaction of nitrogen oxides and fog water droplets. A paucity of toxicologic and epidemiologic data does not allow a clear assessment of the health effects of either nitric acid-based particle.

Although research has clearly demonstrated a potential for nitrogen oxides, particularly nitrogen dioxide, to have serious health consequences, few exceedances of the NAAQS occur (see **Table 1**). In general, health researchers are more concerned with (1) the key role nitrogen oxides play in the photochemical reactions which produce ozone and (2) the presence of nitrogen oxides indoors (both in occupational settings and in homes). Ambient concentrations of nitrogen oxides are generally lower than those found in grain silos and in homes using fossil fuel-consuming appliances.

Carbon Monoxide

Despite an increase in motor vehicle miles traveled over the period of 1990–2000, total emissions for carbon monoxide decreased by 29%. This dramatic change is attributed largely to controls initiated by the Federal Motor Vehicle Control Program. These figures do not reflect the additional decreases in carbon monoxide emissions that have resulted from the use of oxygenated fuels since 1992. Under the Clean Air Act of 1990, oxygenated fuels are required in all areas that do not meet the NAAQS for carbon monoxide during the winter months (when carbon monoxide levels are highest). Preliminary results of the oxygenated fuel program suggest that further decreases in carbon monoxide emissions will be achieved.

In general, ambient exposure to carbon monoxide is directly related to one's proximity to motor vehicle exhaust. Away from highways and industrial combustion processes, ambient carbon monoxide concentrations rarely exceed 1 ppm. Carbon monoxide levels can reach 3 or 4 ppm near roads and 5 ppm in the passenger compartment of automobiles. Heavier traffic conditions are typically associated with peak concentrations of 10–50 ppm. Even greater carbon monoxide concentrations can be encountered by workers in confined spaces such as tunnels. Significant exposures to carbon monoxide can also occur indoors. Levels as high as 10 000 ppm have been recorded in enclosed spaces in which a firefighter might enter. Operation of gasoline-powered equipment within a building can also result in significant

carbon monoxide levels with ill-effects (e.g., Zamboni ice cleaners in skating rinks). Importantly, significant amounts of carbon monoxide are present in cigarette smoke. In nonsmoking human subjects, carboxyhemoglobin levels do not exceed 0.4% if environmental carbon monoxide levels are zero. Carboxyhemoglobin levels in cigarette smokers, however, can range from 5% to 10%.

Carbon monoxide is classified as a chemical asphyxiant. Its detrimental effects are mediated by its ability to combine with hemoglobin and other oxygen-carrying or -utilizing proteins. By binding avidly to hemoglobin and causing the formation of carboxyhemoglobin, the carrying capacity of hemoglobin for oxygen is reduced proportionately. One of the most sensitive measures of ill-effects after carbon monoxide inhalation is neurological testing. As little as 4% carboxyhemoglobin impairs neurologic function in repetitive tasks. In patients with preexisting angina or chronic pulmonary obstruction, increases in carboxyhemoglobin levels of only 2% were found to produce quicker onset of angina and dyspnea, respectively, during exercise. Reduced night and peripheral vision accompany carboxyhemoglobin levels of 10%. As levels exceed 10%, headaches may occur and at carboxyhemoglobin levels of 20–30%, nausea and weakness ensue. Decreases in mental function, collapse, and coma are evident as carboxyhemoglobin exceeds 35%.

Thus, carbon monoxide can produce a wide range of adverse effects. The concentration of carbon monoxide encountered in urban environments is relatively low and may have little effect on normal individuals. Several subpopulations, however, may be sensitive to current ambient exposure levels of carbon monoxide. These groups include individuals with chronic obstructive pulmonary disease, exertional angina, and cardiac arrhythmias. Fetuses may also be affected by carbon monoxide. Carbon monoxide binds more tightly to fetal hemoglobin and is cleared more slowly. Animal studies have demonstrated that maternal carbon monoxide exposure can reduce birth weight and increase neonatal mortality. Epidemiologic findings appear to confirm this effect of environmental carbon monoxide exposure on fetuses, although the confounding influences of smoking and indoor sources of carbon monoxide are hard to eliminate.

Lead

Research on the health effects of chronic, low-level lead exposure is quite extensive and has been garnered from both epidemiologic and animal studies. The most critical of these adverse health effects have occurred in children and include deficits in physical and neurobehavioral development. In adults, small but consistent increases in blood pressure are significantly correlated with increases in blood lead concentrations. Acute, high-dose lead exposures result in more severe toxicological effects.

Exposure to lead can occur via a number of pathways including ingestion (drinking water, food, and soil) and inhalation. Although ingestion of lead contributes the majority of the average individual's body burden of lead, airborne lead has been estimated to be responsible for 7–40% of blood lead. The major sources of airborne lead are gasoline additives, metal smelters, and battery manufacturing/disposal. Total emission for lead has decreased dramatically over the past two decades and has reduced ambient air concentrations by 90% nationwide. The decrease in total emissions and ambient concentrations is a direct result of federal regulations issued by US EPA requiring the removal of lead from gasoline. The dramatic decrease in lead emissions has been paralleled by an equally impressive decrease in average blood lead levels, making this one of the most successful federal intervention programs in the field of environmental health. Over a 4 year period (1976–80), average blood levels decreased from ~ 15.5–$9.5\,\mu g\,dl^{-1}$. Despite the improvement in nationwide airborne lead concentrations, industrial point source release is still a problem. As of 2004, 3 areas in the United States were designated as nonattainment areas in regard to airborne lead and ~ 10 million people resided in counties that do not meet the NAAQS for lead.

Future Directions and Control Strategies

Improvements in air quality in the United States have occurred as a result of federal regulations promulgated by the Clean Air Acts of 1970 and 1990. While the decrease in emissions for some NAAQS pollutants has been impressive (e.g., lead and carbon monoxide), only minor changes have been documented for others (e.g., nitrogen and sulfur oxides and PM_{10}). Moreover, as of 2003, nonattainment regions have been identified for five of the six NAAQS pollutants. Thus, it is important to acknowledge that a major air pollution problem still exists. The lack of significant improvement in various pollutant categories occurs as a result of several factors including economics, technological limitations, inability to identify proper control strategies, and politics. Reduction of pollutant emissions from point sources, in particular, has proven to be difficult to regulate and enforce. The Clean Air Act is not a static regulation, and changes have been made. Recent promulgated changes to the New Source Review have been made to give the industry more flexibility

without damaging the environment, but opponents claim these changes will institute a delay in cleaning the nation's air. Such controversies will arise as cost/benefit analyses and politics enter into the quasi-science of risk assessment.

The reported emissions for the six pollutants with NAAQS are only a portion of the total amount of toxic substances released by mobile and point sources. While regulations and controls set in place to reduce the release of particulate matter, volatile organics, and nitrogen oxides will also reduce the emissions of many air toxics, it is estimated that 1 million tons of air toxics are released in the United States each year. Air toxics are generally defined as hazardous air pollutants, other than the six NAAQS pollutants, with the potential for causing increases in mortality or serious illnesses. The Clean Air Act Amendments of 1990 identify 189 substances requiring regulation. Regulation of these air toxics necessitates technology-based standards for reducing emissions and establishing an accidental release program. The top 10 air toxics, in terms of total emissions, are toluene, methanol, methyl ethyl ketone, xylene, chlorine, hydrochloric acid, carbon disulfide, and chlorinated alkanes and alkenes. Over a 9 year period (1987–95), a sustained downward trend in total emissions of these air toxics was obtained. It must be emphasized that provisions in the Clean Air Act Amendments of 1990 focus on point sources of air toxics emissions rather than individual substances. Thus, key source categories have been identified and are to undergo prompt regulation for reducing hazardous emissions. Examples of key emissions sources for which regulations have been developed include chemical manufacturing plants (which emit as many as 150 of the 189 hazardous air toxics), coke oven batteries, dry cleaning facilities, ethylene oxide sterilization facilities, industrial cooling towers, and chromium electroplating operations.

See also: Clean Air Act (CAA), US; Combustion Toxicology; Ecotoxicology; Environmental Toxicology; Lead; Ozone; Photochemical Oxidants; Pollution, Air Indoor; Pollution, Soil; Pollution, Water; Respiratory Tract.

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Pollution, Air Indoor

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Introduction

Indoor air quality is one of the key determinants of the acceptability of an indoor environment. Indoor air pollution in occupied buildings is often higher than outdoor air pollution. This is true for modern buildings in developed countries when significant sources of indoor air pollution exist, as well as for buildings in developing countries, particularly in rural areas, where people use open stoves for cooking and heating. The World Health Organization (WHO) has estimated that indoor air pollution from the use of solid fuels in 'high-mortality' developing countries is ranked fourth among the 10 leading risk factors as percentage causes of the global disease burden.

Half of the world's population, living mostly in developing countries, is exposed to indoor air pollution, which is estimated to cause 36% of all lower respiratory infections and 22% of chronic obstructive pulmonary disease (COPD). Indoor air pollution is a concern in the developed countries as well, where energy efficiency improvements sometimes make houses relatively airtight, reducing ventilation, and raising pollutant levels. The risk perception of people with respect to indoor versus outdoor air pollution is, however, often characterized by a lack of awareness that the indoor air environment may contain some of the same pollutants found outdoors and quite a number of different ones. Indoor air pollution is, in fact, not a new problem. When early humans discovered fire and used it to heat their shelters, they must have found that one of its undesirable side effects was production of and exposure to smoke. Attempts to provide adequate ventilation may have been made, but success was only partial in that mummified human lungs from the preindustrial age show considerable carbonaceous pigmentation.

It has become increasingly evident that the indoor environment is a significant source of personal exposure to various air contaminants, for example, formaldehyde and other volatile organic compounds (VOCs), some of which can reach fairly high concentrations. However, any health effects from exposure to indoor air pollutants are a function of the total exposure, which is related to the air pollutant concentration, exposure duration and frequency, the actual indoor compartment of exposure, and the population group exposed. For example, even when indoor concentrations are low, exposures may be of long duration and the total, or cumulative, dose can be quite high. This reflects the fact that people can spend upwards of 90% of their time indoors, be it at home, at the office, in a school, or in shopping malls or in cars, buses, trains, or other forms of public transit. In many instances, indoor sources actually provide the bulk of personal exposure to certain airborne toxicants (e.g., aldehydes) and the only source of exposure to others. Furthermore, the population exposed largely indoors is much more diverse than that exposed in occupational environments or even in ambient outdoor air. In addition to healthy adults, it can include infants, children, and people with medical conditions, all of whom may be especially vulnerable to certain toxicants.

For a long time, remaining indoors was considered to afford protection from air pollution, and early studies of indoor air quality were generally concerned with examining the ratios of indoor to outdoor concentrations of various contaminants since it was felt that indoor contaminant levels were controlled primarily by outdoor concentrations. Outdoor pollutants can indeed infiltrate indoors through cracks, windows, doors and other openings in buildings and through ventilation systems, or they be carried indoors by building occupants. However, the relative amount of an outdoor pollutant found indoors depends largely on its physicochemical properties. For example, highly reactive gases like ozone may be removed from the air in or prior to entering an indoor environment, and the resulting indoor concentrations would be much lower than those found outdoors. Indoor versus outdoor (I/O) ratios in homes reported in the literature for total suspended particulate matter (TSP) range from 0.2 to >1.0; for PM_{10} I/O ratios range from 0.4 to 1.5. In the smaller size fractions (the subscript for PM refers to the aerodynamic diameter of the particles, in microns, with the number being the high end of the aerodynamic particle diameter range of interest), annual average I/O ratios for fine particulate matter $<2.5\,\mu m$ ($PM_{2.5}$) was found close to unity in nonsmoking homes.

It is now very clear that indoor air contaminants are not totally derived from outdoor sources, but that numerous contaminants can be directly released into the indoor environment from local sources, such as cooking over an open flame, from smoking, from heating systems, from modern synthetic building and furnishing materials, from consumer products and clothing, and even from natural sources, including the normal biological activities of building occupants. Many of these contaminants have been found to occur in increasing levels over recent years due to the attempt to make homes, and other buildings, more airtight for energy conservation. This reduces the rate of air exchange between the outside (fresh air) and inside environments. For example, older homes may have air exchange rates which are 2 to 10 times greater than those found in newer houses. The result is that in newer houses, levels of contaminants can be many times higher than the concentration of these same materials in the outdoor environment, if they occur outdoors at all.

Buildings are not the only sources for exposure to indoor air contaminants. Many people spend a significant portion, often up to 5%, of their day in transit, and public transportation or walking, running, bicycle riding, etc., provide additional opportunities for exposure to various toxicants. Although the air exchange rate in most forms of public transportation is generally higher than in buildings, in many cases the number of occupants per unit volume of air is much greater. A good example of this is modern aircraft, which also may recirculate as much as 50% of the interior air, leading to enhanced concentrations of

contaminants such as ozone, carbon dioxide, and volatile constituents of heated oil and hydraulic fluids. Further, during ground operations, exhaust fumes and deicing fluids can enter into the air supply system.

The major sources of air pollutants found in buildings are described below. However, the mere presence of a potential contaminant source does not necessarily mean that exposure will ensue. This is because the extent of exposure, if any, often depends on the physical nature of a source or the manner in which it is used; an example of this is asbestos, as will be discussed later. Furthermore, the health significance of exposure to indoor pollutants may not always be clear. While many of these toxicants may have adverse effects under exposure conditions found in occupational and other environments, often much less is known about biological responses with prolonged exposures at concentrations common in indoor environments.

Sources of Indoor Air Contaminants

Combustion By-Products

One very common source of indoor air pollutants is the combustion of biomass or fossil fuels, such as in gas ranges (including pilot lights), wood-burning stoves and fireplaces, and gas and kerosene space heaters. These emit both particles and gases. Particles consist of fine ($PM_{2.5}$) and ultrafine ($PM_{0.1}$) particulate matter (PM) (the subscript number refers to the aerodynamic diameter of the particles, in microns, with the number being the high end of the aerodynamic particle diameter range of interest), carbon soot, various mineral constituents of the fuels, and organic compounds, while emitted gases include carbon monoxide, carbon dioxide, nitrogen dioxide, nitric oxide, and, depending on the fuel used, sulfur dioxide and various organics. The amounts of specific contaminants emitted vary depending on the fuel type, the combustion process used, and the nature of the appliance. For example, properly operated gas heaters and stoves emit little if any PM, while wood burning stoves emit much greater amounts. When properly used and vented, many potential contaminants from combustion sources do not remain within the indoor environment, thus becoming outdoor pollutants. However, because combustion activities tend to be episodic, short-term indoor concentrations can be quite high for unvented or improperly vented systems and interior spaces. This is particularly the case in rural areas of Southeast Asia, Africa and South America, where firewood, charcoal, and cow dung are widely used in open stoves for cooking and heating.

About half of all homes in the United States use natural gas for cooking, a typical example of unvented combustion and a major source of indoor nitrogen dioxide and carbon monoxide, especially in kitchen areas. Other generally unvented combustion sources are gas and kerosene space heaters, found in $\sim$10% of homes in the United States. Emissions from the latter are similar to those from gas-fueled devices, but particles from kerosene heaters consist of carbon onto which may be adsorbed organic chemicals (e.g., hydrocarbons), many of which show significant mutagenic activity. Wood stoves are also used for home heating in many areas, and while they are generally vented outdoors, improper venting or lack of proper seals can also result in significant indoor contamination by organic-coated carbon particles. The actual exposure to contaminants from any of these combustion sources depends on the degree of venting used while the appliance is in operation, and the extent and pattern of its use, but indoor concentrations of nitrogen dioxide and carbon monoxide are generally higher than those outdoors when significant sources are present, especially in the winter when interior ventilation tends to be reduced.

Particulate Matter

During the 1990s, suspended PM was identified as one of the most important indoor and outdoor air pollutants. Due to the different sizes of the particles that lead to different deposition patterns in the human airways, PM is mostly not a single compound like a gas but rather a mixture of compounds. Epidemiological studies of short-term exposures found that each $10\,\mu g\,m^{-3}$ increase in PM_{10} is associated with $\sim$0.4–0.6% increase in daily mortality. Somewhat larger associations are observed for cardiovascular mortality and considerably larger associations were found for respiratory mortality. PM_{10} is also associated with increased hospitalization and related health care visits for respiratory disease and, to a somewhat lesser degree, cardiovascular disease. Associations also exist with lower respiratory symptoms, exacerbation of asthma, and coughing. Small but usually statistically significant declines in lung function have been observed as well. The data used in these estimations were from American and European time series studies. A recent analysis of data in Asia has found a similar association between PM_{10} and total mortality. Similar but somewhat stronger associations have also been established for $PM_{2.5}$ and daily mortality.

A threshold for the onset of health effects due to PM could not be established from these studies. In its Guidelines for Air Quality, the WHO, for the first

time, did not derive guideline values as in past publications but quoted exposure–response relationships.

Evidence from long-term exposure studies indicates that cardiopulmonary health effects are associated with $PM_{2.5}$ and that $PM_{2.5}$ was more closely associated with health outcomes than PM_{10}. Total mortality, in general, is observed to be associated with long-term exposure to PM by ~2–4% per $5\,\mu g\,m^{-3}$ increase in $PM_{2.5}$.

These results were obtained from outdoor air investigations of PM-related health outcomes. In view of the indoor/outdoor ratios quoted above and the similarity of particle size distributions of indoor and outdoor sources, there is little doubt that these associations also apply for indoor environments. These epidemiological studies, although coherent and partially consistent, have important limitations that emerge from the investigation of people who are living in uncontrolled environments. As the biological mechanisms are poorly understood, a better understanding of the health effects due to PM requires contributions from toxicology, exposure assessment, and other disciplines.

Nitrogen Dioxide

Any high-temperature combustion process in air initially generates nitric oxide and some nitrogen dioxide, but the former becomes rapidly oxidized to the latter. Combustion processes may produce other forms of nitrogen-containing compounds, such as nitrous and nitric acid vapors; however, the toxicological significance of these is not certain.

When natural gas ranges are in operation for home cooking, indoor nitrogen dioxide levels are generally higher than those found outdoors and are always above those found in homes using electric ranges. The average daily concentration in homes using gas for cooking purposes can range from 0.05 to $0.95\,mg\,m^{-3}$, but short-term peaks of $1.9\,mg\,m^{-3}$ are not uncommon. The result is that personal exposures to nitrogen dioxide are primarily driven by indoor sources in homes using gas appliances. The highest nitrogen dioxide concentrations occur in inadequately ventilated skating rinks by emissions from gasoline or propane-powered resurfacing machines used to periodically smooth or groom the ice. Personal sampler measurements in children in Sweden showed peak hour concentrations up to 8 mg $NO_2\,m^{-3}$.

Nitrogen dioxide is an upper respiratory tract irritant and has, in some cases, been linked to a 20% increased incidence of acute respiratory infection in children residing in homes using gas for cooking. There is also some indication that nitrogen dioxide, at concentrations found in homes associated with the use of natural gas stoves, may increase symptoms of asthma, wheezing, colds, sore throats, hay fever, and absences from school in children. Studies of long-term exposure of adults to gas stoves found more frequent respiratory symptoms, including a reduction in lung function in women but not in men, with gas use. A follow-up of the consequences of gas stoves, wood stoves or fireplaces on nonsmoking asthmatics showed a close association between use of a gas stove and shortness of breath, cough, or restrictions in activity. However, there is still not enough evidence from epidemiological studies alone to establish whether the observed effects are causally related to NO_2 only. Following the precautionary principle, the WHO has derived a guideline value for NO_2 of $200\,\mu g\,m^{-3}$ for 1 h exposures, and an annual guideline value of $40\,\mu g\,m^{-3}$.

Carbon Monoxide

Carbon monoxide is produced during the incomplete combustion of carbon-containing fuels, such as natural gas, kerosene, and wood. Its production rate by gas ranges is actually greater than that for nitrogen dioxide, and indoor levels can be several times greater than those found outdoors. Concentrations ranging from 2.3 to $17\,mg\,m^{-3}$ have been found in homes using gas for cooking. In some cases, indoor levels are enhanced by carbon monoxide derived from automobiles housed in garages attached to residences or connected to office buildings.

Carbon monoxide binds very strongly to hemoglobin in red blood cells, resulting in the production of carboxyhemoglobin (COHb); this can actually be used as a marker for exposure to carbon monoxide. The presence of COHb impairs the normal transport of oxygen within the blood and can result in adverse effects on tissues, such as those in the cardiovascular and nervous systems, which have high oxygen needs.

Symptoms of acute carbon monoxide poisoning range from headache to death. Prolonged exposure can affect the body due to oxygen deprivation, a condition known as tissue hypoxia. Levels of carbon monoxide encountered indoors have been found to result in disorientation in exposed individuals. Other potential health effects of CO include neurological deficits, neurobehavioral changes, and increases in daily mortality and hospital admissions for cardiovascular diseases. Several studies showed small, statistically significant relationships between CO and daily mortality. Some studies appear to show that the association between ambient CO and mortality and hospital admissions due to cardiovascular diseases persists even at very low CO levels indicating no

threshold for the onset of these effects. It is possible that CO may have more serious health consequences than the COHb formation, and at lower levels than that mediated through elevated COHb levels. The effects of prolonged exposures to indoor concentrations of carbon monoxide on the health of normal individuals are, however, not certain.

The WHO guideline values for CO are 100, 60, 30, and 10 mg m^{-3} for exposure times of 15 min, 30 min, 1 h, and 8 h, respectively.

Smoke from Solid-Fuel Use

Solid fuels used in cooking and heating include wood, coal, lignite, peat, and dung. Half the world's population uses these burning materials. The use of wood for space heating in homes has also increased during the past 25 years in a number of areas in the United States. Smoke from solid fuels is a complex mixture of gases and particles, including PM, carbon monoxide, nitrogen dioxide, sulfur dioxide, and various organic compounds such as polycyclic aromatic hydrocarbons. The amount of each produced depends on burn rate, the type and quantity of wood used, and its moisture content. In developed countries, wood stoves are generally vented outdoors, and the newer ones even operate under negative pressure and should contribute little contamination to the indoor air environment. However, some indoor pollution may occur from faulty venting, leakage, or during nonairtight conditions such as during start-up, stoking, and reloading. There has not, however, been adequate characterization of the influence of wood combustion on indoor air quality. Furthermore, while individual constituents of wood smoke are irritants and carcinogens, the actual health hazards due to indoor exposure are unknown. There is some evidence of an increase in chronic respiratory symptoms, such as cough and wheeze, in children.

In developing countries, evidence on the health impacts of solid-fuel use has emerged. The apparent odds ratios (ORs) comparing the risk of these diseases between people living in houses using unvented biomass fuel and similar households not using such fuels are listed below. All the ORs reported are statistically significant results, mostly of multivariate analyses in which a number of potentially confounding variables were included.

Acute respiratory infections in children are the chief cause of childrens' ill health in the world and strongly associated with indoor use of solid fuels for cooking in a number of studies in Asia and Africa (OR = 2–6). COPD is strongly associated with use of solid fuels in nonsmoking women, often along with *cor pulmonale*, in studies from Latin America, South Asia, and Saudi Arabia (OR = 3.4–15). In many Chinese studies lung cancer was statistically associated with the use of coal for cooking and heating, but not biomass fuels (OR = 3–9).

There is some evidence from studies of solid-fuel use in developing countries indicating a relationship between adverse pregnancy outcomes and smoke exposure. After multivariate analyses, stillbirth has been associated with biomass fuel use by pregnant women in one Indian study (OR = 1.5) and with low birth weight in Guatemala. After multivariate analyses, TB and blindness (cataracts) have been shown to be related to use of biomass fuels in two national and two local studies in India. Unfortunately all these studies relied on the type of stove or fuel as the indicator of pollution.

Environmental Tobacco Smoke

One indoor air pollutant of concern since the 1950s in terms of preventable morbidity and mortality is tobacco smoke, which contains over 4000 different chemical compounds emitted as particles or gases. Tobacco smoke is the largest single source of air contamination in many indoor environments in developed countries and also in developing countries in indoor environments without use of open burning for cooking and heating. Environmental tobacco smoke (ETS) is the term used to describe the smoke found indoors and which consists of a combination of that emitted into air from the burning end of a cigarette, cigar, or pipe (side-stream smoke) plus the smoke that is exhaled by the smoker. Generally active smoking consumes half of a cigarette while smoldering consumes the other half.

The combustion conditions differ when a cigarette is puffed compared to when it smolders, so the actual ratios of chemical constituents in side-stream and mainstream smoke also differ, although qualitatively the materials are similar. Because the temperature of the burning cone is lower as the cigarette smolders than during active puffing, combustion is less complete in side-stream than in mainstream smoke. Consequently, side-stream smoke has higher concentrations of chemical compounds than mainstream smoke. The bulk of ETS actually consists of side-stream smoke. While the amount of these inhaled by the nonsmoker compared to the active smoker is reduced by dilution in room air, ETS is the source of numerous toxic and carcinogenic contaminants in indoor environments; some of the major ones are listed in **Table 1**. Respirable particle levels in the smoking areas of some buildings can be up to 25 times greater than those in nonsmoking areas, reaching concentrations above 300 μg m^{-3}. Nicotine,

Table 1 Major indoor air contaminants derived from environmental tobacco smoke

In vapor/gas phase	*In particulate phase*
Acetaldehyde	
Acetone	Aniline
Acrolein	Benzo(*a*)pyrene
Ammonia	Carbon
Carbon dioxide	Nicotine
Carbon monoxide	Metals (nickel, arsenic, cadmium)
Formaldehyde	Phenol
Hydrogen cyanide	
Nitrogen oxides	
Pyridine	

polycyclic aromatic hydrocarbons, carbon monoxide, formaldehyde, acetaldehyde, acrolein, nitrogen dioxide, and benzene are also significantly elevated in the homes of smokers compared to those of nonsmokers. For example, median nicotine levels monitored weekly in US smoker homes ranged between 1.4 and 3.0 $\mu g\,m^{-3}$, with maximal values between 4.4 and 28.6 $\mu g\,m^{-3}$.

Passive smoking, or involuntary smoking, is the term used to describe the inhalation of ETS by nonsmokers. The amount of smoke to which any individual is exposed is quite variable, depending on the number of sources (i.e., active smokers), the degree of building ventilation that affects dilution, and the presence of any air cleaning devices. The contribution of various indoor environments to personal exposure to ETS varies with the time-activity patterns and the individual susceptibility. For example, infants who do not attend day care are mainly exposed in the homes of smokers. Nonsmoking adults who work in offices together with smokers may be principally exposed in these offices. Studies have shown that exposures to ETS in the home are usually greater than those at the workplace.

Laboratory studies indicate that changes in ventilation rates simulating conditions expected in many residential and commercial environments during smoking do not have a significant influence on the air concentration levels of ETS components, for example, CO, NO_x, aromatic compounds, and nicotine. This suggests that efforts to reduce indoor air pollution through higher ventilation rates in buildings would not lead to a meaningful improvement of indoor air quality and is in agreement with the results of studies carried out in the United States at different hospitality venues.

Personal exposures to ETS can be assessed using biological markers in body fluids, such as saliva, blood, or urine. The presence of ETS components and their metabolites in body fluid of exposed nonsmokers strengthens the plausibility of associations between ETS exposure and disease. Biological markers of exposure to ETS have been used to estimate the prevalence of doses of potential toxic agents inhaled during involuntary smoking. At present, the most sensitive and specific of these markers are nicotine and its major metabolite, cotinine. Nicotine and cotinine are almost never present in body fluids in the absence of ETS exposure. Because the circulating half-life of nicotine is $\sim$30 min, nicotine concentrations in body fluids are representative of very recent exposures. Cotinine remains in the body for $\sim$20–30 h and, therefore, reflects an equilibrium reached by daily exposure to ETS. Its assessment clearly indicates that passive smoking is a significant source of exposure to cigarette smoke, with cotinine levels in nonsmokers approaching 10% of those found in active smokers.

Exposure to ETS has been linked to various diseases and symptoms, particularly in children of smoking parents. Some of the health effects result predominantly from transplacental exposure of the fetus to tobacco smoke components. Health effects on the fetus resulting from maternal smoking during pregnancy include decreased birth weight, growth retardation, or prematurity, spontaneous abortion, perinatal mortality, and congenital malformations. Postnatal health effects of ETS exposure include sudden infant death syndrome, and adverse effects on neuropsychological development and physical growth. ETS has also been evaluated as a risk factor for major childhood cancers. The evidence is, however, limited and does not yet support conclusions as to the causal nature of observed associations.

Studies of involuntary smoking and lower respiratory illnesses in childhood, including more severe episodes of pneumonia and bronchitis, have demonstrated dose–response relationships. Schoolchildren responses to parental ETS include increased acute respiratory infections; increased frequency of chronic respiratory symptoms (i.e., cough, phlegm, and wheezing); middle ear infections; and reduced lung function and rate of lung growth. Exposure to ETS might also cause asthma as a long-term consequence of the increased occurrence of lower respiratory infection in early childhood, other pathophysiological mechanisms including inflammation of the respiratory epithelium, or increased airways responsiveness developed shortly after birth from smoking mothers. While the underlying mechanisms remain to be identified, the epidemiological evidence associating ETS with childhood asthma is increasing.

Effects in adult nonsmokers are not as conclusive in terms of alterations in lung function, but irritation of the eyes and of the upper and lower respiratory tract do occur, and ETS both increases the risk of

developing cardiovascular disease and is a major preventable cause of cardiovascular disease and death.

ETS is a significant risk factor for lung cancer in nonsmokers, and it has been classified as a respiratory carcinogen by IARC. The increased individual risk can be 30–50% depending on the extent of exposure, and exposure to ETS is estimated to be responsible for lung cancer deaths among nonsmokers in the United States. The most recent meta-analysis estimated the excess risk of lung cancer for nonsmokers who lived with a smoker as 26% with a 95% confidence interval between 7% and 47%. This and other estimations illustrate that passive smoking must be considered an important cause of lung cancer death from a public health perspective. The WHO estimated the unit risk for ETS as 10^{-3} per $\mu g\,m^{-3}$, a risk for developing cancer during lifetime only below the unit risks of benzo(*a*)pyrene, chromium(VI), and arsenic.

Volatile and Semivolatile Organic Compounds

The WHO has categorized indoor vapor-phase organic compounds into classes given in the following table:

Category description	*Acronym*	*Boiling point range (°C)*
Very volatile (gaseous) organic compounds	VVOCs	<0 to 50–100
Volatile organic compounds	VOCs	50–100 to 240–260
Semivolatile organic compounds	SVOCs	240–260 to 380–400
Organic compounds associated with particulate matter, particle bound organic compounds	POMs	>380

Some VOCs can be malodorous pollutants, sensory irritants, or hazardous air pollutants. Hazardous VOC air pollutants include acetaldehyde, benzene, carbon tetrachloride, chloroform, ethylbenzene, formaldehyde, hexane, methylene chloride, naphthalene, paradichlorobenzene, pesticides (biocides), styrene, tetrachloroethylene, toluene, trichloroethylene, and xylenes. They are found in essentially all indoor locations, released by off gassing from numerous sources, such as construction and decorating materials, consumer products, paints, paint removers, furnishings, carpets, and from combustion of wood, kerosene, and tobacco. While more than 500 VOCs have been identified in indoor air, ~50 occur most commonly. The major sources for many of these are listed in **Table 2**. In older homes, the total concentration of all volatile organics can range from 0.02 to $1.7\,mg\,m^{-3}$, while in newer homes, levels of 0.5–$19\,mg\,m^{-3}$ have been found. Exposure to certain organic compounds indoors is much greater than that which occurs outdoors, with indoor concentrations of some substances being 10 times higher than those outdoors and with short-term peaks reaching 1000 times higher.

Semivolatile organic compounds, which are solids or liquids at room temperature, are also found in indoor air, derived from pesticides, wood preservatives, floor waxes and polishes, and from combustion sources. These have, however, not been as extensively investigated indoors.

Some VOCs are known human carcinogens (e.g., benzene, vinyl chloride). Others are animal carcinogens and may be human carcinogens (methylene chloride, trichloroethylene, tetrachloroethylene, chloroform, and *p*-dichlorobenzene). The unit risks for these compounds are shown in the table below: (Unit risks reflect the probability of attracting cancer in a hypothetical population during lifetime exposure to $1\,\mu g\,m^{-3}$ of VOCs.)

VOC	*Unit risk* $(\mu g\,m^{-3})^{-1}$	*Source*
Benzene	6×10^{-6}	WHO
Chloroform	4.2×10^{-7}	WHO
p-Dichlorobenzene	6.6×10^{-6}	US EPA
Methylene chloride	4.7×10^{-7}	US EPA
Tetrachloroethylene	6×10^{-6}	US EPA
Trichloroethylene	4.3×10^{-7}	WHO
Vinyl chloride	1×10^{-6}	WHO
Formaldehyde	1.3×10^{-5}	

Many more VOCs are respiratory tract irritants or can affect the central nervous system (e.g., toluene and xylene) at high (occupational) concentrations. Acute effects at lower environmental concentrations are often difficult to observe under controlled conditions. Furthermore, many organic chemicals have distinct odors, which can act as stressor agents affecting response. Exposure to volatile organics is generally assessed by measurement of the chemical in breath samples, but some can also be found in body fluids, such as mother's milk and blood. Other routes of VOC exposure are drinking water (chloroform, trihalomethanes), food and beverages (chloroform, trihalomethanes, tetrachloroethylene, trichloroethylene), and dermal absorption (chloroform).

One of the most common volatile organic contaminants found in indoor air is formaldehyde. It is

Table 2 Common indoor sources of volatile organic compounds

Chemical class	*Examples*	*Typical sources*
Aldehydes	Formaldehyde	See **Table 3**
Hydrocarbons		
Aliphatic	Propane, butane, undecane, pentane	Cooking and heating fuel; aerosol propellants; lubricants; perfume; glues
Aromatic	Benzene, styrene, toluene, xylene	Paint; varnish; glue; cleaners; lacquers; combustion sources, ETS
Halogenated	Chloroform, 1,1,1-trichloroethane, trichloroethylene, methylene chloride, *p*-dichlorobenzene	Pesticides; dry-cleaning solvents; aerosol propellants; degreasing agents; paint strippers
Alcohols	Methanol, hexanol	Window cleaners; paint; adhesives; cosmetics
Ketones	Acetone	Lacquers; polish removers; adhesives
Terpenes	Pinene, limonene	Air fresheners; polishes; fabric softeners

derived from various sources, as shown in **Table 3**, but its use as a bonding resin in pressed wood products, such as plywood, particle board, paneling, and fiberboard commonly found in home and furniture construction, represents the single largest current use. In past years, a major source for formaldehyde was urea–formaldehyde foam insulation injected into the walls of homes. While this use has generally ended in developed countries, very high indoor levels of formaldehyde are still found in homes with such insulation, where concentrations can range from 150 to 500 $\mu g\,m^{-3}$ compared to levels of 40–113 $\mu g\,m^{-3}$ in homes where it was not used. Homes that make extensive use of plywood, such as mobile and prefabricated houses, also have high levels, which have been measured at 1300–5000 $\mu g\,m^{-3}$. It is evident that formaldehyde concentrations vary widely; they depend on the age of the structure, potential sources, and indoor temperature and humidity (e.g., high temperatures enhance off gassing).

Significant quantities for formaldehyde are consumed in the production of other resins or polymers such as polyacetyls, melamine resins, and alkyl resins. Formaldehyde is also used in rubber/latex manufacture, textile treatment other than permanent-press fabrics, dye manufacture and use, photoprocessing chemicals, laboratory fixatives, embalming fluids, disinfectants, and preservatives. Formaldehyde can also be emitted by combustion appliances, wood fires, tobacco smoke, and in indoor chemistry.

Average concentrations of formaldehyde range between 30 and 60 $\mu g\,m^{-3}$ for conventional homes, are at 100 $\mu g\,m^{-3}$ in mobile homes, and range between 50 and 350 $\mu g\,m^{-3}$ in homes with exposure to ETS. At the workplace without occupational exposure similar concentrations of formaldehyde are observed. With occupational exposure, the concentrations may be as high as 1000 $\mu g\,m^{-3}$.

Table 3 Common indoor sources of formaldehyde

- Urea–formaldehyde foam insulation (UFFI)
- Resins used as bonding agents in pressed wood products
  - Particle board
  - Plywood
  - Paneling
- Resins used as water repellants, stiffeners, or wrinkle resistors
  - Paper products
    - Paper towels
    - Grocery bags
    - Waxed paper
  - Permanent press clothing
  - Carpeting
  - Linoleum
  - Plastics
  - Drapery
- Consumer products
  - Cosmetics
  - Shampoo
  - Deodorants
  - Dyes
- Combustion processes
  - Natural gas ranges and heaters
  - Kerosene heaters
  - Tobacco smoke

Formaldehyde can enter the body via the respiratory system, skin, or gastrointestinal tract, but it is primarily absorbed in the respiratory tract where it is rapidly metabolized. It is an upper respiratory tract and eye irritant; may cause respiratory symptoms, reductions in lung function, and headaches; may predispose to asthma; and can also affect the nervous system. It is carcinogenic in laboratory animals, but human carcinogenicity is still an open issue. The WHO guideline value for formaldehyde is 100 $\mu g\,m^{-3}$ as a 30 min average, intended to prevent significant sensory irritation. This guideline value represents an exposure level at which there is a negligible risk of upper respiratory tract cancer in humans. An IARC expert group recently classified formaldehyde as carcinogenic to humans, determining that there is

now sufficient evidence that formaldehyde causes nasopharyngeal cancer in humans.

Other aldehydes, which may be important in the indoor environment, include acetaldehyde, acrolein, and glutaraldehyde. Acetaldehyde is a major by-product of hydrocarbon oxidation when wood or kerosene is burned for heating and cooking in developing countries, and a combustion by-product from unvented gas and kerosene appliances. It is also the predominant aldehyde detected in mainstream and side-stream tobacco smoke. Acrolein is produced and released into the indoor environment as a combustion/chemical oxidation product from the heating of oils and fats containing glycerol, wood combustion, and cigarette smoke. Acrolein emissions in mainstream smoke are significantly lower than formaldehyde emissions but are significantly higher in side-stream smoke.

Acetaldehyde is a relatively mild irritant of the eyes and upper respiratory system. It is toxic to the cilia of respiratory epithelia and may interfere with respiratory clearance mechanisms. Acetaldehyde is also a central nervous system depressant and a proven carcinogen in animals and a potential carcinogen in humans. The tolerable concentration for acetaldehyde according to the Guidelines for Air Quality of the WHO is $2000\,\mu g\,m^{-3}$ for 24 h and $50\,\mu g\,m^{-3}$ as annual mean.

Acrolein is a very potent eye irritant, causing lacrimation at concentrations of $\sim 2.3\,mg\,m^{-3}$ and irritation at concentration as low as $58\,mg\,m^{-3}$. At high concentrations, acrolein can cause significant lung injury, including dyspnea, asthma, congestion, edema, and persistent respiratory insufficiency with decreased lung function. Acrolein is ciliatoxic like formaldehyde, and can suppress pulmonary killing of bacteria. On chronic skin exposure, acrolein can cause contact dermatitis and sensitization. Acrolein can also be a potential carcinogen at least as potent as formaldehyde. The WHO has derived a guideline value of $50\,\mu g\,m^{-3}$ as a 30 min average based on eye irritation in humans.

Exposure concentrations for glutaraldehyde range up to $2\,mg\,m^{-3}$. Exposure to glutaraldehyde can lead to significant prevalence rates of nasal and throat irritation, nausea, and headache. Pulmonary symptoms, such as chest tightening, asthma, and similar symptoms have also been reported for medical workers. Other effects include skin symptoms, reproductive effects, and cancer. An exposure value of $0.21\,mg\,m^{-3}$ has been proposed by the American Conference of Governmental Industrial Hygienists (ACGIH) as a time-weighted average concentration to be at or below in order to protect nearly all workers from adverse effects.

Indoor/Outdoor Air Concentrations and Exposure Estimates for Benzene and Formaldehyde in Europe

Benzene

The EXPOLIS and MACBETH studies were extensive measuring campaigns carried out at the pan-European level to determine indoor/outdoor concentrations for benzene and other pollutants, and to relate them to personal exposure estimates. The results clearly indicated that ambient air concentrations for benzene substantially vary between the northern and southern part of Europe, with higher ambient air levels measured in the cities of southern Europe. This is mainly due to climatic conditions (higher temperature, low wind speed regimes), heavy traffic and often the lack of infrastructure needed to facilitate the movement of the citizens to and from the city. While in Athens (Greece), Murcia (Spain), and Milan (Italy) outdoor concentrations up to $21\,\mu g\,m^{-3}$ were measured, in Copenhagen (Denmark), Helsinki (Finland), and Prague (Czech Republic) outdoor concentrations reach values up to $5\,\mu g\,m^{-3}$. In indoor environments (homes), mean benzene concentrations range from a low of 2.2 to a high of $13.2\,\mu g\,m^{-3}$. Personal exposure monitoring concentrations were found to be often higher compared to those from indoor and outdoor sampling. There is clear evidence that personal exposure to benzene is at least twice as high as the ambient air concentrations.

In the frame of the German Environmental Survey (GerES II), the personal exposure (mean) concentrations were $13.5\,\mu g\,m^{-3}$, similar to those obtained in other cities of Central Europe (Prague, Antwerp). Assuming a 24 h exposure to this concentration a daily intake of 270 μg of benzene is estimated, based on a breathing volume of $20\,m^{-3}$ daily. Another study reported on personal exposure concentrations in the city of Nancy ranging from 9.9 to $55.5\,\mu g\,m^{-3}$, with a mean value $\sim 23.8\,\mu g\,m^{-3}$, which is significantly higher than the (mean) indoor and outdoor concentrations of 10.8 and $4.4\,\mu g\,m^{-3}$, respectively. Using the mean personal exposure concentration of $23.8\,\mu g\,m^{-3}$, a daily intake up to 476 μg of benzene is estimated.

In the United Kingdom, ambient air concentrations of benzene are generally in the range of 1–$6\,\mu g\,m^{-3}$. Mean indoor air concentrations were estimated to be $8\,\mu g\,m^{-3}$ for homes. However, nonoccupational exposed adults receive very high daily doses of 74–528 μg of benzene, which corresponds to an average range of benzene in air of 3.7–$26.4\,\mu g\,m^{-3}$, an amount significantly higher than

the mean outdoor air benzene concentration. Other studies reported that the mean personal exposure for individuals in Hertfordshire, England, was 183.9 μg m^{-3} (24 h). Using the mean outdoor air concentration near homes to predict personal exposures a value of 92.6 μg m^{-3} (24 h) has been obtained. At the pan-European level, and in accordance with the studies carried out, the mean home-indoor concentration for benzene considering all cities included in EXPOLIS and MACBETH studies, is ~9.6 μg m^{-3}; the home-outdoor/urban concentration (mean) is ~7.4 μg m^{-3}. Taking into account the time people approximately spend indoors and in work places (85%) and outdoors (15%), a daily intake of 184.4 μg of benzene results from exposure to indoor and outdoor air. This value corresponds fairly well to those reported from the local measuring campaigns.

From all data available, it can be concluded that personal exposure cannot be estimated from ambient air concentrations. Reducing benzene emissions from mobile sources only will have a rather limited effect on total human air exposure to this compound.

Formaldehyde

Formaldehyde has been one of the most important pollutants in indoor nonindustrial environments. A large body of data exists on measurements for formaldehyde in homes and buildings in Europe. Indoor air concentration levels for formaldehyde range from a few μg m^{-3} up to 70 μg m^{-3}, while mean outdoor concentrations of ~10 μg m^{-3} were measured. In air pollution episodes formaldehyde concentrations can reach high values (up to 80 μg m^{-3}) even at locations far from emission sources. However, in almost all measurements formaldehyde indoor concentrations exceed by several times (5–20 times) the outdoor levels, indicating strong emission sources inside buildings and homes. According to a WHO study, exposure of humans to formaldehyde is mostly determined by its concentration indoors. A daily intake of 20 μg results from the exposure to ambient air, while indoor and workplace concentrations has been estimated to amount to ~0.5–2 mg day^{-1}.

Indoor/Outdoor Air Concentrations and Exposure Estimates for Benzene and Formaldehyde in the United States

Several studies have been carried out in the United States to determine indoor/outdoor air concentration levels for priority pollutants and to assess personal exposure estimates. They have shown higher indoor than outdoor concentrations for the main pollutants, especially for VOCs.

Indoor (mean) concentrations for benzene range from 8.2 to 17 μg m^{-3}. 'Typical values' for indoor as well as for outdoor environments were up to 5 μg m^{-3}. For formaldehyde mean indoor concentrations reach values up to 92 μg m^{-3}, while 'typical values' for outdoor air concentrations of 4 μg m^{-3} are reported. Indoor/outdoor (*I*/*O*) ratios, based on typical air concentration levels, of 2 and of 50 for benzene and formaldehyde, respectively, are calculated. Daily exposure estimates are based on the assumption that people spend ~90% of its time in indoor environments and 10% outdoors. For benzene daily personal exposures vary between 108 and 177 μg m^{-3} for 24 h periods, ~20% lower than the mean exposures estimated for European citizens. For formaldehyde personal exposures range from 1080 to 2000 μg m^{-3} over 24 h, rather similar to European exposure estimates.

Asbestos and Other Man-Made Vitreous Fibers

Asbestos is a class of fibrous silicate minerals, each type of which differs in fiber shape and chemical formulation. It was widely used for decades because of its properties as a heat and sound insulator and fireproofing material and can be found in older floor and ceiling tiles, roofing felt and shingles, dry wall patching compounds, fireproofing insulation sprayed around steel beams, and the insulation of boilers and pipes. While it can no longer be used for most applications in new buildings, it is still a major indoor contaminant in many older ones, including homes and schools. However, the mere presence of asbestos in an indoor environment does not indicate exposure. If the asbestos-containing item is intact and fibers do not escape into the air, there is no exposure, and in many cases it is better to leave the material in place if it is well contained. However, much asbestos-containing material is old and in poor condition or damaged and may be friable (i.e., sheds fibers into the air). Asbestos fibers can be released during renovation of older buildings.

Actual indoor air concentrations of asbestos range from below 100 to several thousand fibers per m^3. Exposure to certain types of asbestos fibers is associated with specific respiratory diseases. These are asbestosis, a form of lung fibrosis, and two types of malignancies, namely, mesotheliomas, which are tumors of the lung pleura or peritoneum, and bronchial carcinomas. There is a strong weight of evidence that asbestos shorter than 5 μm do not cause cancer in humans. Evidence from occupational case–control studies indicates that the relative risk of mesothelioma is related to asbestos fibers longer than 5–10 μm. For lung fibrosis or asbestosis, the role of

fibers below 5 μm is not as clear. WHO estimated that with a lifetime exposure to 1000 F m^{-3} in a population of whom 30% are smokers the excess risk due to lung cancer would be of the order 10^{-6}–10^{-5}. For the same lifetime exposure, the mesothelioma risk for the general population would be in the range 10^{-5}–10^{-4}.

Synthetic or man-made vitreous fibers, such as continuous filament fiber glass, glass wool fibers, rock wool fibers, slag wool fibers, refractory ceramic fibers, and glass microfibers, used as asbestos substitutes for many applications seem to pose much less of a public health risk. The potential for deep lung penetration is greatest for refractory ceramic fibers and glass microfibers; both of these are primarily used in industrial applications. In two large epidemiological studies in the 1980s, there have been excesses of lung cancer in rock/slag wool production, but not in glass wool, glass microfiber, or continuous filament fiberglass workers. Although concomitant exposure to other substances may have contributed to the observed increase in lung cancer, the fibers appeared to be the principal determinants of risk. More recent cohort and case–control studies have not found increased respiratory cancer risk for rock or slag wool exposure or for refractive ceramic fibers. In spite of many epidemiological and experimental studies, the debate on man-made vitreous fibers is still controversial.

Radon

Radon (Rn-222) is an odorless and colorless natural radioactive gas. It is produced during the radioactive decay of radium-226, itself a decay product of uranium-238 found in many types of crustal materials, that is, rocks and soils. Rn-222 has a short half-life (3.8 days) and decays into a series of solid particulate products, known as radon progeny or radon daughters, all of which have even shorter half-lives ($\sim$30 min or less). Other isotopes of radon also occur naturally, but due to differences in half-life and dosimetry their health significance is minimal compared to that from exposure to Rn-222.

The main source of indoor air radon is the soil and rock beneath a building, from which the gas penetrates indoors, primarily through cracks or openings in the foundation or basement, including drain and utility access areas. Some well (ground) water in areas having high soil radium content may also be a source of indoor radon, as may natural gas or building materials containing radium. Often radon levels indoors tend to be highest in the lowest levels of a building, from which the gas can then permeate the entire structure. Arithmetic mean radon concentrations in European countries range from $\sim$30 to 140 Bq m^{-3}. In Russia, radon levels range between 19 and 230 Bq m^{-3}; in the United States average levels are around 50 Bq m^{-3}. Because of the skewed distribution of radon levels the geometric mean concentrations range 20–50% lower. Many countries have set an action level of 200 Bq m^{-3} at which mitigation measures should be taken to reduce radon levels at home. In the European Community, the action level is 400 Bq m^{-3}. The highest acceptable level of residential radon has been set by the US EPA at 150 Bq m^{-3}, but $\sim$5–10% of homes in the United States exceed this benchmark.

The risk from radon exposure is essentially due to inhalation of its progeny, which can attach to abundant sources of particles in indoor air that then act as carriers of these radioactive particles into the respiratory tract. Radon accounts for up to 50% of the total internal dose from all natural background radiation sources and this, in turn, is due almost completely to two of its progeny, namely, polonium-218 and polonium-214, which decay via the release of α-particles. Alpha particles, while lodged in the airways of the lung can damage the cells lining the airways, thus inducing lung cancer.

Radon exposure in the home likely substantially increases lung cancer risk in either nonsmokers or smokers. According to a nationwide Swedish epidemiological study of lung cancer due to radon exposure, the attributable proportion of lung cancer related to residential radon exposure ranges between 2% and 5% for lifetime exposure to 25 Bq m^{-3}, 5–9% for lifetime exposure to 50 Bq m^{-3}, and 9–17% for lifetime exposure to 100 Bq m^{-3}. The WHO estimated that these attributable proportions of lung cancer correspond to a unit risk of $3–6 \times 10^{-5}$ per Bq m^{-3}. The (US) National Academy of Sciences, in 1998, has estimated that between 15 400 and 21 800 lung cancers per year in the United States can be attributed to radon exposure. Furthermore, the individual risk may increase if other cancer-associated factors, especially cigarette smoke, are also present.

Biological Agents

Indoor air can contain a wide variety of biological contaminants; some examples are presented in **Table 4**. While many of these are nonpathogenic, others induce disease by infection of the respiratory tract or by immunologic means, such as allergy.

Biological agents in indoor environments include fungi, bacteria, allergens from dust mites, cockroaches and animal dander, and toxic components such as endotoxins and mycotoxins. Biological agents and

Table 4 Common indoor biological contaminants

Bacteria
Bacillus subtilis
Escherichia coli
Klebsiella pneumoniae
Legionella pneumophila
Mycobacterium spp.
Pseudomonas aeroginosa
Salmonella typhosa
Staphylococcus aureus
Streptococcus albus
Streptococcus spp.
Viruses
Fungi
Alternarium
Aspergillus spp.
Penicilium funiculosum
Thermophilic actinomycetes
Insects and insect parts
Cockroach
Mites
Dander

some of the diseases they can cause are often associated with moisture and dampness in buildings, in heating, ventilation, and air conditioning (HVAC) system components, porous materials, gypsum boards, and other locations with inadequate humidity control. Diseases potentially caused by biological agents in indoor environments include:

- Legionnaire's disease (pneumonia);
- humidifier fever (acute influenza-like symptoms);
- hypersensitivity pneumonitis (acute fever and cough, fibrosis of lung);
- asthma (intermittent episodes of wheezing, coughing, difficulty in breathing);
- pulmonary hemosiderosis (bleeding in the lungs);
- acute febrile illness (influenza, cold);
- tuberculosis; and
- lung cancer.

Biological agents are disseminated in indoor air by various means. Depending on the organism, this includes human actions (such as sneezing and coughing); via mechanical devices which result in the aerosolization of water spray containing these agents, such as humidification systems and whirlpool baths; via air movement induced by ventilation systems and by air currents derived from convective radiant heating systems; or by dusting or vacuuming of contaminated carpets or furniture. The risk of developing an infection or allergy from exposure to indoor air is often greater than that from outdoor air due to reduced ventilation in confined spaces resulting in the buildup of microorganisms or allergens to effective localized levels.

Infectious Agents

Infectious agents found in indoor air include viruses, bacteria, fungi, and protozoans. Viruses are internal cell parasites and can exist outside living cells for only a short period. On the other hand, bacteria, fungi, and protozoans can exist for extended durations on nonliving material. While bacteria are primary pathogens for humans, fungi and protozoans are generally opportunistic, that is, they produce disease only in compromised individuals, such as those with reduced defenses due to concurrent disease or use of certain medications.

Infectious disease can be produced by any pathogen able to be aerosolized and subsequently transported into the respiratory tract at the appropriate concentration. Some common diseases which may result from airborne transmission in indoor environments are listed in **Table 5**. The rate of infection within any environment is a function of the viability and virulence of the pathogen, its concentration in the inhaled air, and characteristics, such as droplet size, of the carrier aerosol within which it is contained. Some biological agents produce disease at low concentrations, while others must accumulate to a higher level. Furthermore, individual susceptibility to infection depends on a number of factors, such as age and health, as well as concomitant exposure to chemical pollutants.

While there are a number of potential sources of infectious agents in the indoor environment, humans are the principal one for pathogens responsible for most airborne viral diseases and many bacterial diseases. Nonliving sources can also harbor infectious agents. A good example is the bacterium, *Legionella pneumophila*, which becomes airborne from contaminated cooling system water and is responsible for Legionnaire's disease. Another example is humidifier fever for which episodes have been associated with inhalation of aerosols from humidifiers contaminated with gram-negative bacteria and protozoa. Endotoxin from *Flavobacterium* and from a *Pseudomonas* species was shown to be the potential agent of two humidifier fever outbreaks.

Pathogenic fungi generally derive from outdoor air, but their spores are able to penetrate into buildings through air spaces or intake vents, and interior growth can then occur on damp surfaces. Fungi and bacteria growing in the HVAC system have been implicated in outbreaks of hypersensitivity pneumonitis. Causative agents included *Cladosporium*, thermophilic actinomycetes, *Bacillus subtilis*, and *Penicillium* species. In large buildings with complex HVAC systems and with many potential sites of

Table 5 Some diseases potentially spread by indoor air exposure

Viral
Chickenpox
Colds
Influenza
Smallpox
Measles
Bacterial
Legionnaire's disease
Tuberculosis
Brucellosis
Fungal
Histoplasmosis
Cryptococcosis
Coccidiomycosis
Protozoan
Pneumocystis carinii
Acanthameoebosis

microbial growth, outbreaks of the disease often cannot be ascribed to a single agent.

Allergens and Immunologic Agents

Indoor air may contain biological agents capable of eliciting an allergic response. An allergic response is characterized by production of a specific immunoglobulin (antibody) termed IgE. Allergic sensitization is an important risk factor for asthma, particularly in children and young adults. Occupational asthma can develop among adults exposed to sensitizers. Very common indoor allergens are dust mite, cockroaches, animal danders from pets, and mold. The indoor allergens, except for mold, are often present in greater concentrations in residential than in other buildings. Mold flourish in any building with inadequate moisture control.

Another type of immunologically mediated lung disease is hypersensitivity pneumonitis. This is acutely characterized by flu-like symptoms, including fevers, cough, and chills, but in a chronic state may result in a slow, progressive decline in pulmonary function. A number of antigenic materials can produce hypersensitivity pneumonitis. While they are mostly complex organic particles, a common indoor antigen involved in its pathogenesis is the thermophilic actinomycetes. These organisms are found in decomposing organic matter and contaminate indoor environments through ventilation and humidification systems.

Allergic asthma may be exacerbated by exposure to antigens found in indoor air, including house dust, fungal spores, and molds. The house dust mite (*Dermatophagoides farinae*), which exists in bedding and in the stuffing of upholstered furniture, contains a potent allergen which occurs at high concentrations in house dust and then becomes airborne during cleaning activities. Inhalation of dust contaminated with these mites can increase the severity of asthma or perhaps even the risk of its inception.

Pulmonary hemosiderosis or pulmonary hemorrhage has been observed in a number of young infants (most under 6 months old), in the eastern neighborhoods of Cleveland, who have been coughing up blood due to bleeding in their lungs. Some infants have died and more infants continue to get ill. This bleeding appears to be caused by something in their home environments, most likely toxins produced by an unusual fungus called *Stachybotrys chartarum* or similar fungi.

Tuberculosis (TB) is caused by any of the human, bovine, or avian types of the tubercle bacillus *Mycobacterium tuberculosis*. Indoor levels of *M. tuberculosis* are generally low, but since TB is infectious, the clinically relevant exposure may be only a few bacteria. Globally, TB is on the increase, especially in developing countries.

Lung cancer through exposure in the indoor environment can be caused by carcinogenic mycotoxins, secondary metabolites produced by fungi mycelium and spores. Molds include *Aspergillus flavus*, which produces aflatoxin, a potent carcinogen, and *Aspergillus versicolor*, which produces a precursor for aflatoxin. *Aspergillus versicolor* is common in buildings with poor humidity.

Sick Building Syndrome and Multiple Chemical Sensitivity

Most indoor environments are contaminated by a combination of both microorganisms and particles and gases, but little is known regarding health effects from exposure to such complex mixtures even though biological responses to the inhalation of contaminated indoor air may depend on interactions between individual substances. Examples of some potential interactions in the risk of developing lung cancer are those between radon and ETS and between asbestos and ETS; between ETS and PM in the induction and/or exacerbation of respiratory infection in children; and between allergens and ETS in the exacerbation of asthmatic symptoms. Exposure to mixtures of indoor air pollutants does appear to be associated with two clinical conditions, namely, sick building syndrome (SBS) and multiple chemical sensitivity.

There have been numerous reports of a spectrum of nonspecific health complaints from occupants of various buildings, including schools, hospitals, and, most often, modern offices. Complaints include respiratory tract infection, irritation of the eyes, nose,

and throat, headaches, neurological reactions, nausea, lethargy, and dizziness. The range and severity of the symptoms varied greatly depending on the sensitivity of exposed individuals. While a causative role of the indoor environment was strongly suggested when it became clear that the symptoms generally abated upon leaving the building, in most cases no specific cause for them has been found. The term used for this collection of clinical signs is SBS, or building-related symptoms (BRS) as suggested by the American Conference of Governmental Industrial Hygienists. BRS is estimated to occur in about one-third of all buildings in the United States, especially in those that have been made 'tight' for energy conservation. This, in turn, allowed for the accumulation of contaminants from numerous indoor sources.

A single chemical is most likely not responsible for SBS but, rather, it probably reflects exposures to various chemicals, which can differ at different sites. Because many VOCs produce similar symptoms to those noted in BRS, they are suspected to be potential causative agents. However, there seem to be some contributions from a wide range of other factors in the environment such as temperature, humidity, and cleanliness of offices, personal control over the environment, noise, and lighting. In addition, biological agents may contribute to BRS.

BRS are to be distinguished from what have been termed building-related illnesses (BRI). These latter have definite etiological agents and specific clinical manifestations, for example, hypersensitivity pneumonitis associated with molds and thermotolerant bacteria. Other examples of BRI are rhinitis induced by sensitizers or irritants, allergic fungal sinusitis, asthma exacerbated by VOCs, molds and bacteria, allergic or irritant contact dermatitis induced by molds and/or VOCs, allergic or irritant conjunctivitis induced by molds and/or VOCs, and CNS toxicity induced by CO, VOCs, heat, and noise. The symptoms of BRI do not abate when leaving the building, and medical treatment is generally necessary.

Multiple chemical sensitivity (MCS) or multiple chemical intolerance (MCI) is a term used to describe a variety of symptoms associated, in some cases, with exposure to indoor air contaminants. Individuals with this syndrome seem to respond to very low levels of chemicals, and the condition can involve various organ systems. It appears to be induced by a wide variety of agents, but once induced it can be triggered by low-concentration exposures to numerous other chemicals. Indoor air pollutants not only appear to set off symptoms in the chemically intolerant, but several studies suggest that some pollutants or pollutant mixtures may also initiate the condition. This phenomenon has been described in more than a dozen countries, including the United States, Canada, Australia, and nine European countries. Among the chemicals reported as initiating exposures were organophosphate and carbamate pesticides in the United States and organic solvents in Europe. The fact that people in different countries have different cultural practices and time-use patterns, live in buildings made out of different construction materials, have different ventilation practices and uses of chemicals indoors and yet share a toxicant-induced loss of tolerance (TILT) is a compelling anomaly that is still the cause of much debate.

Management of Indoor Air Quality

Control and improvement of indoor air quality can be achieved by proper design and construction of buildings. Design considerations include site selection for the building, building envelope design, ventilation, commissioning levels of pollutants, selection of materials, and combustion appliances. Indoor air pollution control in existing buildings includes the management of pollutant sources, operation and maintenance of ventilation systems, and air cleaning. Resolving indoor air-related problems includes addressing occupant complaints and symptoms, applying building diagnostic procedures, and conducting building and health surveys from the end of building managers. Governments can help to improve indoor environment quality by developing and implementing integrated strategies for the indoor environment, strengthening public education, and supporting research and technology development. Once an understanding of the nature of the problem is obtained, remediation generally involves some combination of the following: changes in ventilation; source removal, substitution, or modification; air purification; or changes in human behavior. While details are beyond the scope of this entry, some examples of these approaches will suffice. Increased ventilation to allow dilution of indoor air with fresh outdoor air or recirculated indoor air can reduce levels of combustion by-products, biological agents, and radon gas; removal of sources or substitution of less hazardous materials for asbestos insulation and organics in consumer products and furnishings can reduce contamination by these agents; source modifications, such as reduction of contaminant emission rates through design changes or containment of emissions by some barrier, can reduce levels of combustion by-products, radon, and volatile organics; and behavioral modifications can reduce cigarette smoke exposure to nonsmokers. Management of the quality of the indoor environment concerns not only indoor air quality but also thermal comfort, lighting, and noise protection.

In addition to the management procedure mentioned above, in developing countries, management of indoor air pollution is a very important task due to the use of open stove cooking and heating indoors. Two types of interventions play a role: technical interventions include change of kitchen layout, improvement of stoves and use of fuel alternatives. Social–behavioral interventions refer to change of behavior with respect to cooking traditions and practices, and cultural patterns; they also refer to issues in population groups (e.g., women's involvement, education, workload and time constraints) and the community (needs, training, sustainability of intervention, access to cleaner fuels).

See also: Asbestos; Combustion Toxicology; Diesel Fuel; Fuel Oils; Pollution, Air; Respiratory Tract; Tobacco Smoke; Volatile Organic Compounds (VOC).

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Pollution, Soil

Thomas E McKone

Introduction

Soil is the thin outer zone of the earth's crust that supports rooted plants and is the product of climate and living organisms acting on rock. A true soil is a mixture of air, water, mineral, and organic components. The relative mix of these components determines both the value of the soil for agricultural and other human uses and the extent to which chemicals or biological organisms added to soil will be transported and/or transformed within the soil. Soils are characteristically heterogeneous. A trench dug into the soil zone typically reveals several horizontal layers having different colors and textures. These layers and their generic structure are illustrated in **Figure 1**. These multiple layers are often divided into three major horizons – (1) the 'A' horizon, which encompasses the root zone and contains a high concentration of organic matter; (2) the 'B' horizon, which is unsaturated, is below the roots of most plants, and contains a much lower organic carbon content; and (3) the 'C' horizon, which is the unsaturated zone of weathered parent rock consisting of bedrock, alluvial material, glacial material, and/or soil of an earlier geological period.

In an ecological sense, soils exist where the atmosphere, the hydrosphere, the geosphere, and the biosphere all converge. Thus, contaminants in soil can impact human health and the environment through a complex web of interactions. The sections below provide an introduction to three issues related to toxicology and soil – (1) the potential for soil

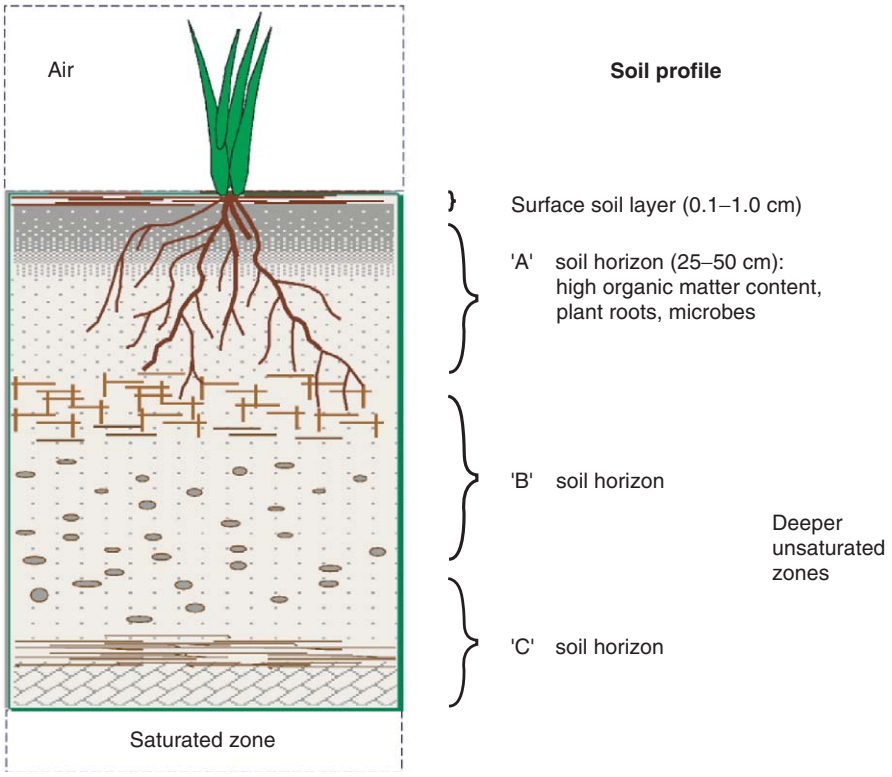

Figure 1 The typical horizontal profile and structure of soil in the unsaturated zone.

contamination by chemical, biological, and radioactive contaminants; (2) the potential fate, including both transport and transformation processes, for contaminants in soil; and (3) the types of direct and indirect human and animal contacts with soil contaminants that can result in risks to human health.

Soil Contamination

Throughout the world soils are contaminated to some extent from local, regional, and global pollution sources. Frequently, this contamination is the result of human and natural activities that involve the direct application of contaminants to soil. However, soil contamination also results from the transfer by rain and dry deposition of contaminants from air; by the transfer of contaminants through sewage-sludge (biosolids) applications; from the use of contaminated water for irrigating farms, gardens, or lawns; or by the soil itself through natural physical or biological agents that provide a source of contamination. Metal species and radionuclides released from combustion processes or from volcanoes and persistent organic pollutants migrate globally in the atmosphere and result in low levels of soil contamination as a result of deposition from the atmosphere. Pesticide use and the disposal of radioactive, biological, and chemical wastes can lead to much higher but localized levels of soil contamination. Some sources of contamination, such local high concentrations of toxic elements, the natural production of radon in soils, and the replication of toxic organisms are not external but internal to the soil. In the sections below, sources of soil contamination are identified and discussed.

Direct Application of Contaminants to Soil

Direct releases to soil occur in the form of pesticide, herbicide, and fertilizer applications; burial or land farming of domestic and industrial wastes; applications of sewer sludge to agricultural lands; and chronic releases from motor vehicles, resulting from the wear of brakes and tires as well as oil leaks. In addition, accidental discharges to the soil from storage tanks and miscellaneous spills during the transport of toxic substances can also occur. Contaminant releases to soil are normally quantified in terms of mass per unit area per unit time. For example, pesticide applications to agricultural fields can range from under 1 to over 20 $\mathrm{kg\,ha^{-1}}$.

Deposition from the Atmosphere

Contaminants in the atmosphere can be transferred to soil either directly through dry deposition, wet deposition, and vapor partitioning or indirectly

through deposition to plants, whose parts fall onto the soil. Dry deposition is the process by which particulate matter settles out of the atmosphere and onto soil and plant surfaces. Contaminants that are attached to these particles will be transferred to soil through this deposition process. Atmospheric contaminants on particles are also washed out of the air to soil with rain or snow in the wet deposition of the particles. Contaminants dissolved in the gas phase of air and not bound to particles can also be transferred to soil through a combination of wet deposition and chemical partitioning. Contaminants dissolved in air that are water soluble are easily washed out during rain and snow. This is wet deposition of a gas phase. In addition, contaminants that are water soluble can be transferred from air to soil through partitioning, which involves the diffusing of chemical from solution in air to solution in the soil water. Similarly, contaminants that are relatively insoluble in water but highly lipid soluble can be carried from air to soil through partitioning into the organic phases of soil. In this process, the contaminants diffuse from solution in air to solution in the organic phase of soil. Finally, contaminants in air can be transferred from air to vegetation surfaces by dry deposition, wet deposition, and by partitioning into the lipid and water phases of plants. When the plants decay, lose leaves, or are mowed, residual contamination is transferred to soil.

Use of Contaminated Water for Irrigation

The use of contaminated water supplies to irrigate farmlands, gardens, and lawns can result in the accumulation of persistent compounds in the irrigated soil. Organic contaminants with low water solubility, when introduced to the soil, will migrate to the organic carbon-phase of the soil where they can be retained for relatively long periods. Some metal species can also accumulate and persist in soil if their soil chemistry favors the binding of these contaminants into the mineral phase.

Use of Sewage Sludge on Agricultural Lands

A large fraction of the sewage sludge produced in many regions of the world is used as soil amendments often after treatment to reduce the content of harmful microorganisms. Sewage sludge is the semisolid residue from municipal wastewater treatment plants. Sewage sludge contains nutrients and organic matter that can improve soils. They also contain contaminants and pathogens that are discharged to the sewer system from homes, businesses, industries, and streets. Controversy surrounding both the practice of land application and the science behind the regulations as well as allegations of illness and even death resulting from use of sewage sludge prompted the US Environmental Protection Agency to commission a study by the National Research Council (NRC) of the US National Academy of Sciences on the health risks of sewage sludge. The NRC completed its report in 2002.

Contaminant Sources Internal to the Soil

In some cases the source of soil contamination is the soil itself. For example, soils rich in toxic elements such as arsenic, lead, mercury, and cadmium provide their own source of contamination. In addition, soils rich in uranium and its radioactive decay product radium provide continuous long-term sources of the radioactive gas radon in soil. The radon can diffuse from soil into the air of buildings or into groundwater, with resulting radiation exposures to human and animal populations. Other possible sources of contamination internal to soil itself are biological organisms, which are either themselves health threatening or which produce toxic chemicals.

Transport and Transformation of Soil Contaminants

There are a number of competing processes that impact the fate of a physical, chemical, or biological contaminant found in soils. When a contaminant is added to or formed in a soil column, there are a number of mechanisms by which it can be transported out of the soil column to other part of the environment, be destroyed, or be transformed into some other species. Therefore, once a contaminant has been identified in the soil column, one must also determine whether that substance will (1) remain or accumulate within the soil column, (2) be transported by dispersion or advection within the soil column, (3) be physically, chemically, or biologically transformed within the soil (i.e., by hydrolysis, oxidation, etc.), or (4) be transported to another part of the environment through a cross-media transfer (i.e., volatilization, runoff, groundwater infiltration, etc.). The purpose of this section is to provide an overview of the processes by which contaminants are transported in and out of soil layers and to provide a summary of typical transformation processes. **Table 1** summarizes processes by which contaminants are transferred to and from soils.

The Composition of Soil

In terms of their ability to transport, sequester, or transform harmful substances, we regard soils as composed of three major phases – gases, liquids, and

Table 1 Processes by which contaminants are transferred to and from soils

Gains	*Losses*
Deposition from air	Volatilization to air
Washout from air by rainfall	Resuspension of soil particles
Dry deposition of air particles	Mass transfer (diffusion and advection) downward to groundwater
Mass transfer (diffusion and advection) upward from groundwater	Transfers to vegetation
Contaminant sources	Soil solution runoff
	Erosion (mineral runoff) to surface water
	Chemical/physical transformation

solids. The fraction by volume that each of these phases contributes to total soil volume varies with soil type and with depth. The volume fraction of soil that is gas varies from a value of 10% typical in clay soils to 25% typical in sandy soils. The volume fraction of gas in soil decreases as one moves from the 'A' down through the 'C' horizon. The water phase of soil, the 'soil solution', consists mostly of water but includes dissolved minerals and nutrients. The volume fraction of soil that is liquid ranges from 10% typical of sandy soils to 40% typical of clay soils. The solid phase of soil makes up from 50% to 80% by volume of the soil composition and from 75% to 90% by mass of the soil. Soil solids include mineral (i.e., the parent rock) and organic components, including humic acids and decaying matter. The mineral component of soil is in the range of 70–90% by mass. The organic phase of soil is defined by the organic-carbon content of the soil. The organic-carbon content of soil ranges from much less than 1% by mass for desert and/or sandy soils to as much as 5% by mass for clay soils and even as high as 10% by mass for carbon rich soils such as peat bogs.

Transport Processes in the Soil Column

In order to understand how chemical species are transported in soil, it is important to recognized that the soil column needs to be viewed as having at least three distinct reservoirs for contaminants. These reservoirs are – (1) the surface-soil layer, (2) the rooting zone, and (3) the deeper unsaturated zone. The nature of these soil components is described below. These layers are illustrated in **Figure 1**.

The Ground-Surface-Soil Compartment Studies of radioactive fallout in agricultural land-management units reveal that, in the absence of tilling, particles deposited from the atmosphere accumulate in and are resuspended from a thin ground- or surface-soil layer with a thickness in the range 0.1–1 cm. The ground-surface-soil layer is at the top of the 'A' soil horizon. The ground-surface-soil layer has a lower water content and higher gas content than underlying layers. Contaminants in this surface-soil layer are more likely than deeper-soil contaminants to be transported horizontally by mechanical runoff and soil-solution runoff to nearby surface waters. Surface-soil contaminants are susceptible to wind erosion, volatilization, photolysis, biodegradation, and transfer to plant surfaces by rainsplash. In contrast to contaminants in deeper soil, surface-soil contaminants are susceptible to chemical transformation by sunlight. Surface-soil contaminants are transferred to and from air by diffusion and resuspension/deposition and transferred to and from the rooting-zone soil by diffusion and leaching.

The Rooting-Zone Soil Root-zone soil includes the 'A' horizon below the surface layer. The roots of most plants are confined within the first meter of soil depth. In agricultural lands, the depth of plowing is 15–25 cm. In addition, the diffusion depth, which is the depth below which a contaminant is unlikely to escape by diffusion, is on the order of a meter or less for all but the most volatile contaminants. Soil–water content in the root zone is somewhat higher than that in surface soils. The presence of clay in this layer serves to retain water. Contaminants in root-zone soil are transported upward by diffusion, volatilization, root uptake, and capillary motion of water; transported downward by diffusion and leaching; and transformed chemically primarily by biodegradation or hydrolysis.

The Deeper Unsaturated Soil The deeper unsaturated soil includes the soil layers below the root zone and above the saturated zone, where all pore spaces are filled with water. This compartment can encompass both the 'B' and the 'C' soil horizons. The soil in this layer typically has a lower organic carbon content and lower porosity than the root-zone soil. Contaminants in this layer move downward to the groundwater zone primarily by capillary motion of water and leaching. Chemical transformation in this layer is primarily by biodegradation.

Transformation

The transformation of toxic substances in soil can have a profound effect on their potential for human exposure and accumulation by biota. Transformation processes in soil include physical processes such

as radioactive decay; chemical processes such as photolysis, hydrolysis, and oxidation/reduction; and biological processes such as microbial transformations. All of these processes can significantly reduce the concentration of a substance or alter its structure in such a way as to enhance or diminish its toxicity.

Radioactive Decay Radioactive elements are made up of atoms whose nuclei are unstable and give off atomic radiation as part of a process of attaining stability. The emission of radiation transforms radioactive atoms into another chemical element, which may be stable or may be radioactive such that it undergoes further decay.

Photolysis Most organic contaminants are capable of undergoing photolytic decomposition. Such decompositions can be partial, resulting in the formation of stable by-products, or complete, resulting in the destruction of the compound or organism. Although the atmosphere attenuates solar radiation before it reaches the earth's surface, the solar radiation generally sufficient to break bonds in many compounds at this surface. Phototransformation in soil impacts only those contaminants on the soil surface. However, in agricultural lands that are tilled, contaminants in the tilling horizon ($\sim$15–20 cm) can be brought to the surface where phototransformation occurs. Phototransformations can result in relatively short half-lives (e.g., hours to days) for contaminants such as pesticides that are applied directly to crops or surface soils.

Hydrolysis Hydrolytic transformation of organic chemicals can be a significant destructive process for toxic compounds that are present in the aqueous phase of soils. Hydrolysis is most important for chemicals that have functional groups (e.g., amides, esters, carbamates, organophosphates), which can be rapidly altered (e.g., minutes to days) in the presence of water. For amides and carbamates, hydrolytic cleavage yields aromatic and aliphatic amines with increased likelihood of toxic activity. Conversely, hydrolytic degradation of compounds that contain stable constituents (e.g., halogenated compounds such as carbon tetrachloride) can have half-lives of several thousand years. Because hydrolytic reactions are driven by the availability of hydrogen and hydroxide ions, the pH of the soil can have a dramatic influence on the rate of hydrolysis for any given compound.

Oxidation and Reduction Many inorganic and organic chemicals can undergo oxidation or reduction reactions in soil. An indicator of a compound's ability to be oxidized or reduced is provided by its oxidation potential (E^o), which is the voltage at which it is transformed to its reduced state. A similar measure of a soil's ability to reduce a compound is provided by the redox potential (pE), which is a measure of electron activity. Redox potentials are relatively high and positive in oxidized environments (e.g., surface waters), and low and negative in reduced environments (e.g., aquatic sediments and the subsurface soil layers). These environmental conditions are especially important for inorganic chemicals that are rarely present in their elemental form in the environment. Arsenic, for example, exists primarily in its oxidized form (arsenate) in the atmosphere and in surface waters and in its reduced form (arsenite) in sediments.

Microbial Transformation Due to their broad range of enzymatic capabilities, microorganisms are capable destroying other microorganisms and transforming many inorganic and organic compounds. The chemical transformations can result in the partial degradation of a compound (e.g., conversion of trinitrotoluene to dinitrotoluene), mineralization (i.e., complete transformation to carbon dioxide and water), or synthesis of a stable product (e.g., formation of methyl arsenicals from arsenate). While these processes generally result in the detoxification of the parent compound, toxic products may also be formed. For example, the microbial metabolism of aromatic amines can result in the formation of toxic by-products.

Human Contact with Soil

Human contacts with soil can be multiple and complex. **Table 2** lists a matrix of potential human

Table 2 The matrix of exposure pathways that link humans with contaminated soils through direct and indirect contacts

Exposure routes	*Exposure pathways linking contaminated soil with human contact*
Ingestion	Direct soil ingestion by humans
	Ingestion of fruits, vegetables, and grains contaminated by transfer from soil
	Ingestion of meat, milk, and eggs contaminated by transfer from soil to plants to animals
	Ingestion of meat, milk, and eggs contaminated through soil ingestion by animals
	Ingestion of groundwater contaminated by soil
Inhalation	Inhalation of soil vapors that migrate to indoor air
	Inhalation of soil particles transferred to indoor air
Dermal contact	Dermal contact with soil

contacts with soils that can result in human uptake of soil contaminants through inhalation, ingestion, and dermal exposure routes. In the sections below we consider what is known about some of these exposure pathways and how they might be assessed in a risk assessment or other health-effects study.

Direct Soil Ingestion

Both adults and children continuously ingest small amounts of soil through inadvertent hand-to-mouth activities. Children who spend a great deal of time outdoors have been observed to contact and ingest soil through their repeated exploration and contact with surfaces and their frequent hand-to-mouth activities. But even adults through activities such as gardening, outdoor labor, and cleaning are also subject to inadvertent soil ingestion. Some individuals have been observed to intentionally ingest rather large quantities of soil. The ingestion of nonfood substances such as soil is called pica. Geophagia is the intentional, chronic, and often addictive consumption of earth. Although they are not activities common to the population at large, pica and geophagia can result in very large consumptions of soil contaminants and put the groups who engage in these activities at much higher risk of exposure to soil contaminants.

Several studies have been conducted to characterize ranges of soil ingestion by children. Some studies make use of measurements of soil levels on children's hands in combination with observations of hand-to-mouth activity to estimate soil uptake. The reliability of this method has improved recently by the introduction of videotaping combined with computer-based evaluation of the tapes to record hand-to-mouth activity. Another approach to soil ingestion measurement makes use of tracer elements in feces. Both feces of children and soil in their play yard are analyzed for elements such as aluminum, silicon, and titanium – elements thought to be poorly absorbed in the gut. Assuming no nonsoil sources of these elements, and a fecal excretion rate, soil ingestion for each child is estimated on the basis of the mass of each tracer element in feces relative to that in soil. Hospitalized children who have little contact with soil are often used as control groups.

Transfer of Soil Contaminants to Vegetation and Food Products

Soil contaminants in both the rooting zone and the surface-soil layer can be transferred to edible parts of vegetation by a number of processes. Contaminants in the rooting zone are transferred to plants through root uptake. The partitioning of contaminants between soil and root tends to increase with increasing contaminant concentration, since the root membrane on most plants restricts uptake to dissolved species. Contaminants in the rooting zone can be transferred to surface soil by plowing and tilling or by the activities of burrowing animals such as worms, ants, and rodents. Contaminants in surface soil can be transferred to edible plant parts through resuspension/deposition, rainsplash, and volatilization/partitioning. Resuspension/deposition is the process in which soil particles are blown by the wind up from the soil surface and then fall back onto the leaves of vegetation where the soil contaminants can be retained for some time on the leave surfaces or absorbed by the plant into the leaf tissues and possibly transported to other parts of the plant. Rainsplash is a process in which the impact of falling rain drops onto the soil surface causes soil particles to scattered into the air with impact onto plant surfaces. Volatilization/partitioning is a two-step process in which contaminants with a sufficiently high vapor pressure are volatilized from the soil and then collect into the waxy surface or the water portion of leaves through air/lipid or air/water exchange.

In the current scientific literature, plant/soil bioconcentration ratios (BCRs) are used to express a concentration ratio that relates the concentration measured in edible vegetation to a concentration in the soil supporting that vegetation. The plant–soil BCR expresses the ratio of contaminant concentration in plant tissues, roots, stems, leaves, seeds, and fruit, in milligram per kilogram (plant fresh mass) to concentration in soil. There are different protocols for expressing soil concentration among the different researchers who have measured plant–soil BCRs. Some express soil concentration in the soil solution, milligram per liter, whereas others use the soil dry mass concentration milligram per kilogram.

Contaminants in vegetation can be transferred to food products that are derived from the vegetation. The level of contamination of vegetative food products often depends on which part of a plant is being consumed. Translocation, which is the process by which a contaminant is transferred from one part of a plant to another, can result in significant differences in contaminant concentration between the total plant and the part of the plant being consumed; that is, the fruit or seeds. In addition, ingestion of contaminated soil and the ingestion of soil-contaminated pasture or grains by food producing animals can lead to the contamination of animal-based food products; that is, meat, milk, dairy products, and eggs.

Dermal Contact with Soil

Dermal exposure to contaminants in soil can occur during a variety of activities, such as construction

work, gardening, and recreation outdoors. Adults who work outdoors in activities such as construction, farming, or gardening can have rather high soil loadings on their skin. Children playing outdoors can also have rather large soil loadings on their skin. Lipid-soluble chemicals have a strong tendency to move from a soil layer on the skin surface to the lipid-rich outer layer of human skin. However, the rate at which this transfer takes place is often very slow and could require hours or even days to reach an equilibrium state. Estimating doses that result from dermal contact with a contaminated soil involves a number of often difficult-to-measure parameters, including the contaminant concentration in soil, the soil-to-skin adherence factor, the chemical-specific absorption factor for the skin–soil system, the exposure frequency, and the exposure time. The exposure frequency expresses how often, that is, days per year, an individual is involved in an activity the results in soil contact. The exposure time is a measure of how long, in hours, the soil is in contact with skin during an exposure activity.

Dose estimates for soil contact include a great deal of uncertainty. This uncertainty arises because we must deal with the transport of chemicals within the skin layer; the interaction of the soil layer on the skin with the skin surface; the dynamic conditions always involved in scenarios addressing interaction of the skin surface with chemicals, soil, air, and water; and addressing the level of protection provided by clothing.

Inhalation of Soil Particles Suspended as Dust

Soil contaminants that are bound to soil particles can be resuspended and inhaled along with the fine particles to which these contaminants are attached. The inhalation of suspended particles can take place both outdoors and inside buildings. Exposure assessors and toxicologists now recognize that fine and coarse particles in the indoor environment are attributable to both air and soil sources and enter the indoor environment by processes such as penetration through windows and cracks and soil tracking. Soil tracking is the process by which soil particles are carried into the indoor environment by shoes and clothing of human occupants as well as on the feet and fur of pets.

Contaminant Vapor Transport into Buildings

The vapors of volatile contaminants, such as radon and volatile organic compounds, can be transported through diffusion from the soil pore spaces into buildings. Three principal factors are needed to define the ratio of contaminant concentration in indoor air to observed contaminant concentration in soil gas. These are (1) the distance between the contaminant source and the building foundation, (2) the permeability of the soil, and (3) the area of cracks in the foundation relative to the total area of the foundation.

Groundwater Contamination

Soil contaminants can be transformed by physical, chemical, and/or biological processes. Those that are not transformed can be carried to groundwater in areas of net recharge. Once contaminants move from soil into groundwater these contaminants can contact humans through a number of exposure pathways – such as direct water ingestion, dermal uptake in showers/baths, irrigation of crops, feeding food-producing animals.

Summary

The purpose of this article is to consider the nature of soils, how soils are contaminated by human activities, how these contaminants are transported and transformed in the soil column, and the types of human activities that could result in human exposure to soil contaminants. Soils are complex systems that exist at the interface among atmosphere, biosphere, hydrosphere, and lithosphere. A true soil includes gas, water, mineral, and organic components. Potential human contacts with soil can result in inhalation, ingestion, and dermal uptake of soil contaminants through both direct and indirect exposure pathways. The magnitude and persistence of exposure depends not only on the level of soil contamination, but also on the physical and chemical properties of soil, the chemical properties of the contaminant, and the frequency and duration of human activities such as occupational and recreational activities or use of home-grown food, which result in direct and indirect soil contacts. Toxicologists should be aware of the complex nature of soils, of the potential of soil contamination, and of types of direct and indirect contacts that human populations have with soil.

See also: Pollution, Air; Pollution, Water.

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Pollution, Water

Ruth Custance

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Water is a major transporter of toxic chemicals in the environment. Our view of the hazards associated with water pollution varies considerably depending on how the water is to be used. Chemicals in water used for drinking or bathing result in direct exposure to humans and, if doses of chemicals derived from these activities are sufficiently high, these exposures can lead to toxic effects. However, chemicals introduced into streams and lakes frequently result in exposures by less direct means, for example, by eating fish obtained from these waters. The nature of chemicals that are involved in exposure via drinking water and that of chemicals involved in exposure through food derived from contaminated water are frequently quite different. Generally, chemicals that are found in drinking water tend to have significant water solubility and low affinities for clay and organic matter found in soils. Those chemicals that are obtained in food derived from water are generally much less soluble in water and have very high solubility in fats. These properties account for their accumulation in fish tissues. Many of the chemicals which bioaccumulate are also poorly degraded in the environment. This further contributes to their accumulation. In many cases, the impact of these latter chemicals on wildlife is more important than their effects on human health.

Chemicals are introduced into water in a variety of ways. In the past, the focus has been on industrial pollution. When industrial outfalls into bodies of water were less well-controlled than they generally are today, these point sources were important. While it is important to recognize that point sources of chemicals can still be responsible for local or regional problems in water quality, nonpoint sources of water pollution contribute much more to chemical contamination of water on a national basis. Chemical contamination of groundwater has occurred as a result of poor chemical disposal practices in the past or the leaking of storage vessels such as underground tanks and landfills. The types of chemicals that occur in groundwater are chemicals that are very mobile in soils. As with other point sources, the impact of these sites is local rather than national in scope. However, significant portions of entire groundwater basins used for drinking water have been contaminated in various regions of the country with the more mobile compounds especially the fuel oxygenate, methyl tertiary butyl ether (MTBE). Because, cleanup of groundwater is technically very difficult and expensive. Every effort must be made to prevent this type of contamination. While the actual impact on water used for drinking is relatively small when considered on a national basis compared to other types of water pollution, the problem of uncontrolled hazardous waste sites does occur in all regions of the country and produces a great deal of public concern. In addition, there have been instances where for more mobile compounds such as perchlorate, widespread contamination of groundwater and surface water used for drinking water has occurred as a result of releases from hazardous waste sites. Nonpoint sources would include chemicals used in

agriculture, such as fertilizers and various pesticides; fallout from products of incomplete combustion, such as the automobile; and chemicals that are washed into streams, rivers, and lakes by runoff of urban areas. Thus, the types and numbers of chemicals that can contaminate water from these sources are practically endless.

In recent years, efforts have been undertaken to conserve water due to the growing demand for a limited resource. As a result, water reuse is being employed, especially in the Western United States. Water reuse is when wastewater generated from a community is reclaimed for a beneficial use such as irrigation for ornamental or agricultural crops, decorative water features, industrial application and with advanced treatment potable water. The use of reclaimed water may expose the public to chemical and microbial contamination from the wastewater stream. Therefore, for each type of beneficial reuse, treatment standards are being established to protect public health.

Chemicals are also deliberately added to water. In the treatment of wastewater, and more particularly in the treatment of drinking water, a variety of chemicals are added for purposes of disinfecting, clarifying, and preventing corrosion of pipes. Moreover, as water is distributed to consumers, the surfaces it contacts have the potential of contaminating the water. These surfaces may be the water mains and pipes in a municipal distribution system, or they may be the surface of a plastic bottle in which the water is purchased in a supermarket.

Because of the complex sources of chemicals in water, contaminants of water will be discussed as they are introduced into water; those introduced into ambient water, chemicals introduced during the treatment of water, and contaminants associated with the distribution of water.

Contaminants of Ambient Water

Natural Contaminants

It is important to recognize that the bulk of the chemicals found in water are of natural origin. Many of these chemicals are innocuous at even the highest concentrations that might be found in freshwater. Some are essential minerals and metals that are important to the normal physiology of the body. These would include sodium, chloride, magnesium, calcium, bicarbonate, carbonate, sulfate, and iron. Occasionally, these materials are present at concentrations that will cause gastrointestinal disturbances (e.g., diarrhea induced by sulfates and nausea and vomiting due to copper).

Occasionally, water will come into contact with natural deposits of potentially hazardous chemicals. A relatively frequent contaminant of groundwaters in the Western United States is arsenic. Usually the concentrations are below $100\,\mu g\,l^{-1}$, but there are concerns that such concentrations may represent a cancer hazard. At higher doses, of course, arsenic is clearly toxic to a variety of organ systems. Less frequently, river water may erode deposits of asbestos. While asbestos is recognized as being carcinogenic when it is inhaled, there has been no convincing evidence that ingested asbestos presents such a hazard. This may be partly due to the small size of the asbestos fibers that are found in water. Fibers in excess of $5\,\mu m$ appear to be most dangerous.

Surface waters (i.e., streams and lakes) or groundwater influenced by surface water also contain a complex mixture of organic chemicals. These may range from a fraction of a $mg\,l^{-1}$ up to 10s of $mg\,l^{-1}$. Some of these chemicals are simple sugars, amino acids, and low-molecular-weight organic acids that are normal biological substrates. The bulk of these organic compounds, however, are humic substances. Humic substances consist of humic and fulvic acids which are polymers of small-molecular weight products of biological decay that form over time. The size of the humic acid molecules can be quite large and they can involve very complex and individual structures. Fulvic acids are significantly smaller and tend to be more soluble. The properties of these substances vary considerably in different climates. They are responsible for the dark color seen in many standing waters. In themselves, these chemicals do not pose health hazards. However, they do serve as substrates for reactions with various oxidant chemicals used in the treatment of drinking water.

Agricultural Chemicals

Agricultural chemicals have a high probability of affecting water supplies if they have a significant water solubility, are not rapidly degraded, and have a low affinity for soils. Fortunately, most chemicals currently used in agriculture do not fit this category. However, the large volume used of certain chemicals that are mobile in soils does result in adverse impacts on both surface water and groundwater. The most widespread example of this is nitrates derived from the use of fertilizers. The concentrations of nitrate in surface waters frequently exceed drinking water standards during certain times of the year. A more pervasive problem, however, is the relatively widespread contamination of groundwater by nitrate. These concentrations will remain high for years to

come, even if practices introducing them into the groundwater were stopped today.

Much of the public fear of agricultural products is focused on the use of various pesticides. Many of these compounds are highly toxic. Fortunately, those which are the most toxic and likely to contaminate water, the organophosphorus pesticides, are generally degraded in water. These chemicals would include parathion, methyl parathion, terbufos, and malathion. These chemicals have been found in water, but generally at low concentrations. On the other end of the spectra are the very water-insoluble compounds, such as DDT, chlordane, dieldrin, and lindane, that have high affinity for soils and are found primarily in particulate matter in water. Paraquat is a very dangerous contact herbicide that appears to be very immobile in soils and has rarely, if ever, been found in ambient waters. Generally, these particulates are removed from water before it is used for human consumption and any chemical remaining in the water is at very low concentrations ($<0.01\,\mu g\,l^{-1}$). In addition, the water-insoluble pesticide's affinity for soils minimizes their impact on groundwater. There are, however, a small number of pesticides, such as aldicarb (Temik) and diazinon, that are very mobile in soils and which can be significant contaminants of water. The other group of chemicals that are of concern are low-molecular weight halogenated compounds that are used as soil fumigants. These would include ethylene dibromide, dibromochloropropane, and 1,3-dichloropropene. In agricultural regions, these chemicals have been widely detected in groundwater, which is of concern due to their reproductive toxicity at low doses. The former two chemicals have recently been banned by the US Environmental Protection Agency (EPA). Herbicides such as atrazine, butylate, chloramben, DCPA (dacthal), MCPA, dicamba, metolachlor, metribuzin, picloram, prometon, pronamide, propachlor, propazine, simazine, and 2,4,5-T have also been detected in surface water and/or groundwater supplies. The last compound is no longer in use in the United States because it was contaminated with low levels of a very toxic chemical, 2,3,7,8-tetrachlorodibenzodioxin.

Industrial Chemicals

Industrial contamination of water occurs as the result of directly introducing contaminated wastewater into a body of water or lake or from improper disposal of chemicals to the land. The chemicals most frequently found in water from both of these activities are chemicals that are used in very high volume or are very mobile. The nature of surface waters contamination is more likely to depend on the nature of the industry impacting a particular body of water. The soil surrounding a disposal area frequently acts as an effective barrier to contamination to many chemicals found in hazardous waste sites.

Probably, the most frequent contaminants of water from these two sources are spilled liquid fuels, such as gasoline, kerosene, and diesel oil, and low-molecular-weight solvents such as trichloroethylene, tetrachloroethylene, 1,1,1-trichloroethane (methyl chloroform), benzene, toluene, various xylene isomers, MTBE and aliphatic hydrocarbons. Other solvents may also be found in high concentrations in groundwater where they have been disposed of in large quantities. These would include solvents no longer in common use such as carbon tetrachloride or chloroform. The toxicology of these chemicals varies widely. Acutely and at very high doses most solvents depress the central nervous system (CNS). It is very unlikely that such concentrations would be achieved as a result of environmental contamination. Some of these chemicals have a high probability of producing liver or kidney damage as delayed effects or under conditions of more chronic exposure (e.g., carbon tetrachloride, chloroform, 1,1,2,2-tetrachloroethane, and 1,1,2-trichloroethane). Others present a specific hazard of producing delayed and cumulative nervous system deficits (e.g., methyl butyl ketone, *n*-hexane, trichloroethylene, and toluene). Again, these effects may be observed at levels of exposure encountered occupationally but would be rare from generalized environmental contamination. Generally, the major concern of concentrations found in the aquatic environment is with those chemicals that produce cancer (e.g., benzene, vinyl chloride, dichloromethane, trichloroethylene, tetrachloroethylene, and carbon tetrachloride). Among these chemicals, only benzene and vinyl chloride have induced cancer in humans. Estimates of the cancer risks that arise from environmental exposures to these chemicals are quite controversial.

Around military bases and weapons laboratories of the US Department of Energy, there have been frequent incidents of groundwater contamination by explosives, specialized fuels, and radionuclides. Among the explosives identified in these circumstances are trinitrotoluene, HMX, and RDX. Radionuclides would include tritium, plutonium, and technetium.

Recently a group of chemicals have been identified called 'Emergent Chemicals' that are important regarding water quality. The term 'Emergent Chemicals' is used by the EPA and other regulatory agencies to identify a group of chemicals found in ground water and/or surface water that cause great concern with respect to drinking water supplies. Although detected at points of many monitoring wells, these chemicals

typically do not have State or Federal Regulatory standards due to their recent detection and the lack of health effects data. In addition, analytical methods to detect these compounds at sufficiently low concentrations may not be available or are under development. Finally, due to their chemical properties many of these compounds cannot be treated using standard water treatment processes. As a result, these chemicals pose a challenge to evaluate their occurrence and significance to public health.

One of the first chemicals to be identified in this group was MTBE, a fuel oxygenate that was added to gasoline to meet requirements of the Clean Air Act. Other chemicals that are used as fuel additives include tertiary butyl alcohol (TBA) and tertiary amyl methyl ether (TAME), however MTBE became the most popular due to production, blending, and cost considerations. While MTBE was considered less toxic than other fuel additives, MTBE and the other ethers are highly soluble and very mobile in water, and not readily biodegradable. The widespread use of these compounds resulted in significant and widespread groundwater contamination. Another important chemical that has been detected in drinking water sources is perchlorate. Perchlorate is an anion commonly associated with the solid salts of ammonium, potassium, and sodium perchlorate. The use of perchlorate includes the manufacture of air-bag inflators, matches and flares, paints photography, pyrotechnics, rubber, and tanning and finishing leather. Ammonium perchlorate has been used most significantly in Department of Defense (DoD) applications, as a component of explosives and rocket propellant. Because perchlorate salts readily dissolve in water, perchlorate contamination is being discovered in groundwater and surface water, especially near perchlorate production facilities and facilities that use large quantities in the manufacture of rocket propellants or devices such as flares. One of the more significant findings is the presence of perchlorate throughout the Lower Colorado River basin, a significant source of drinking water and irrigation water for the Southwestern United States. It is thought that the perchlorate is present due to releases from a former perchlorate-manufacturing plant near Lake Mead. Perchlorate can limit the uptake of iodide, an essential nutrient, by the thyroid gland. As a result, large doses of perchlorate were used therapeutically to treat hyperthyroidism. Current research indicates that at concentrations that may be present in the environment, perchlorate exposure can result in reduced levels of iodide in the thyroid which disrupts the thyroid hormones that regulate metabolism and growth. Certain populations are particularly susceptible, such as pregnant women and infants, to adverse health effects when this occurs. The toxicity of perchlorate and its effects on public health at concentrations typically measured in groundwater or surface water is under review by various regulatory agencies.

Attention is also being given to pharmaceuticals and endocrine disrupting chemicals (EDCs) that are being detected in water. EDCs are synthetic chemicals and natural plant compounds that may affect the endocrine system through mimicking the steroid hormones, estrogens and androgens, by binding to hormone receptors or influencing cell signaling pathways. Another way EDCs can act is by blocking or altering hormone binding to homornal receptors. Many of these substances have been associated with developmental and reproductive effects in wildlife and laboratory animals. Some proven environmental estrogens used as pesticides, most notably *o*,*p*′-DDT, toxaphene, and dicofol, have been banned from use in most western industrial countries but are still used in many developing nations. Other proven estrogenic compounds are still being used worldwide in plastics manufacturing (phthalates) and agriculture (endosulfan).

Chemicals Introduced during Treatment of Drinking Water

Chemicals are used for a variety of purposes in the production of drinking water. Also, chemicals are added to water for a variety of purposes. Reservoirs are frequently treated with herbicides to prevent overgrowth of vegetation. Disinfectants are added as barriers to the spread of waterborne infectious disease. Other oxidants (potassium permanganate and chlorine dioxide) are utilized to remove unwanted color or to remove or prevent the formation of chemicals that impart a bad taste or odor to the water. A variety of chemicals are utilized in the clarification of water. Alum and ferric chloride are utilized to aggregate particulate material in the water (i.e., coagulation). A variety of other polymeric chemicals are used to neutralize surface charge that prevents coagulation and settling of particulates and are referred to as coagulant aids. Lime is added to soften water and acids, bases, and buffers are added to adjust the pH and to control corrosion. It is impossible to catalog all the chemicals that are used in the treatment of water. For a more complete list, the interested reader is referred to the NSF International listing of chemicals that meet the NSF standards as drinking water additives (see Further Reading).

Disinfectants

In most of the world, disinfectants are reactive chemicals introduced into water to prevent the

spread of waterborne infectious diseases. In situations in which sanitation is poor, the need for disinfection is very obvious. However, the large amounts of water that are needed in metropolitan areas inevitably means that microbial contaminants are introduced into some of the source water. Chemical disinfectants provide an economical and simple technology for controlling these contaminants. On the other hand, disinfectants vary in their toxicological properties. Therefore, it is important to establish that these chemicals can be used safely at effective concentrations. Chlorine presents no specific toxicological problems at effective concentrations. Monochloramine (i.e., chlorine + ammonia) is relatively safe at the concentrations that are used, but it is a much poorer disinfectant than chlorine. Ozone presents no particular toxicological threat because it is not sufficiently stable in water to reach the taps at which the water is consumed. However, this is also a disadvantage because there is no residual disinfectant to prevent the outgrowth of microorganisms in the mains, service lines, and pipes that distribute the water. As a consequence, a second disinfectant is usually added after an initial treatment with ozone. Another chemical that has been proposed for use as a disinfectant is chlorine dioxide. This chemical is a very effective disinfectant, but it does produce thyroid disorders in experimental animals. Moreover, it degrades to two chemicals, chlorite and chlorate, that produce hemolytic anemia and methemoglobinemia. While it is probable that chlorine dioxide can be used safely for drinking water disinfection, there is less margin of safety with its use and the concentration needed for disinfection. Moreover, close attention must be paid to the amounts of chlorite and chlorate, which are inevitable by-products of this compound, that are produced in the distribution system and occur at the tap.

In 1974 it was discovered that the use of chlorine in the disinfection of water leads to the formation of a group of compounds referred to as the trihalomethanes. This group of compounds includes chloroform, bromodichloromethane, dibromochloromethane, and bromoform. The relative concentrations of the members of this class depend on the concentration of bromide in the water being disinfected. In recent studies, it has become clear that the trihalomethanes are only one class of by-products and that there are small concentrations of a wide variety of chemicals produced with chlorination. However, it should be recognized that all chemical disinfectants are reactive compounds and, as a consequence, all will produce unintended by-products as a result of their use.

Disinfectant by-products are produced by reaction of the disinfectant with other chemicals in the water. The bulk of these chemicals are of natural origin. Humic and fulvic acids are the most common organic chemicals present. These are formed by the natural decay of biological material and are in themselves harmless. As indicated previously, the bromide concentration in the water also influences the type of by-product that is formed. Chlorine and ozone oxidize bromide to hypobromous acid, which acts to add bromine to various chemicals. Under conditions of high pH (alkaline conditions), ozone can further react with hypobromite ion to produce bromate. Differences in pH also affect the levels of other chemicals that are produced. Acid pH results in the formation of a variety of mutagenic chemicals at very low concentrations when chlorine is utilized as the disinfectant, whereas high pH gives rise to higher concentrations of the trihalomethanes.

Epidemiological data suggest that chlorination of drinking water does increase the probability of developing cancer of the bladder and of the large intestine. The elevation of these cancers above background is relatively small. Consequently, the differences may be caused by other risk factors that were not identified. Animal studies do indicate that some of the chemicals that are produced with chlorination are capable of producing tumors, but the tumors have been more commonly found in the liver and kidney. Moreover, the actual risk predicted from the animal studies is much less than that suggested by the epidemiological studies. These differences may indicate that the results of the epidemiology studies were not correct. However, many of the chemicals produced by chlorination have yet to be evaluated in experimental animals. This is a very important question because many of the by-products that have yet to be studied in experimental animals are also produced by other disinfectants such as ozone. The modifications that should be made in the use of disinfectants will not be clear until the toxicological effects of these compounds have been established.

The types of chemicals produced by disinfectants and some specific examples are provided in **Table 1**. The reader should not be deceived by the fact that the list of by-products associated with chlorination is much longer than that of other disinfectants. This is the result of more thorough study, not necessarily an actual reflection of the numbers of by-products that are formed by each process.

Other Chemical Treatments of Water

An NSF International publication (NSF Listings, 1994) provides a complete list of products that have

Table 1 Classes of disinfectant by-products

Disinfectant	*Inorganic*	*Organic*	
		Halogenated	*Nonhalogenated*
Chlorine	Chlorate	Trihalomethanes Haloacetates Haloacetonitriles Haloaldehdydes Haloketones Halofuranones Chloropicrin	Aldehydes Carboxylic acids
Monochloramine		Cyanogen chloride	
		Others generally thought to be the same as chlorine, but of lower concentration	
Chlorine dioxide	Chlorite Chlorate	Not well-characterized	
Ozone	Bromate Hydrogen peroxide	Bromomethanes Bromoacetates Bromoaldehydes Bromoketones Iodinated analogs	Aldehydes Carboxylic acids

Table 2 Chemicals that are used as direct additives to drinking water

Chemicals	*Purpose*
Alum (aluminum salts) Iron (iron salts) Cationic polymers Nonionic polymers Anionic polymers Starch	Coagulation and flocculation for removal of particulate
Phosphates Polyphosphates Orthophosphates	Antiscalants, corrosion control, sequestering agents
Copper salts	Antifouling, algicides
Chlorine Calcium hypochlorite Sodium hypochlorite Ozone Chlorine dioxide Potassium permanganate Hydrogen peroxide	Oxidants (also disinfectants)
Calcium oxide (lime) Calcium hydroxide	Softening, pH adjustment
Potassium hydroxide Sodium hydroxide Hydrochloric acid Sodium bicarbonate	pH adjustment
Sodium flouride	Dietary supplement

been approved for use as direct additives to drinking water by NSF's certification program. The number of specific products used is too large to summarize easily in limited space. Consequently, a partial list of the active ingredients that are representative of products used for specific purposes is provided in **Table 2**.

Most chemicals that are direct additives to drinking water present little hazard to health. Many of these chemicals also have been used as food additives and have been subjected to appropriate levels of toxicological testing. Other additives, such as starch, are natural foodstuffs and would be generally regarded as safe, especially at the low concentrations that would be expected to reach the tap.

Polymeric chemicals are a somewhat special case. These are most frequently introduced as direct additives as coagulant aids. By virtue of their function, these polymers are almost quantitatively removed from the water during normal treatment. Even if applied inappropriately, these chemicals are of such high molecular weight that they would not be absorbed and are almost certainly not a threat to health if they have been properly tested. A potential difficulty with these chemicals is that they may contain varying amounts of the monomers used in their synthesis or other incompletely reacted material of lower molecular weight. Some of the monomeric compounds are quite toxic. Acrylamide is an example of one of these compounds that is neurotoxic, carcinogenic, and a reproductive toxin. Epichlorhydrin, vinyl chloride, and vinylidene chloride are additional examples of these chemicals. For this reason, the amount of unreacted monomer present in the product is closely regulated by certification agencies such as NSF International.

Chemicals Introduced during the Distribution of Water

Water used for human purposes is delivered in a variety of ways. It is placed in a container to be transported or it is forced by gravity into a system of mains, service lines, and pipes to deliver it to individual users. In both cases the water contacts a surface. Water is a very effective solvent and will invariably extract some chemicals from these surfaces. The surfaces that water contacts are metal, plastic, concrete, or a paint or other type of coating that is applied to the surface. In addition to pipes and containers, there are reservoirs and holding tanks in which similar problems are involved.

The chemicals leached from these surfaces depend on the corrosive properties of the particular water as well as the chemical nature of the surface. Hard water tends to deposit a mineral layer on the inside of pipes and on other surfaces that essentially limits the access of water to the surfaces. On the other hand, soft water, particularly at lower pHs, can actively dissolve toxic metals such as lead or cadmium from pipes or solder. Copper in pipes is also frequently leached from pipes at high concentrations when the water has corrosive properties. Asbestos-cement has been used widely in water mains. The extraction of the asbestos fibers from these surfaces is also very much increased at lower pH and with soft water. The use of lead pipe and solder in household pipes has pretty much been abandoned in the United States. However, alloys of lead are still utilized in many faucets and brass fixtures (e.g., submersible pumps). Rather high concentrations of lead can result if water stands in these fixtures overnight. As a result it is always wise to avoid using the water first drawn from the tap in the morning for human consumption. Low levels of lead exposure *in utero* or in the first few years of life have been associated with delayed CNS development in humans and experimental animals.

Plastic pipes are polymeric in nature (e.g., polyvinyl chloride). Within the pipe are traces of the monomers used in the manufacture of the pipe (e.g., vinyl chloride). In addition, there are a variety of other chemicals added during the manufacture of the pipe as lubricants to facilitate their manufacture or stabilizers to prevent the breakdown of the pipe. In Europe, lead has been used as the stabilizer for pipes, whereas various organic tin compounds have been utilized in the United States. Lead is widely recognized as being toxic. Inorganic tin has a very limited toxicity, but this is not the form of tin that is used. Some of the organic tin compounds are potent nervous system toxins (e.g., trimethyl or triethyl tin), while others appear to adversely affect the immune system (dioctyl tin). The forms of tin used in polyvinyl chloride pipe, however, are primarily monomethyl and dimethyl tin, which are much less active as neurotoxins than the trimethyl tin. There will be some extraction of all these chemicals from the pipe when it is first put into service. However, the concentrations that are found in the water decrease sharply with continued use of the pipe. This is only partially due to the depletion of the chemical from the pipe because continuous water flow will form an impermeable barrier (e.g., calcium carbonate) on the interior of the pipe that minimizes leaching from its surface.

Paints and coatings can be utilized on any surface in a distribution system all the way to the pipes in the consumer's home. However, most coatings are applied to storage tanks and water mains. In the past years, some rather dangerous coatings have been used. Coal tar paints were frequently utilized in the first several decades of this century. These paints contain very high concentrations of polycyclic aromatic hydrocarbons (PAHs). Generally, this does not pose much of a problem because the solubility of these compounds in water is quite limited. This is particularly true of most of those which are carcinogenic. However, when the coating begins to degrade with age, it tends to come off the surface as small particles. These very small particles can contain very high concentrations of benzo(*a*)pyrene and other PAHs and have been shown to be carcinogenic when introduced into the stomach of mice. Fortunately, the coal tar paints have been largely replaced by asphalt paints, which contain very much smaller concentrations of PAHs. However, many distribution systems throughout the country have mains which predate this conversion. Another suspect practice of the past was the use of red lead paint in water tanks. Fortunately, this product has also been abandoned.

Summary

The sources of water pollution are diverse. Some of this pollution occurs in the general environment and involves both point and nonpoint sources. Pollution of this kind can impact human health both directly, when the water is consumed for drinking purposes, and indirectly through accumulation of chemicals in foodstuffs derived from the water. The chemicals seen from these two sources have very different characteristics. There are new and emerging chemicals being detected in drinking water supplies that are important for public health. These chemicals are difficult to evaluate due to the lack of/or uncertainty regarding the available toxicity information. In addition, these chemicals can be difficult to treat using standard water treatment processes. Despite the fact that there is contamination of ambient water, most contamination of drinking water by chemicals occurs during its treatment and distribution. While there is no conclusive evidence that these sources of chemicals adversely affect health, it is important to keep this issue in mind in the development of new processes for treating drinking water and new materials for distributing drinking water.

See also: Ecotoxicology; Effluent Biomonitoring; Environmental Processes; Environmental Toxicology; Organophosphates; Pesticides; Pollution, Air; Pollution, Soil; Polycyclic Aromatic Hydrocarbons (PAHs); Polymers.

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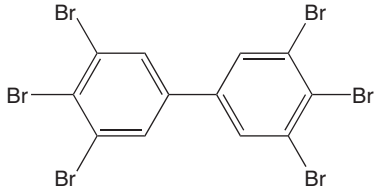
Relevant Website

http://www.epa.gov – Office of Water (US Environmental Protection Agency) – Activities, Documents, and News Online.

Polybrominated Biphenyls (PBBs)

Alan L Blankenship

- Representative Chemicals: Polybrominated biphenyls (PBBs) are members of the polyhalogenated diaromatic hydrocarbon (PHDH) class of compounds. PBBs are a complex mixture of individual compounds which are brominated with between one and 10 bromines in various combinations of positions to create a total of 209 possible congeners. The 10 positions are numbered 2–6 on one ring and 2′–6′ on the other ring. Positions 2, 2′, 6, and 6′, adjacent to the biphenyl bond are called *ortho* positions; 3, 3′, 5, and 5′, *meta* positions; 4 and 4′, *para* positions. Commercial products were mainly composed of hexa-, octa-, or deca-brominated homologs. Environmental contamination with PBBs is likely to have occurred mainly from two commercial products, FireMaster BP-6 and FireMaster FF-1. The principal components in both of these commercial products were 2,2′,4,4′,5,5′-hexabromobiphenyl or PBB-153 (54–68%) and 2,2′,3,4,4′,5,5′-heptabromobiphenyl or PBB-180 (7–27%)
- Chemical Abstracts Service Registry Numbers: CAS 67774-32-7; CAS 59536-65-1
- Synonyms and Commercial Products: Polybromobiphenyls; FireMaster BP-6; FireMaster FF-1; Bromkal 80; Flammex B 10; Adine 0102; Berkflam B 10
- Chemical Formula: $C_{12}H_nBR_m$ (where $n = 0$–9 and $m = 10$–n)
- Chemical Structure:



Uses

Polybrominated biphenyls (PBBs) are inert, stable chemicals used primarily as additive flame retardants to suppress or delay combustion. In their use as flame retardants, PBBs were added to polymer materials, but were not chemically incorporated into the polymer matrix and therefore could migrate out of the polymer matrix with time. Hexabromobiphenyl was used as a fire retardant mainly in thermoplastics in electronic equipment housings. Smaller amounts were used as a fire retardant in coating and lacquers, and in polyurethane foam for auto upholstery. After the voluntary ban of hexabromobiphenyl in the late 1970s, polybrominated diphenyl ethers (PBDEs) and other flame retardants were used as replacements.

Background Information

A significant incident of environmental contamination by PBBs occurred in 1973–74 when approximately 650 pounds (290 kg) of FireMaster BP-6 (principally composed of 2,2′,4,4′,5,5′-hexabromobiphenyl or P-153) were accidentally mixed with cattle

food that was distributed to a number of farms in the lower peninsula in Michigan. As a result, by June 1975, 412 farms had been quarantined. Use of PBBs as well as disposal of contaminated feed, animal carcasses (poultry, dairy cattle, swine), and animal products (dairy, meat, eggs) contributed to environmental contamination. About 50% of the original amount of PBBs mixed with feed was estimated to have been excreted in the feces of the exposed animals and remained on the farms in places of fecal deposition and manure disposal. PBB levels in surface soil samples from seven dairy farms in Michigan that spread contaminated manure on the fields ranged from 35 to 1260 $\mu g\,kg^{-1}$, while the concentrations in surface soil of control farms (that did not use contaminated manure) were $<25\,\mu g\,kg^{-1}$.

Exposure Routes and Pathways

In the past, PBBs were released to the environment during their manufacture and also during the disposal of commercial and consumer products containing PBBs. As a result, the general population may have been exposed to low levels of PBBs by inhaling contaminated air, ingesting contaminated water and food, and using consumer products containing PBBs. Prior to 1973, workers manufacturing fire retardants likely had the greatest potential for substantial exposure to PBBs. However, following the accidental contamination of cattle feed with PBBs in Michigan and the subsequent contamination of meat and dairy products, ingestion of contaminated foods became the exposure pathway of primary concern.

Although the episode in Michigan involving contaminated feed occurred in May 1973, incorporation of PBBs into foods was not identified until April 1974. Thus, PBB-containing meats, milk, butter, eggs, and cheese entered the human food chain for almost a year before the PBBs were identified. Concentrations of PBBs (on a fat basis) in milk samples collected from contaminated farms soon after PBBs were found ranged from 2.8 to 595 $mg\,kg^{-1}$. Concentrations of PBBs in other products processed from the contaminated milk were as follows: butter, 1–2 $mg\,kg^{-1}$; cheese, 1.4–15.0 $mg\,kg^{-1}$; and canned milk, 1.2–1.6 $mg\,kg^{-1}$. In 1974, the concentrations of PBBs in eggs from contaminated farm premises were as high as 59.7 $mg\,kg^{-1}$. The levels of PBBs in poultry and cattle tissues from a contaminated farm in 1974 were 4600 $mg\,kg^{-1}$ and up to 2700 $mg\,kg^{-1}$, respectively. With the seizure and destruction of the contaminated farm animals and products, the levels of PBBs in consumer products showed a steady decline. For example, in 1975, among 18 milk samples, 13 cheese samples, and 14 butter samples taken in Michigan, only three butter samples exceeded the FDA guidelines of 0.3 $mg\,kg^{-1}$ PBBs in fat. In 1975, PBBs were detected in 245/2040 meat samples collected in Michigan, with only 24 samples containing PBB levels $>0.3\,mg\,kg^{-1}$ fat. Although 95% of 1430 meat samples collected in Michigan in 1976 contained detectable PBBs, only one sample contained $>0.6\,mg\,kg^{-1}$, and a market basket survey in Michigan showed detectable PBBs in only 1/102 meat samples.

Currently, however, since PBBs are no longer produced, exposure of the general population to PBBs will likely only be from historical releases. Based on temporal data, it would appear that environmental levels have decreased substantially since the 1970s and current exposure, if any, will likely be at low levels.

Historical monitoring and body burden data indicate that low-level exposures to PBBs were limited to the population within the state of Michigan. The level of exposure to PBBs was slightly higher for the people residing in the lower peninsula of Michigan and highest among people residing in the immediate vicinity of the contaminated dairy farms, where people consumed contaminated meat, eggs, and dairy products. Consumer exposure from using PBB-containing plastic products (e.g., typewriters, calculators, projector housings, and movie equipment cases) is expected to be very low since the PBBs were incorporated into the plastic and their mobilization probably occurred only under conditions such as combustion.

Toxicokinetics

Data regarding the toxicokinetics of PBBs in humans are limited to information derived from cases of accidental ingestion of food contaminated with PBBs and cases of occupational exposure by the inhalation and dermal routes. These data provide qualitative evidence that PBBs are absorbed in humans by the inhalation, oral, and dermal routes. Absorption of PBBs from the gastrointestinal tract in animals can be inferred from the numerous reports of adverse effects and increased residue levels in tissues following oral administration of these compounds; however, few quantitative data exist. Limited quantitative data in animals indicate that some PBB congeners are well absorbed after oral exposure. For example, by comparing the amount of radioactivity in the feces of rats administered a single oral dose of 1 mg ^{14}C-2,2′,4,4′,5,5′-hexabromobiphenyl per kilogram with that monitored after a single intravenous injection of the compound, it was estimated that greater than 90% of the oral dose was absorbed over a 24 h

period. In contrast, with the high absorption rate for the hexabromobiphenyl congener, available data suggest that other congeners such as octabromobiphenyl may be less well absorbed by rats after administration of a single dose. For example, within the first 24 h after dosing with octabromobiphenyl, 61.9% of the dose was found in the feces, although it is unclear how much octabromobiphenyl may have been absorbed and undergone biliary excretion. Subsequent experiments in ruminants revealed that approximately half of an oral PBB dose is excreted unchanged in the feces 7 days after dosing and 23% is excreted in the milk within 95 days postdosing.

In blood, ~80% of PBBs are bound to protein and 20% are associated with lipids. The distribution pattern of PBBs does not appear to differ significantly between humans and animals and among animal species. Due to their lipophilic nature, PBBs, especially the highly brominated congeners, tend to accumulate in lipid-rich tissues. In general, relatively greater amounts of PBBs are usually found in the liver, adipose, skin, and breast milk. In rats treated by gavage with one or four daily doses of ^{14}C-2,2′,4,4′,5,5′-hexabromobiphenyl, initial concentrations of radioactivity were highest in muscle, liver, and adipose tissue, but later redistribution to adipose tissue (4–7 days after the last dosing) resulted in lower concentrations in liver and muscle. In rats dosed daily with ^{14}C-2,2′,4,4′,5,5′-hexabromobiphenyl over a 30 day period, the rank order of residue concentrations in fluids and tissues on day 31 were (in increasing order): blood, muscle, liver, skin, and adipose.

Certain components of PBB mixtures are metabolized by the microsomal monooxygenase system catalyzed by cytochrome P450 of the type induced by phenobarbital. The rate of metabolism of some PBB congeners depends on the bromine substitution pattern. PBB congeners of low bromine content are transformed into hydroxylated derivatives that are predominately eliminated in the urine. However, highly brominated congeners appear to undergo little or no metabolic transformation and are either retained or excreted unchanged in the feces.

Serum half-life values have been estimated using human data from the Michigan PBB cohort. A median half-life of 12–13 years was estimated. Just like polychlorinated biphenyls (PCBs), PBBs are capable of crossing the placental barrier and can concentrate in breast milk. Infants born to and nursing from PBB-exposed mothers may uptake and accumulate PBBs. Lactation constitutes the most important route of excretion of PBB in lactating women. Numerous studies reported PBB levels in breast milk from Michigan women. PBB levels in breast milk on a lipid basis ranged from undetected to 92 667 $\mu g\,kg^{-1}$, with a median of 250 $\mu g\,kg^{-1}$, in a group of parturient women from Michigan. Regression analysis of the data revealed that on a lipid basis, PBBs are 107–119 times more concentrated in milk than in serum.

There is limited information regarding excretion of PBBs in experimental animals. In rats gavaged with ^{14}C-2,2′,4,4′,5,5′-hexabromobiphenyl for 22 days, between 10% and 20% of the daily dose was excreted daily in the feces; this value was predominantly the result of elimination of unabsorbed PBB. In monkeys, the main route of excretion of hexabromobiphenyl residues was also in the feces. Between 60% and 70% of the administered dose was excreted in the feces in the first 11 days after dosing; urinary excretion was minimal.

Mechanism of Toxicity

The mechanism of toxicity for PBBs has been extensively studied, but is not completely understood. Many PBBs, PCBs, polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and other structurally related halogenated aromatic hydrocarbons are believed to share a common mechanism of action strongly related to similarities in their structural configuration. Most of what is known regarding the mechanism of action of these compounds is based on structure–receptor binding relationships, structure–induction relationships, and structure–toxicity relationships. The mechanism for some congeners is related to the enhancement of gene expression triggered by initial binding to the cytosolic aryl hydrocarbon (Ah) receptor.

However, there are also likely physicochemical differences in PCBs, PBBs, and PBDEs due to the higher atomic weight and considerably larger molecular volume of bromine compared to chlorine. These differences contribute to dissimilar physical/chemical properties that can influence the relative bioavailability, absorption, tissue accumulation, receptor interactions, and toxicities of the chemicals.

Acute and Short-Term Toxicity (or Exposure)

The toxicological properties of PBBs are very similar to those of structurally related PCBs.

Animal

In a dairy cow herd that accidentally consumed FireMaster BP-6 in their diet, feed intake and milk production dropped to about half of normal levels. Initial symptoms noted in the herd included hematomas, hoof and hair abnormalities, and weight

loss. While the cause of these effects is generally attributed to PBB exposure, there is some controversy because these signs of toxicosis were not reproduced in controlled experiments with PBBs.

In rodents, oral doses of PBB cause liver hypertrophy, fatty liver, and scattered necrosis. In addition, neurological effects of PBB poisoning have been demonstrated in rats. Specifically, offspring from rats fed PBBs at a dose of 2 mg kg^{-1} during gestation and lactation showed signs of neurological damage and growth retardation.

The calculated 90 day LD_{50} for FireMaster FF-1 in rats is 149 and 65 mg kg^{-1} day^{-1} for male and female rats, respectively. However, in mink, the calculated dietary LD_{50} is 0.47 and 0.61 mg kg^{-1} day^{-1} for male and female minks, respectively, based on exposure to FireMaster FF-1 for 63–294 days.

Human

Epidemiological studies conducted following the Michigan incidence revealed no acute symptoms from the consumption of PBB-contaminated food. For long-term exposure the only symptoms that were at least partially attributed to PBB consumption included chloracne, blurred vision, and fatigue.

Chronic Toxicity (or Exposure)

Although available studies on chronic effects in humans are largely inconclusive, the animal data suggest that the PBBs can cause reproductive and developmental toxicity, and affect the liver, thyroid, and immune system. Hepatic effects in rodents and other laboratory animal species exposed orally to FireMaster PBBs in chronic-duration studies range from microsomal enzyme induction and liver enlargement to fatty changes and necrosis. Altered vitamin A homeostasis, primarily manifested as decreased hepatic storage of vitamin A, is another established effect of PBBs in animals. Thyroid effects, ranging from decreases in serum levels of serum T4 and serum triiodothyronine (T3) to histological and ultrastructural changes in the follicles, have been produced in rats in chronic-duration studies at doses as low as 1.3 mg kg day^{-1}.

Based on the results of the oral studies of FireMaster FF-1 in mice and rats, there is sufficient evidence to conclude that PBBs are carcinogenic in animals and potentially carcinogenic in humans. For example, in oral studies with mice and rats, hepatocellular adenomas, carcinomas, and/or liver neoplastic nodules were induced following single or repeated (intermediate- and chronic-duration) exposures. PBBs as a group have been classified as possibly carcinogenic to humans by IARC (Group 2B). This classification is based on sufficient evidence for carcinogenicity to animals and inadequate evidence of carcinogenesis in humans. The EPA has not classified the carcinogenicity of PBBs.

In Vitro Toxicity Data

PBBs induce cytochrome P450 isozymes from the CYP1A family. The ability to induce CYP1A enzymes is related to the binding affinity of congeners to the Ah receptor. In a study that attempted to utilize this biochemical effect as a biomarker of effect in humans, caffeine was used as a metabolic probe for induction of CYP1A activity. In a caffeine breath test of Michigan subjects from populations with varying concentrations of PBBs in serum, the correlation was poor between PBB concentration in serum and measures of caffeine metabolism. The authors concluded that there was substantial variability in enzyme activity possibly due to polymorphisms of the genes that regulate metabolizing enzymes and factors such as exposure to cigarette smoke, age, nutrition, hormone use, and hepatic disease.

Environmental Fate

Based on the similarity in structure and physicochemical properties between PBBs and PCBs, the environmental partitioning behavior of PBBs is generally very similar to that of PCBs, for which there are much more data. In general, PBBs are persistent, lipophilic, and tend to bind to particulate matter. The log octanol/water partition coefficients ($\log K_{ow}$) for PBB congeners vary by congener but are generally in the range of 5.53–8.58. The log carbon matter partition coefficients ($\log K_{oc}$) for PBB congeners vary by congener but are generally in the range of 3.33–5.09. Studies have shown that adsorption to and transport of sediments and particulates is a major transport mechanism of PBBs in aquatic systems. In sediments, PBBs can be reductively debrominated through a mechanism similar to that of reductive dechlorination of PCBs, dependent upon the presence of dehalogenating microorganisms. PBBs may be transported from water to aquatic organisms by direct bioconcentration as well as through diet. In fathead minnows (*Pimephales promelas*), experimentally derived bioconcentration factors BCFs of ~18 000 were derived for hexabromobiphenyl mixtures. Much less is known about the environmental fate of PBBs in terrestrial systems. Available data indicate that translocation of PBBs from soil into plants is not significant.

See also: Polychlorinated Biphenyls (PCBs).

Further Reading

Agency for Toxic Substances and Disease Registry (2002) *Toxicological Profile for Polybrominated Biphenyls and Polybrominated Diphenyl Ethers*. Atlanta, GA: US Department of Health and Human Services, Public Health Service.

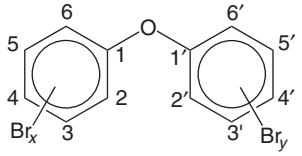
Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Polybrominated Biphenyls.

Polybrominated Diphenyl Ethers (PBDEs)

Alan L Blankenship, John Newsted, and Paul Jones

- Representative Chemicals: Heptabromodiphenylether (HepBDE); Nonabromodiphenyl ether (NoBDE); Tetrabromodiphenyl ether (TeBDE); Hexabromodiphenyl ether (HeBDE); Octabromodiphenyl Ether (OBDE); Pentabromodiphenyl ether (PeDBE); Decabromodiphenyl ether (DeBDE)
- Chemical Abstracts Service Registry Numbers: CAS 68928-80-3 (HepBDE); CAS 63936-56-1 (NoBDE); CAS 40088-47-9 (TeBDE); CAS 36483-60-0 (HeBDE); CAS 32536-52-0 (OBDE); CAS 32534-81-9 (PeDBE)
- Synonyms: Heptabromodiphenyl ether; Heptabromodiphenyl oxide; Nonabromodiphenyl ether, 2,3′,4′-Tribromodiphenyl ether; Tribromodiphenyl ether; Tetrabromodiphenyl ether; Hexabromodiphenyl ether; Octabromodiphenyl ether; Octabromodiphenyl oxide; Pentabromodiphenyl ether pentabromodiphenyl oxide; 4,4′-Dibromodiphenylether bis-*p*-bromophenyl ether; Dibromodiphenyl ether; *p*,*p*′-1,1′-oxybis (2,3,4,5,6-pentabromobenzene); Berkflam b 10E; Bis(pentabromophenyl) ether; BR 55N; Bromkal 82-ode; Bromkal 83-10de; DE 83R; DBDPO; Decabromodiphenyl ether; Pentabromodiphenyl ether; Saytex 102; Saytex 102E; Tardex 100
- Chemical/Pharmaceutical/Other Class: Brominated aromatic
- Chemical Formula: $C_{12}H_3Br_7O$
- Chemical Structure:



Uses

Polybrominated diphenyl ethers (PBDEs) are used as additive flame retardants in a wide range of products including thermoplastics. The most important limitations on their use are incompatibilities that affect the physical properties of the polymers and the tendency for additives to be fugitive. These flame retardants are added to polymer materials instead of being chemically incorporated into the matrices and as a result are much more prone to leaching or escape from the finished polymer product than reactive flame retardants. The major uses of the PBDEs are in: high-impact polystyrene, acrylonitrile butadiene styrene (ABS), flexible polyurethane foam, textile coatings (not clothing), wire and cable insulation, electrical/electronic connectors, and other interior parts. These applications account for 80–90% of the consumption of PBDEs in the United States. PBDEs are used in resins, polymers, and substrates at levels ranging from 5% to 30%. In consumer products, resins containing PBDE are typically used in interior parts, minimizing the potential for exposure of the public. Currently, technical DeBDE is the most widely used PBDE flame retardant worldwide, followed by ODBE.

Exposure Routes and Pathways

The widespread use of PBDEs has resulted in their ubiquitous presence in the environment. In occupational environments, exposure to DeBDE may occur through inhalation of dusts. Atmospheric inhalation exposure to PBDEs is expected to be low, since their vapor pressures are in the range of 10^{-7} mmHg. Particulates in the respirable range are expected to be formed during the grinding of solids. Dermal exposure may occur during filtration, drying, drumming/bagging, size reduction, and maintenance. Exposure to PBDEs can also take place during processing (incorporation into various polymers) and final production. Exposure of the general public may occur via inhalation of ambient air, ingestion of fish, and dermal contact with products such as television enclosures or textiles containing PBDEs.

Toxicokinetics

Studies conducted with rats suggest that PBDEs are poorly absorbed by oral, inhalation, or dermal

routes. In rats given oral doses of DeBDE and OBDE, ~91% and 62% of the administered dose, respectively, was found in the feces within 24 h indicating these compounds were not significantly absorbed by the rats. In rats fed DeBDE no organ or tissue contained more than 0.26% of the administered dose. However, absorption of PBDE is affected by degree of bromination; lower brominated PBDEs tend to be better absorbed. The elimination half-life of PeBDE from rats ranged from 19 to 110 days. However in rats given a mixture of OBDE and DeBDE, the elimination half-life of DeBDE in the feces was estimated to be <24 h while for OBDE there was an initial phase half-life of <24 h followed by a second phase half-life of >16 days. Little or no metabolism of PBDEs was observed in this study.

Mechanism of Toxicity

Some PBDEs have been shown to bind to the Ah receptor and as a result, may have some limited 'dioxin-like' activity. However, recent evidence suggests that trace levels of polybrominated dibenzo-p-dioxins, dibenzofurans, and biphenyls may have been present in PBDEs at sufficient concentrations to elicit the observed 'dioxin-like' toxicity in the experiments discussed below. In one Ah receptor activation study, the PBDEs with the greatest activity were penta- and hexa-BDE congeners while tri- and tetra-BDE congeners had the least potency. These results were similar to those observed in a receptor binding study where binding affinities of PBDEs ranged from 10^{-2} to 10^{-5} times that of dioxin. In this study, 2,3,4,4′-penta-BDE had the greatest affinity (2% of the TCDD affinity) for the Ah receptor while DeBDE did not bind to the receptor. The order of affinity of PBDE congeners to the Ah receptor was similar to that observed for several other end points including cytochrome P450 induction (EROD) and immunotoxicity as measured by splenic PFC responses to SRBC antigen. PBDEs have also been shown to disrupt thyroid function. Depending on dose, duration, and PBDE congener, the chemicals have been shown to disrupt production, transport, and disposition of thyroid hormones. Evidence for these effects include: (1) histological changes in the thyroid, (2) decreased serum thyroxine (T_4) levels with no changes in serum TSH, and (3) the structural similarity of several PBDEs to T_4. Estrogenic and antiestrogenic activities of several PBDE congeners and three hydroxylated PBDEs have been evaluated *in vitro*. Eleven of 17 PBDE congeners have showed estrogenic activity in the ER-CALUX assay. All PBDE congeners were at least 250 000 times less potent than 17β-estradiol (E_2). However, some hydroxylated PBDEs showed estrogenic potencies that exceeded E_2. These results indicate that pure and hydroxylated congeners of PBDEs can be agonists of estrogen receptors and that the metabolism of PBDEs may produce more potent pseudoestrogens.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute toxicity of DeBDE for laboratory animals is low. It is not an irritant to skin or eyes in rabbits and is not chloracnegenic in rabbits and is not a human skin sensitizer. The combustion products of flame-retarded polystyrene containing DeBDE were tested for acute toxicity and comedogenicity. The rat oral LD_{50} of the soot and char was $>2000\,mg\,kg^{-1}$ body weight. In short-term toxicity studies on rats and mice, DeBDE at dietary levels of 100 or $50\,g\,kg^{-1}$ did not induce adverse effects. A one-generation reproduction study on rats showed no adverse effects with dose levels of $100\,mg\,kg^{-1}$ body weight. DeBDE did not cause any teratogenic effects in the fetuses of rats administered a dose of $100\,mg\,kg^{-1}$ body weight. Developmental toxicity studies have shown no evidence of teratogenicity of DeBDE, OBDE, and PeDBE in rats and rabbits, although fetotoxic effects that included variations in skeletal ossification were observed at maternally toxic doses.

Chronic Toxicity (or Exposure)

Animal

In a carcinogenicity study of DeBDE with rats and mice, an increase in the incidence of adenomas was found in the livers of male rats receiving $25\,g\,kg^{-1}$ and female rats receiving $50\,g\,kg^{-1}$. In male mice, increased incidences of hepatocellular adenomas and/or carcinomas were found as well as an increase in thyroid follicular cell adenomas/carcinomas (combined). Female mice did not show any increase in tumor incidence. There was equivocal evidence for carcinogenicity in male and female rats and male mice only at dose levels of $25–50\,g\,kg^{-1}$ diet. The International Agency for Research on Cancer (IARC) has concluded that there was limited evidence for the carcinogenicity of DeBDE in experimental animals. The very high dose levels, lack of genotoxicity, and minimal evidence for carcinogenicity indicate that DeBDE, at the present exposure levels, does not present a carcinogenic risk for humans.

Human

A morbidity study of extruder personnel blending polybutyl-eneterephthalate containing DeBDE during

an exposure period of about 13 years did not reveal any deleterious effects. Additional studies of this group showed that the immune system of the exposed persons was not adversely affected over this time.

In Vitro Toxicity Data

Cytogenetic examination of bone marrow cells showed no increase in aberrations in maternal and neonatal rats following maternal oral exposure to a DeBDE and NoBDE mixture. *In vitro* assays found that DeBDE did not induce gene mutations in several bacterial tests (Ames assays) or in mammalian cells. DeBDE also did not induce chromosomal aberrations in Chinese hamster ovary cells. However, exposure to the congeners 2,2′,4,4′-tetra-BDE, 3,4-diBDE, and 2-monoBDE caused increased recombinogenic activity at the HGPRT locus in several cell lines.

Environmental Fate

An estimated vapor pressure of 4.7×10^{-12} mmHg indicates DeBDE will exist solely in the particulate phase in the ambient atmosphere. Particulate-phase DeBDE will be removed from the atmosphere by wet and dry deposition. Direct photodegradation may be fairly rapid based upon studies with sunlight irradiation. If released into soil, DeBDE is expected to be immobile based upon an estimated K_{oc} of 692 000. Volatilization from moist soil surfaces is not expected to be an important fate process based upon an estimated Henry's law constant of 1.2×10^{-8} atm^3 mol^{-1}. No data were located showing the biodegradation of this compound in soil or water environments; this compound was not biodegraded over 14 days in a single screening biodegradation test. If released into water, DeBDE is expected to adsorb to suspended solids and sediment based upon the estimated K_{oc}. Volatilization from water surfaces is not expected to occur based upon this compound's estimated Henry's law constant. BCF values ranging from 0.3 to <50 suggest bioconcentration in aquatic organisms is low to moderate. The fate of DeBDE in the environment needs further study; in the absence of sunlight, the compound persists in soils and sediments while in sunlight, DeBDE readily degrades to the lower brominated congeners, such as tetra- and hexabrominated biphenyl ethers, which readily bioaccumulate.

Other Hazards

Formation of brominated dioxins and furans on combustion of PBDE containing products may be a hazard.

See also: Bromine; Polybrominated Biphenyls (PBBs).

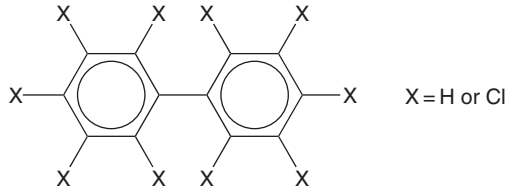
Further Reading

Agency for Toxic Substances and Disease Registry (2002) *Toxicological Profile for Polybrominated Biphenyls and Polybrominated Diphenyl Ethers*. Atlanta, GA: US Department of Health and Human Services, Public Health Service.

Polychlorinated Biphenyls (PCBs)

Swarupa G Kulkarni and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 1336-36-3
- Synonyms: Arochlor; Chlorodiphenyls; Clophen; Fenchlor; Kanechlor; Phenochlor; Pyralene
- Chemical Structure:



Uses

Polychlorinated biphenyls (PCBs) were used in electrical capacitors, electrical transformers, vacuum pumps, and gas transmission tribunes. They were also used as hydraulic fluids, plasticizers, adhesives, fire retardants, wax extenders, lubricants and cutting oils, inks, dedusting agents, etc. PCBs are no longer commercially produced in the United States but are still found in the environment. PCB's have been found in at least 500 of the 1598 National Priorities List Sites identified by the Environmental Protection Agency (EPA).

Exposure Routes and Pathways

Most exposures are environmental or occupational with the delayed symptoms being the first indication that an intoxication has occurred.

Toxicokinetics

PCBs and polybromated biphenyls are absorbed by all routes. Dermal absorption varies depending on the compound, concentration, and species but is in the 15–56% range. PCBs are chemically inert and the more highly chlorinated compounds are resistant to metabolism. The liver is the primary site of metabolism and the primary mechanism is hydroxylation and conjugation with glucuronic acid and is inversely proportional to the chlorine content. PCBs are primarily distributed to the adipose tissues. During pregnancy, one-tenth of the maternal serum level can be found in cord blood and 107–119 times the serum level can be found in human milk. Excretion is variable depending on the species and inversely related to the chlorine content. PCBs are excreted in breast milk.

Mechanism of Toxicity

The exact mechanism of action by which PCBs cause their toxicity is unclear. They are potent enzyme inducers and affect thiamine utilization.

Acute and Short-Term Toxicity (or Exposure)

Animal

In laboratory animals exposed orally or cutaneously to sublethal levels of various PCB mixtures, common findings are severe atrophy of 1° and 2° lymphoid organs, lower circulatory immunoglobulin levels, and decreased specific antibody responses following immunization with antigens. Both augmentation and suppression of cell-mediated immunity on exposure to PCBs have been reported.

Human

PCBs have a low acute toxicity but they accumulate in the environment and in animal and human tissues; the potential for chronic or delayed toxicity is significant. The most dramatic case of PCB poisoning occurred in West Japan in 1968 (Yusho accident) when rice oil contaminated with PCBs poisoned more than 1600 people. Fatigue, headache, increased sweating of the palms, itching, visual disturbances, numbness of the extremities, subcutaneous facial edema, joint swelling and pain, cough, intermittent abdominal pain, and menstrual changes were noted. However, the symptoms may not be purely due to PCB toxicity since the oil also contained dibenzofurans and quaterphenyls, which are known to be toxic. Fifteen cases of reproductive and fetotoxic human effects were observed in the Yusho epidemic. Decreased immunoglobulin levels were observed.

PCBs are mildly irritating to the eyes and skin. Facial edema, eye discharge, swollen eyelids, conjunctival hyperemia, and visual and hearing disturbances may result. Increases in diastolic and systolic blood pressures are possible. Neurobehavioral and psychomotor impairment have been seen after occupational exposure. Gastrointestinal disturbances and diarrhea have been noted. Clinical hepatitis has been seen in the Yusho epidemic. PCB exposure can cause elevation of serum triglycerides. Chloracne, which may occur from either dermal contact or systemic absorption, is a specific skin reaction associated with cyclic halogenated compounds and is characterized by distinct cystic, skin-colored lesions and comedones, both of which may become inflamed and infected. Edematous swelling of the limbs has been reported. Pruritis was observed in 14% of the exposed persons following exposure to combustion products of PCBs. Small elevation in urinary uroporphyrin levels and decreased coproporphyrin levels in a small number of humans accidentally exposed to PCBs have been reported.

Chronic Toxicity (or Exposure)

Animal

Liver damage is a consistent finding in animal studies. PCBs are carcinogenic in animals causing liver tumors in rats.

Human

Long-term exposure to PCBs may cause embryo toxicity including fetal death, fetal resorption, cleft palate, dilated renal pelvis, and hypoplasia of the thymus. Males may be more susceptible to the teratogenic effects than females. It may cause reproductive and fetotoxic effects. Mammalian reproductive effects include changes in the estrus cycle, implantation failure, increased abortions, low birthweight offspring, and decreased postnatal survival. PCBs are considered potential human carcinogens. A slight increase in melanoma of the skin in men occupationally exposed to PCBs has been reported. Renal adenocarcinoma in workers chronically exposed to PCBs has occurred.

Clinical Management

Most exposures are environmental or occupational with the delayed symptoms being the first indication that intoxication has occurred. There is no specific treatment, only supportive treatment. Emesis is of no use since ingestion of PCBs will not be recognized until long after emesis is of any value. Vomiting may cause aspiration. On ingestion, activated charcoal

mixed with a saline cathartic or sorbitol may be used. On ocular exposure, the eyes should be flushed. On dermal exposure, multiple soap and water washings are necessary. On inhalation exposure, emergency airway support and 100% humidified supplemental oxygen with assisted ventilation may be needed. If a cough or difficulty in breathing develops, the victim should be evaluated for respiratory tract irritation, bronchitis, and pneumonitis.

Environmental Fate

PCBs have been identified in at least 500 of 1598 hazardous waste sites proposed for inclusion on the EPA National Priorities list. Before being banned and before the Clean Water Act regulated wastewater discharges, PCBs could be found, often at high levels, in wastewaters from industries handling PCB equipment. These wastewaters either were discharged directly to surface waters or sent to municipal sewage treatment plants. Urban industrial areas are more likely to have higher PCB contamination than rural areas. While not highly volatile, PCBs, especially the less chlorinated ones, will partition into the air. Atmospheric transport is the most important mechanism for dispersion of PCBs. Those PCBs with a high degree of chlorination are much more persistent in the environments than those with lower degrees of chlorination.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value (ACGIH TLV) for chlorodiphenyl (42% chlorine) is $1\,mg\,m^{-3}$ time-weighted average (TWA). The ACGIH TLV for chlorodiphenyl (54% chlorine) is $0.5\,mg\,m^{-3}$ TWA. ACGIH has not established a short-term exposure limit for chlorodiphenyl.

The Occupational Safety and Health Administration permissible exposure limit (OSHA PEL) for chlorodiphenyl (42% chlorine) is $1\,mg\,m^{-3}$ PEL – TWA, with skin notation. The OSHA PEL for chlorodiphenyl (54% chlorine) is $0.5\,mg\,m^{-3}$ PEL – TWA, with skin notation.

The EPA has set a limit of 0.0005 mg of PCBs per liter of drinking water ($0.0005\,mg\,l^{-1}$). Discharges, spills, or accidental releases of 1 pound or more of PCBs into the environment must be reported to the EPA. The Food and Drug Administration (FDA) requires that infant foods, eggs, milk and other dairy products, fish and shellfish, poultry, and red meat contain no more than 0.2–3.0 parts of PCBs per million parts (0.2–3.0 ppm) of food. Many states have established fish and wildlife consumption advisories for PCBs.

Miscellaneous

PCBs are mixtures of different congeners of chlorobiphenyl. The arochlors are characterized by four-digit numbers. The first two digits indicate that the mixture contains biphenyl (12), triphenyls (54), or both (25 and 44); the last two digits give the weight percentage of chlorine in the mixture. For example, Arochlor 1242 contains biphenyl with ~42% chlorine.

Physical properties vary by product because of the varied composition. For example; Arochlor 1242 is a clear mobile liquid; Arochlor 1254 is a light yellow, viscous liquid; and Arochlor 1260 is a light yellow, soft sticky resin. PCBs are heat stable and resistant to biologic degradation as well as acids, bases, oxidation, and other chemical reactions.

See also: Environmental Hormone Disruptors; Neurotoxicity; Psychological Indices of Toxicity; Skin.

Further Reading

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Faroon OM, Jones D, and deRosa C (2001) Effects of polychlorinated biphenyls on the nervous system. *Toxicology and Industrial Health* 16: 305–333.

Relevant Website

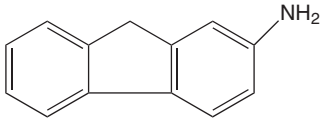
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Polychlorinated Biphenyls (PCBs).

Polycyclic Aromatic Amines

Shayne C Gad

This article is a revision of the previous print edition article by Jayne E Ash and Shayne C Gad, volume 2, pp. 576–577, © 1998, Elsevier Inc.

- Representative Chemicals: Flouren-2-amine; 3,3′-Dichlorobenzidine
- Chemical Abstracts Service Registry Number: CAS 153-78-6
- Synonyms: 2-Aminofluorene; Fluorene
- Chemical Structure



Uses

Polycyclic aromatic amines occur naturally in coal tar. They are by-products of the coal refining process. They were used in the 1930s as an insecticide.

Exposure Routes and Pathways

Dermal contact, ingestion, and inhalation are possible routes of exposure.

Toxicokinetics

Polycyclic aromatic amines are readily absorbed into the body via the gastrointestinal tract, where metabolic activation takes place. Aryl amines are *N*-hydroxylated and subsequently glucuronidated via uridine diphosphate (UDP)-glucuronosyl transferase or sulfated by sulfotransferases, *N*-acetylation of the amine, and *O*-acetylation of the *N*-hydroxy amine can occur.

Mechanism of Toxicity

N-Hydroxy metabolites within the gastrointestinal tract transform fluoren-2-amine into a mutagen or carcinogen. A number of polycyclic aromatic amines are potent bladder carcinogens. As noted above, sequential hydroxylation and glucuronidation leads to urinary excretion, with metabolites in the urinary bladder. While glucuronidation enhances excretion via the urine, a glucuronidase in the bladder hydrolyzes the glucuronide and under acidic conditions *N*-hydroxylarylamines are formed. A spontaneous conversion of the amine leads to an arylntrinium ion, which can initiate tumor formation. Sulfate esters can degrade to electrophilic nitrinium ion–carbonium ion, which can form adducts with macromolecules.

Acute and Short-Term Toxicity (or Exposure)

Animal

Polycyclic aromatic amines have relatively low acute toxicity potential. The oral and intraperitoneal values of LD_{50} of acetylaminofluorene in mice are 810 and 470 mg kg^{-1}, respectively.

Human

Little information is available regarding acute toxicity of polycyclic aromatic amines in humans.

Chronic Toxicity (or Exposure)

Animal

A number of polycyclic aromatic amines are carcinogens in animals. 2-Acetylaminfluorene is a teratogen, mutagen, and carcinogen. It is tumorigenic in rats at 2420 mg (TD, oral). Dietary exposure to 2-acetylaminofluorene in rats led to tumors of the liver, bladder, renal pelvis, ear canal, colon, lung, pancreas, and testis. Tumors of the liver, bladder, and kidney have been observed in mice exposed to dietary 2-acetylaminofluorene. Bladder and liver tumors have been observed in other laboratory animals exposed to 2-acetylaminofluorene.

Human

Carcinogenic properties are dependent on individual rates of acetylation. Persons who are slow acetylators are more susceptible to bladder cancer from aromatic amines, as generally are workers in industrialized countries. Nutrition is also implicated in the development of cancer by polycyclic aromatic amines.

Clinical Management

The victim should be removed from exposure.

Environmental Fate

Polycyclic aromatic amines may be transported as vapor or adsorbed onto particulates. Due to low water solubility, polycyclic aromatic amines are

not transported in water but adsorb onto soil and sediments. Leaching is negligible. Bioaccumulation is not considered a concern.

Ecotoxicology

Little information is available concerning the ecotoxicity of this class of chemicals.

See also: Carcinogenesis; Oil, Crude.

Further Reading

Hodgson E and Goldstein JA (2001) Metabolism of toxicants: Phase I reactions and pharmacogenetics. In: Hodgson E and Smart R (eds.) *Introduction to Biochemical Toxicology*, 2nd edn., pp. 67–113. New York: Wiley.
Kitchen KT (1999) *Carcinogenicity*. New York: Dekker.

Relevant Website

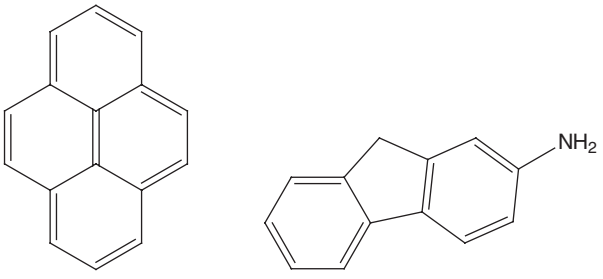
http://www.cdc.gov – National Institute of Occupational Safety and Health.

Polycyclic Aromatic Hydrocarbons (PAHs)

Shayne C Gad and Samantha E Gad

This article is a revision of the previous print edition article by Shayne C Gad and Jayne E Ash, volume 2, p. 577, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Numbers: CAS 129-00-0 (Pyrene); CAS 153-78-6 (Fluoren-2-amine)
- Chemical Formula: $C_{16}H_{10}$ (for pyrene); $C_{13}H_9NH_2$ (for fluoren-2-amine)
- Other Compounds: Benzo[*a*]pyrene; 3-Methylcholanthrene
- Chemical Structures:



Uses

Pyrene is used in biochemical research. Polycyclic aromatic hydrocarbons (PAHs) occur naturally in coal tar, fossil fuel combustion, forest fires, and open flame grilled meats. PAHs are found in cigarette smoke and in diesel emissions, when asphalt surfacing and tar roofing, and also in aluminum and coke plants. Pyrene was used in the 1930s as an insecticide.

Exposure Routes and Pathways

Dermal contact, ingestion, and inhalation are possible exposure routes.

Toxicokinetics

PAHS are readily absorbed via the gastrointestinal tract and then metabolically transformed to more reactive forms. These toxicants are typically converted into more reactive metabolites through phase I biotransformations, and then converted into more readily excretable conjugates via phase II processes.

Mechanism of Toxicity

Pyrene increases photosensitivity and suppresses the immune system. P450 metabolism of a number of PAHs leads to carcinogenic and mutagenic potential. PAHs have different toxicity profiles; some are more toxic than others. However, the mechanism of toxicity often relies on adduct formation with macromolecules following biotransformation.

Acute and Short-Term Toxicity (or Exposure)

Animal

In animals, pyrene is a mild dermal irritant and primary irritant. The oral LD_{50} is $2.7\,g\,kg^{-1}$ in rats and $800\,mg\,kg^{-1}$ in mice.

Human

Photosensitization of skin and eyes can be caused by dermal exposure and inhalation causing skin effects including erythema and lesions.

Chronic Toxicity (or Exposure)

Animal

Several PAHs have been shown to cause reproductive and developmental effects in rodents. Genotoxic properties have been found *in vitro* and *in vivo*.

Human

Toxic dermal effects are increased by exposure to ultraviolet light. Lesions on sun exposed skin may progress to skin cancer. Respiratory effects include cough, chronic bronchitis, and naematuria. Workers exposed to high airborne concentrations of some PAHs have shown increased rates of cancer and is therefore considered a probable carcinogen. Pyrene produces a carcinogenic effect from exposure to skin as well as a presence in bloodstream. It also produces immunodepression. Benzo[*a*]pyrene is found in relatively high levels in the environment and is a probable mutagen and teratogen; it has caused severe and long lasting hyperplasia and metaplasia as precancerous lesions.

Clinical Management

The victim should be removed from exposure. Exposed skin and eyes should be thoroughly flushed with tepid water. Supportive therapy should be provided.

Environmental Fate

The PAHs are produced by the incomplete combustion of fossil fuels, wood, and other organic material. These compounds are largely adsorbed onto smoke particles/aerosols and are a major component of industrial air pollution. Partitioning between water and air, between water and sediment, and between water and biota are the most important of the distribution processes. Even though most of these toxicants are released into the atmosphere, considerable amounts are found in water. These toxicants can enter the aquatic environment in many ways but mostly through large oil spills. Their affinity for organic matter in sediment, soil, and biota is high, and these compounds therefore accumulate in organisms in water and sediments. In *Daphnia*, accumulation of PAHs from water is correlated with their octanol–water partition coefficient. In organisms that actively metabolize these chemicals, absorbed concentrations are not correlated with the partition coefficient. Biomagnification is not observed with these toxicants. PAHs undergo photodegradation, microbial degradation, and metabolism in higher organisms. Hydrolysis plays essentially no role in their degradation. These chemicals are photooxidized in air and water in the presence of radicals; for example, OH, NO_3, and O_3. The reaction of two- to four-ring structures with NO_3 leads to nitro-derivatives, which are known mutagens.

PAHs exhibit toxic properties at low concentrations and several have been listed as priority pollutants to be monitored in industrial effluents, natural waters, soils, and sediments. They enter soil systems and natural waters via wastewater effluents from coke and petroleum refining industries, accidental spills and leakages, rainwater runoff from highways and roadways, or from intentional disposal in the past. Low aqueous solubilities of PAHs and high octanol–water partition coefficients (K_{OW}) often result in their accumulation in soils and sediments to levels several orders of magnitude above aqueous concentrations. PAHs can be potent carcinogens, and their presence in groundwater, streams, soil, and sediments may constitute a chronic human health hazard.

There has been tremendous interest in understanding the fate and transport of PAHs in subsurface environments that are largely microaerobic or anaerobic. Little is known about anaerobic biotransformation of these contaminants, particularly in the context of soil and ground water contamination. Aerobic transformation of PAHs associated with soil and groundwater often leads to rapid depletion of dissolved oxygen and this eventually decreases the redox potential (E_h). Such decrease in the redox potential can result in favorable growth environments for denitrifying, sulfate-reducing, or even methanogenic ($E_h < -0.3\,V$) microbial populations. Nearly 10–15% of the bacterial population in soil, water, and sediments consists of anaerobic organisms. Anaerobic transformations may, therefore, play a significant role in oxygen-depleted natural habitats.

Ecotoxicology

Marine organisms adsorb and accumulate PAHs from water. Concentrations up to $7\,mg\,kg^{-1}$ have been noted in organisms living near industrial effluents, and average levels in aquatic animals at contaminated sites were 10–500 $\mu g\,kg^{-1}$. Average levels of these toxicants in aquatic organisms at sites with unspecified sources of PAH were 1–100 $\mu g\,kg^{-1}$, but high concentrations (up to $1\,mg\,kg^{-1}$) were found in some species, for example, lobsters in Canada. Concentrations of PAHs in insects ranged from 0.7 to $5.5\,mg\,kg^{-1}$. In heavily contaminated locations, concentrations of benzo[*a*]pyrene in earthworm feces may reach $2\,mg\,kg^{-1}$.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration permissible exposure limit for benzo[*a*]pyrene is $0.2\,mg\,m^{-3}$.

See also: Absorption; Benz[*a*]anthracene; Carcinogenesis; Methylcholanthrene, 3-; Respiratory Tract.

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http://www.inchem.org – Selected Non-Heterocyclic Polycyclic Aromatic Hydrocarbons (Environmental Health Criteria 202 from the International Programme on Chemical Safety.

Polyethylene Glycol

Hon-Wing Leung

- Chemical Abstracts Service Registry Number: CAS 25322-68-3
- Synonyms: α-Hydro-ω-hydroxypoly-(oxy-1,2-ethanediyl); Macrogol; PEG; Carbowax; Jeffox; Nycolin; Pluracol E; Poly-G; Polyglycol E; Solbase; Polyox
- Chemical/Pharmaceutical/Other Class: A distribution of liquid and solid polymers of varying molecular weights (from 200 to several million) corresponding to an average number of oxyethylene groups
- Chemical Structure:

$$H(OCH_2-CH_2)_nOH$$

Where n = average number of oxyethylene groups

Uses

Polyethylene glycols are widely used in food, cosmetics, and topical pharmaceuticals (e.g., ointments and suppository base).

Exposure Routes and Pathways

Ingestion and skin contact are the most common routes of both accidental and intentional exposures.

Toxicokinetics

The absorption of orally administered polyethylene glycols is dependent on their molecular size. While 50–65% of liquid polyethylene glycols (molecular weight up to 600) are absorbed, only from 0% to 2% of solid polyethylene glycols (molecular weight more than 1000) are absorbed. High-molecular-weight polyethylene glycols are retained in the blood circulation for a longer period than low-molecular-weight polyethylene glycols.

Polyethylene glycols are not appreciably metabolized. Ethylene glycol is not known to be a metabolite. The distribution of the higher members of polyethylene glycols within the body is extracellular, whereas the lower-molecular-weight members of the series diffuse intracellularly to a considerable extent. Polyethylene glycols tend to accumulate in the muscle, skin, bone, and the liver to a higher extent than the other organs, irrespective of the molecular weight.

Liquid polyethylene glycols are rapidly excreted in the urine, while the higher-molecular-weight members are mainly eliminated in the feces.

Mechanism of Toxicity

Many years of human experience in the workplace and in the use of consumer products containing polyethylene glycols have not shown any adverse health effects, except for administering high doses to sensitive or unhealthy persons. Nephrotoxicity associated with the topical treatment of burn patients with polyethylene glycols may reflect the compromised function of the patients' kidneys rather than the direct toxic effects of polyethylene glycols.

Acute and Short-Term Toxicity (or Exposure)

Animal

Polyethylene glycols have a very low level of acute toxicity to animals. They do not produce appreciable irritation to the rabbit skin and are only mildly irritating to the rabbit eyes.

Human

There have not been any reports of acute toxic or irritative effects in humans exposed to polyethylene glycols. The lowest-molecular-weight members (200–300) have been observed to produce at most only a

mild sensitization reaction in a very small percentage of individuals in skin patch testing studies.

Chronic Toxicity (or Exposure)

Animal

Subchronic feeding and drinking water studies in rats and dogs revealed that polyethylene glycols have very low toxicity. Nephrotoxicity and hepatotoxicity have been observed in monkeys and dogs respectively, after continuous intravenous infusion of high doses of polyethylene glycol. Chronic feeding and skin painting studies in rat and mouse, respectively, do not indicate any significant incidence of tumor production.

Human

No epidemiological studies or case reports of ill effects in healthy humans attributable to chronic exposure to polyethylene glycols were found in the available literature.

In Vitro Toxicity Data

Polyethylene glycols are negative in a battery of genotoxicity tests.

Clinical Management

Since polyethylene glycols are of very low acute toxicity and nonirritating, emergency care is not anticipated. There is no specific antidote for polyethylene glycols. Treatment of overexposure should be directed at the control of symptoms and the clinical condition of the patient.

Environmental Fate

Like other polymeric substances, polyethylene glycols are not readily biodegradable. However, owing to their hydrophilicity, they have a low potential to bioaccumulate.

Ecotoxicology

Polyethylene glycols have a very low order of toxicity to aquatic organisms including daphnids and fish.

Exposure Standards and Guidelines

The American Industrial Hygiene Association has set a workplace environmental exposure limit of $10\,\mathrm{mg\,m^{-3}}$ as an aerosol for polyethylene glycol.

See also: Ethylene Glycol; Polymers.

Further Reading

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Polymers

Samantha E Gad

Background

Polymers are macromolecules formed by the chemical bonding of five or more identical units called monomers. These monomers are then connected repeatedly to form isomers which are then strung together repeatedly to form polymers. In most cases the number of monomers is quite large (3500 for pure cellulose) and often is not precisely known. In synthetic polymers, this number can be controlled to a predetermined extent (e.g., by shortstopping agents). Combinations of two, three, or four monomers are called dimers, trimers, and tetramers respectively, and are known collectively as oligomers. Such oligomers are not polymers.

A partial list of polymers by type includes the following:

I. Inorganic: siloxane, sulfur chains, black phosphorus, boron–nitrogen, silicones.

II. Organic

1. Natural
a. Polysaccharides: starch, cellulose, pectin, seaweed gums (e.g., agar), vegetable gums (e.g., arabic)
b. Polypeptides (proteins): casein, albumin, globulin, keratin, insulin, DNA
c. Hydrocarbons: rubber and gutta percha (polyisoprene), also called elastomers
2. Synthetic
a. Thermoplastic polymers: nylon, polyvinyl chloride, polyethylene (linear), polystyrene,

polypropylene, fluorocarbon resins, polyurethane, acrylate resins
b. Thermosetting polymers: polyethylene (cross-linked) phenolics, alkyds, polyesters
3. Semisynthetic cellulosics (rayon, methylcellulose, cellulose acetate) and modified starches (e.g., starch acetate).

Further examples of natural polymers also include collagen, chitosan, and polyhydroxyalkanoates. Additional synthetic polymers include poly(glycolic acid) (PGA), poly(lactic acid) (PLA), copolymers of PGA and PLA, and polydioxananone.

Uses

In many materials, processing conditions can induce the polymer chains to link with each other along the length of the chain to produce a wide variety of mechanical properties, varied in order to suit current biomedical applications. Polyethylene (PE) is used in a variety of different applications. Depending on its linking, PE can be elastic and flexible, or hard and smooth. Low-density PE is used as tubing in catheters, while ultra-high-molecular-weight PE is commonly used in total hip or knee replacements; the smooth surface allows for low friction with other materials and therefore increases the durability of the artificial joint.

Polymers can be categorized in a number of ways. Homopolymers, for example, consist of only one repeating monomer unit. The most commonly encountered homopolymers are listed in **Table 1**.

Copolymers are produced by the simultaneous polymerization of two or more dissimilar molecules. Examples include polyvinyl acetate, polyesters, and polyamides. Synthetic elastomers (such as SBR synthetic rubber, made from styrene and butadiene) are also copolymers. This pattern continues with the terpolymers (such as acrylonitrile–butadiene–styrene (ABS)), which consist of three different monomers.

Hydrogels are another type of polymer structure comprised of a hydrophilic cross-linked network that swells in water. They can exist as homopolymers, copolymers, or multipolymers and are generally biocompatible and have low degradation. Hydrogels can be produced with a wide range of swelling characteristics determining solute diffusion rates, surface properties, refractive indexes, and mechanical characteristics. Cellulose derivatives swell to a higher degree than other polymers.

This ability of hydrogels to swell and dehydrate depending on composition and environment is used for the controlled release of drugs. Soft contact lenses also have hydrogel content which allows gas exchange to the eye. These may be used in future applications in blood contact applications and for wound-healing and artificial cartilage and skin.

Natural and synthetic biodegradable polymers are used for purposes that require only temporary stability to support tissue ingrowth. These polymers degrade when placed in the body while allowing functional tissue to grow in its place. This process takes advantage of polymers' properties of hydrolytic instability, hydration, molecular backbone cleavage, loss of molecular weight, and solubilization. These sutures degrade slowly into by-products that the body can remove itself through natural functions, allowing time for the wounded tissue to complete its own healing process and eliminating the need for a second operation to remove them.

Table 1 Commonly used homopolymers in medical devices

Polyacrylates	Polyamides
Polybutylene	Polychloroprene
Polyethylene	Polypropylene
Polysiloxanes	Polystyrene
Polysulfones	Polytetrafluoroethyene
Polyvinyl chloride	

Biocompatibility

For most devices, we are concerned only with the synthetic organic polymers. The principal class of natural polymers of concern is the elastomer class. The chief class of inorganic polymers of concern is the silicone class.

Partially as a reflection of their high molecular weights, true polymers themselves are not generally absorbed into the body, are not irritating, and are not sensitizers. As shown in **Table 2**, polymers themselves generally have very low toxicities.

The principal concerns with the biocompatability of polymers are additives, residual monomers, and contaminants that are leachable in the body. Plastic extractables include such chemicals as base polymers, fillers, lubricants, plasticizers, antioxidants, pigments, and slip agents. They may also include reaction

Table 2 Oral lethalities of common polymers

Polymer	*Rat LD_{50} value ($g\,kg^{-1}$ body weight)*
Polyethylene	>8
Polypropylene	>8
Polychloroprene latex	>40
Chlorosulfonated polyethylene	>20
Polyvinyl acetate	>25
Polyacrylonitrile	>3
Polyacrylamide	>8.2
Aromatic polyamides	>7.5

products or degradants formed during the device manufacturing process. They can reduce the purity or potency of a drug solution; create turbidity, precipitates, and particles; and even increase toxicity.

Residual monomers are those remaining individual building-block units in homopolymers, copolymers, terpolymers, etc. that are not successfully incorporated into the plastic during the synthesis process. Technically, we should also include dimers, trimers, and other small-chain fragments that are left in the polymer mass but are not chemically bound to it. Many factors help determine how much residual monomer will be left in a polymer and how available such residuals are to a surrounding biological matrix. Moreover, some of the monomers are quite active biologically. When testing a plastic for biocompatibility, biologically available (leachable) residual monomers are a significant concern. Examples of toxic monomers (and their principal toxicities) that can be found in polymers include the following:

- acrylonitrile: human carcinogen (liver, brain);
- vinyl chloride: human carcinogen (liver);
- formaldehyde: animal carcinogen (nasal); and
- methylene dianiline; suspect human carcinogen.

A wide variety of other chemical entities are specifically incorporated into plastics to achieve desired goals of structure, performance, and processing ease. A short list of the major categories of additives is provided in **Table 3**.

Such additives are available and significant biologically. A historical example is diethylhexylphthalate, a once widely used plasticizer that was found both to be an animal carcinogen and to migrate readily from plastic bags and tubing to the blood and intravenous solutions they contained.

The result of the additives and contaminants being in plastic is that a range of toxic materials may be leached from many plastics. A short list of some of the more significant toxic materials is provided in **Table 4**.

A number of tests are available for the chemical characterization of medical device materials to establish material safety and biocompatibility. These tests include infrared analysis, aqueous and nonaqueous physicochemical tests, high-performance liquid and gas chromatography, atomic absorption spectroscopy and inductively coupled plasma spectroscopy, and a variety of mechanical/physical tests.

The United States Pharmacopeia (USP) describes a group of tests used to characterize the plastic components of pharmaceutical containers and medical devices to avoid use of materials that may release water-soluble chemicals into the drug products or tissue fluids they contact. USP limits can be used to establish specifications for raw materials.

These aqueous physicochemical tests are designed to determine the presence of water-soluble substances without regard to their identity. Results are presented as a set of four values, showing the results for test type together with the corresponding USP limits (see **Table 5**). These aqueous extract tests are intended to serve as the basis for design specifications.

The USP recommends isopropyl alcohol (IPA) for conducting physicochemical tests of elastomeric closures used for pharmaceutical containers. IPA can dissolve many chemicals that are insoluble in water. The extract is analyzed for nonvolatile residue and residue on ignition. Turbidity and ultraviolet absorption tests are performed to detect the presence of extractables without specifically identifying their chemical makeup. Results are presented as a set of five values for each of the end points (see **Table 6**). USP limits do not yet exist for these tests, but they are not necessary for establishing specifications for the acceptance of materials.

Table 3 Additives used in plastics

Plasticizers	UV absorbers	Lubricants
Blowing agents	Antioxidants	Fillers
Colorants	Release agents	Emulsifiers
Flame and fire retardants	Stabilizers	Accelerators
Curing agents	Antistatic agents	

Table 4 Identified toxic materials in polymers

Aluminum	Ketones and hydrocarbons
Acrylonitrile (monomer)	Lead
Arsenic	Mercaptobenzothiazole
Benzene	Methyl chloride (monomer)
Benzoic peroxide	Methylene chloride
Bisphenol A	Methylene dinaline
Cadmium	Nickel
Carbon tetrachloride	PAHs on carbon black
Dibutyl tin	Pyrene
Epoxy curing agents	Tin
Ethylene dichloride	Tricresyl phosphate
Ethylene oxide	Triphenyl phosphate
Formaldehyde	

Pyrolysis

All plastics emit toxic and irritant fumes with increasing temperatures. However, the evolution rate and composition of the fumes emitted vary for different plastics and are strongly temperature dependent. Some common examples include thermoplastics such as polyvinyl chloride (PVC), polyethylene (PE), polypropylene (PP), polystyrene (PS), ABS copolymer, and polytetrafluoroethylene (PTFE). When

Table 5 Testing for water-soluble substances in polymers

Polymer	*Nonvolatile residue (mg)*	*Residue on ignition (mg)*	*Heavy metals (ppm)*	*Buffering capacity (ml)*
ABS	1	<1	<1	<1
Polyurethane	1	⩽1	⩽1	<1
Polycarbonate	1	⩽1	⩽1	<1
Polyisoprene	9	⩽1	⩽1	<1
Polyvinyl chloride	1	<1	<1	1
Polyethylene	<1	<1	⩽1	<1
PTFE	<1	<1	⩽1	<1
Polystyrene	<1	<1	⩽1	<1
Polypropylene	<1	<1	⩽1	<1
Silicone	1	⩽1	⩽1	<1
USP limits	15	5	1	10

Note: Results of aqueous extraction physicochemical testing on polymers commonly used in medical devices.

Table 6 Physicochemical tests for polymer contaminants using isopropanol extraction

Polymer	*Nonvolatile residue (mg)*	*Residue on ignition (mg)*	*Turbidity (NTU)*	*Maximum optical density*	*Wavelength of max. optical density (nm)*
ABS	46	<1	4.18	>2.0	241
Polyurethane	119	<1	21.38	>2.0	244
Polycarbonate	<1	<1	0.04	>2.0	227
Polyisoprene	223	<1	24.38	>2.0	250
Polyvinyl chloride	123	1	0.24	>2.0	297
Polyethylene	20	<1	7.08	>2.0	241
PTFE	<1	<1	0.00	0.0	
Polystyrene	66	1	8.10	1.2	290
Polypropylene	20	<1	13.10	0.1	
Silicone	444	248	0.70	0.1	

Note: Results of alcohol extraction testing on polymers commonly used for medical devices.

Table 7 Inhalation lethalities of common polymers

Polymer	*Rat LC_{50} value (mg l^{-1}) 30 min*
Polyethylene	75.5
Polyacrylamide	45.7
Polystyrene	56.6
Nylon 6/6	58.1
Polysulfone	63.2
Chlorinated polyethylene	87.5

heated to destruction the parent monomers of a polymer are often one of the pyrolysis products. CO has been a concern, also PVCs since they can give off HCl during a fire, and polyurethane because of HCN. The health effects of hot-wire cutting of PS foams, and PVC and PE films have been studied. **Table 7** shows pyrolysis lethal concentrations of several polymers.

The manufacture of these polymers offers no opportunity for excessive heating. However, in the process of fabrication, reclaiming clad metal, wire coating and stripping, nonstick cookware, scavenging melts, coatings, fires, incineration, and machining, thermal exposure or thermal abuse might occur. When subject to the normal melt processing temperatures, most plastics would produce complex mixtures of small quantities of toxic vapors, usually at concentrations considerably below their exposure standards. However, irritant aerosols and gases can also be produced which may cause complaints of sensory irritation if the process is not controlled properly.

PTFE, also known as Teflon is a synthetic polymer (CF_2CF_2) with antistick (lubricant) properties that is used as a coating for nonstick cookware, domestic boilers, irons, ironing board covers, solid fuel burners, and heat lamps. Problems arise when pans boil dry or unfilled saucepans are heated. Frying temperatures normally range between 100°C and 200°C. Above 280°C, a polymer undergoes chemical decomposition (pyrolysis). PTFE, as well as butter or corn oil, can produce pyrolysis products that can cause death in birds. When PTFE undergoes pyrolysis, both gaseous and particulate materials are given off, including fluorinated compounds, which are toxic to animals and humans with birds being most susceptible. In humans, exposure to fumes can lead to a transient, febrile, flu-like syndrome called polymer-fume fever. Polymer-fume fever is caused by inhaling the fumes from a hot polymer and is characterized by typical flu symptoms (chills, spiking

fever, achy feeling, tightness of chest, headache, cough, weakness in legs, and malaise). These symptoms last 18–48 h before complete recovery without any residual effects or after effects. No animal species has yet been found that responds to PTFE or poly FEP fume the same way as humans. No similar events or fume fevers have been reported when other polymer fumes are inhaled. There are no known deaths from polymer-fume fever.

See also: Biocompatibility; Combustion Toxicology; Pollution, Water.

Further Reading

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Kroschwitz JI (1990) *Concise Encyclopedia of Polymer Science and Engineering*. New York: Wiley.

Shefter VO (1995) *Handbook of Toxic Properties of Monomers and Additives*. Boca Raton, FL: CRC Press.

United States Pharmacopeia XXIII.

Waritz RS (1975) An industrial approach to evaluation of pyrolysis and combustion hazards. *Environmental Health Perspectives* 11: 197–202.

Relevant Website

http://www.devicelink.com – Albert DE. The Growing Importance of Materials Characterization in Biocompatibility Testing, Canon Communications LLC © 2002. Originally published in *Medical Device & Diagnostic Industry*, March 2002.

Potassium

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 7440-09-7
- Synonyms: Kalium
- Chemical/Pharmaceutical/Other Class: Alkali earth metal
- Chemical Formula: K^+

Uses

An essential element for humans, potassium is used in foods and as a salt substitute. It is a major essential element for plants as well and is, therefore, a constituent of most fertilizers. Potassium and its compounds are used in specific medicinal preparations and in cement and glass manufacturing. Potassium bromate is used in hair waving products. Potassium permanganate, a powerful oxidizing agent, is used in the photographic and chemical industries. A dilute solution is used for special dermatological applications.

Background Information

Potassium forms 2.50% of earth's crust and was first isolated in 1807. It is one of the most reactive metals. Radioactive decay of ^{40}K to ^{40}Ar is used as a tool in geological dating.

Exposure Routes and Pathways

The primary exposure pathway is through ingestion of food; sources include milk, meat, and a variety of fruits. Many salt substitutes contain potassium chloride.

Toxicokinetics

Potassium salts are more than 90% absorbed, but blood levels are controlled by hemostatic mechanisms. Climate plays a role in potassium blood levels; people in warm climates have ~30% more potassium in their blood than people in very cold climates.

All tissues of the body contain potassium. It is found mainly in the muscle followed by the skeleton. Excretion of potassium via urine is also controlled by hemostatic mechanisms; the kidney regulates this so that there is normally no major loss of this essential element. The amount of potassium excreted depends on the chloride ion concentration and the adrenal hormone secretion level.

Mechanism of Toxicity

Potassium is a cofactor and activates a large variety of enzymes, including glycerol dehydrogenase, pyruvate kinase, L-threonine dehydrase, and ATPase. Its acute toxicity is primarily due to its action as an electrolyte. Excessive or diminished potassium levels can disrupt membrane excitability and influence muscle cell contractility and neuronal excitability.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral, intraperitoneal, and intravenous LD_{50} values of potassium chloride in rats are 2600, 660, and 142 mg kg^{-1}, respectively. Signs of acute toxicity may include convulsions and seizures, cardiac arrythmias, dyspnea, cyanosis, nausea, and vomiting.

Human

Excess intake of potassium, reduced renal excretion of potassium, or both can lead to hyperkalemia, which can lead to serious arrhythmia and death. The toxicity of excess potassium can be exacerbated by aldosterone antagonist drugs. Slow-release potassium tablets in overdose are a frequent cause.

Periodically, solutions containing a relatively high concentration of a potassium salt are sold as a nutritional supplement. In light of the fact that ingestion of additional potassium can upset the sodium–potassium ratio, potassium supplements are only indicated on the advice of a physician. Unusually high intake of potassium can cause abnormal EKG readings (T-waves will be evaluated and P-waves depressed). Ventricular fibrillation can result and lead to cardiac arrest. A large increase ($\sim$18 g day^{-1}) may produce neuromuscular weakness or paralysis.

Potassium permanganate is a mucous membrane irritant. Taken internally, it can be corrosive to the stomach. It is poorly absorbed, but it can cause nervous system symptoms and increased methemoglobin levels.

Chronic Toxicity (or Exposure)

Animal

Little is known regarding chronic effects of potassium exposures in animals.

Human

Potassium perchlorate can induce aplastic anemia, which can be fatal.

Clinical Management

Intravenous injection of calcium gluconate can antagonize the cardiac effects of excess potassium. Also, intravenous injection of sodium bicarbonate and glucose will help diminish the effects of potassium hemodialysis overdose, while dialysis can be used to remove excess serum potassium.

See also: Lye; Sodium.

Further Reading

Cotton AE, Wilkinson G, Murillo CA, and Bochmann M (1999) *Advanced Inorganic Chemistry*, 6th edn., pp. 92–110. New York: Wiley.

Goyer RA, Klaassen CD, and Waalkes MP (1995) *Metal Toxicology*. San Diego CA: Academic Press.

Saxena K (1989) Clinical features and management of poisoning due to potassium chloride. *Medical Toxicology and Adverse Drug Experience* 4: 429–443.

Potassium Iodide

Elizabeth J Scharman

- Chemical Abstracts Service Registry Number: CAS 7681-11-0
- Synonyms: SSKI, Iosat®, Thyro-Block®
- Chemical/Pharmaceutical/Other Class: Antithyroid agent; Antisporotrichotic agent
- Chemical Formula: KI

Uses

In patients with hyperthyroidism, potassium iodide is used in the treatment of thyrotoxicosis and to decrease the vascularity of the thyroid before the thyroid gland is surgically removed. Potassium iodide is also used to protect the thyroid by blocking the uptake of radioactive iodine, for example, during radionuclide therapy with I-131 or following an accident at a nuclear power facility. Potassium iodide can be given orally for the treatment of cutaneous sporotrichosis. Although used as an expectorant, clinical evidence regarding efficacy for this indication is lacking.

Exposure Routes and Pathways

Ingestion is the route of both accidental and intentional exposure to potassium iodide.

Toxicokinetics

Data on the percentage bioavailability, volume of distribution, and half-life of potassium iodide are not

available. Iodides are distributed in extracellular body water. Limited information reports that potassium iodide is readily absorbed and that iodide is concentrated in the thyroid and salivary glands, gastric mucosa, choroid plexus, placenta, and breast milk with 90% being renally excreted and the remainder being excreted in sweat, feces, and breast milk.

Mechanism of Toxicity

Adverse effects are the result of hypersensitivity reactions to the iodide component or the result of iodine accumulation following chronic administration. In patients with renal impairment, potassium concentrations may increase.

Acute and Short-Term Toxicity (or Exposure)

Human

Potassium iodide is unlikely to result in acute toxicity. Manifestations of a hypersensitivity reaction may include angioedema, cutaneous and mucosal hemorrhage, urticaria, fever, arthralgia, enlarged lymph nodes, and eosinophilia. In patients with chronic urticaria or systemic lupus erythematosus, hypocomplementemic vasculitis may be precipitated.

Chronic Toxicity (or Exposure)

Animal

Chronic feeding studies in minks showed shorter gestational periods and fewer animals per litter compared with controls. At the highest doses tested (1000 ppm), no animals whelped.

Human

Potassium concentrations may become elevated in patients with renal impairment. Signs of potassium excess include confusion, muscle weakness, and dysrhythmias. Chronic iodine toxicity, iodism, is manifested by symptoms that include stomatitis, laryngitis, metallic taste, salivation, tenderness of parotid and submaxillary glands, gastric irritation, diarrhea, headache, coryza, sneezing, productive cough, eye irritation, eyelid swelling, and acneiform eruptions.

Toxicity is usually the result of chronic administration.

In Vitro Toxicity Data

Studies using the alkaline comet assay have not found potassium iodide to produce DNA damage.

Clinical Management

Allergic reactions should be treated appropriately with supportive care, maintenance of airway, breathing, and circulation, and antihistamines plus steroids as needed. Discontinue potassium iodide administration and provide symptomatic and supportive care. The extent of iodide adsorption to activated charcoal has not been determined. Plasma iodide levels do not guide therapy; the potassium level should be checked.

See also: Charcoal; Gastrointestinal System.

Further Reading

Dolan TF Jr. and Gibson LE (1971) Complications of iodide therapy in patients with cystic fibrosis. *Pediatrics* 79: 684–687.

Eeckhout E, Willemsen M, and Deconinck A (1987) Granulomatous vasculitis as a complication of potassium iodide treatment for Sweet's syndrome. *Acta Dermato-Venereologica* 67: 362–364.

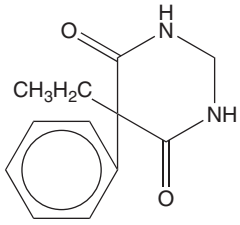
Potentiation *See* Chemical Interactions.

Primidone

S Rutherfoord Rose

- Chemical Abstracts Services Registry Number: CAS 125-33-7
- Synonyms: Primaclone; Hexamidinum; 2-Desoxyphenobarbital; 5-Ethylperhydro-5-phenylpyrimidine-4,6-dione; Mysoline®; Midone®; Dilon®; Mylepsin®; Liskantin®; Majsolin®; Sertan®
- Chemical/Pharmaceutical/Other Class: Primidone is a desoxybarbiturate; a congener of phenobarbital

- Chemical Formula: $C_{12}H_{14}N_2O_2$
- Chemical Structure:



Uses

An anticonvulsant, primidone is used in the treatment of generalized tonic–clonic seizures and partial focal seizures. It is often used in combination with phenytoin or carbamazepine.

Exposure Routes and Pathways

Ingestion is the route of exposure. Toxicity results from acute or chronic overdosage of tablets or oral suspension.

Toxicokinetics

Following therapeutic doses, primidone is usually well absorbed (bioavailability ranges from 70 to 95%) with peak plasma concentrations occurring in 3–6 h. Primidone is converted by the liver to two metabolites: phenobarbital and phenylethylmalonamide (PEMA). Both metabolites are active and phenobarbital is thought to be primarily responsible for primidone's anticonvulsant activity. Phenobarbital appears in the blood 2–4 days after beginning primidone therapy. The volume of distribution averages $0.6\,l\,kg^{-1}$, but there is much interindividual variation. Approximately 20% of primidone and PEMA are bound to plasma proteins, but the binding of phenobarbital is ~50%. Primidone crosses the placenta and is excreted in breast milk.

The majority of a dose is excreted in the urine as PEMA; ~15% as phenobarbital. The plasma elimination half-lives of primidone, PEMA, and phenobarbital are ~8–10, 24–36, and 100 h, respectively. The metabolism of primidone is enhanced with chronic therapy, with a reduced half-life of 4–7 h. An elimination half-life of 6.2 h has been documented following overdose.

Mechanism of Toxicity

Primidone and PEMA appear to have weak anticonvulsant activity compared to that of phenobarbital. Both primidone and phenobarbital contribute to central nervous system depression, probably through enhancement of GABA activity in the brain and the resulting decreased neuronal excitability.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute poisoning in animals may cause lethargy, incoordination, loss of reflexes, coma, or respiratory depression. Treatment is based on supportive care in consultation with a veterinarian.

Human

Acute intoxication resembles barbiturate toxicity. Clinical effects include dose-related central nervous system depression, nystagmus, ataxia, nausea and vomiting, dizziness, vertigo, and irritability.

Chronic Toxicity (or Exposure)

Animal

Toxic effects similar to those seen in humans are found when higher than therapeutic doses are used.

Human

With chronic exposure, side effects may include rash, thrombocytopenia, leukopenia, and a lupus-like disorder. Chronic therapy is likely to result in tolerance, and withdrawal symptoms if primidone therapy is abruptly stopped. Doses in excess of 1500 mg (twice the maximum recommended daily dose) should be considered toxic. Less common side effects are hypotension, hypothermia, and dermal bullae. Encephalopathy has been observed in an epileptic patient with high plasma levels and poor renal function. With plasma concentrations exceeding $80\,\mu g\,ml^{-1}$, primidone may precipitate and cause crystalluria. Plasma levels $>10\,\mu g\mu ml^{-1}$ are associated with toxic effects. The therapeutic range is reportedly $5–10\,\mu g\,ml^{-1}$, but clinical effects correlate more closely with phenobarbital blood levels.

In Vitro Toxicity Data

Mutagenicity studies using mammalian polychromatic erythrocytes have been positive.

Clinical Management

The most important aspect of treatment for acute overdose is provision of airway maintenance and ventilation. Hypotension and hypothermia should be

corrected if present. Decontamination should be accomplished with oral activated charcoal. There are no antidotes. Patients with high plasma phenobarbital levels may be treated with multiple oral doses of activated charcoal and/or urinary alkalinization to enhance phenobarbital excretion. At therapeutic levels, hemodialysis has been shown to increase primidone clearance from 30 to 98 ml min^{-1}. Phenobarbital is also removed by hemodialysis. Primidone, PEMA, and phenobarbital can be removed by hemoperfusion. There is no evidence that extracorporeal drug removal has a beneficial clinical effect with respect to morbidity or mortality.

See also: Charcoal.

Further Reading

Bertino JS Jr and Reed MD (1986) Barbiturate and non-barbiturate sedative hypnotic intoxication in children. *Pediatric Clinics of North America* 33(3): 703–722.

Sigg T and Leikin J (1999) Massive crystalluria in a patient taking primidone (letter). *Annals of Emergency Medicine* 33: 726–727.

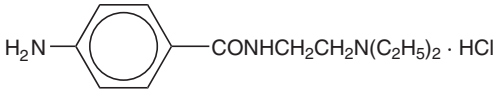
Probabilistic Analysis *See* Monte Carlo Analysis.

Procainamide

Christopher P Holstege

This article is a revision of the previous print edition article by Daniel J Cobaugh, volume 2, pp. 586–588, © 1998, Elsevier Inc.

- Chemical Name: Procainamide
- Chemical Abstracts Services Registry Number: CAS 51-06-9
- Synonyms: 4-Amino-*N*-[2-(diethylamino)ethyl] benzamide monohydrochloride; Amisalin; Novocamid; Procamide; Procanbid; Procan-SR; Procapan; Pronestyl
- Chemical/Pharmaceutical/Other Class: Class IA antiarrhythmic
- Chemical Structure:



Uses

Procainamide is used in the management of ventricular tachydysrhythmias.

Exposure Routes and Pathways

Reports have shown toxicity by both oral and parenteral routes.

Toxicokinetics

The bioavailability from immediate-release capsules ranges from 50% to 95%. Sustained-release formulations are designed to deliver release of procainamide over 12 h. In overdose, absorption may be delayed, especially if sustained-release preparations are involved. Procainamide's volume of distribution (V_d) is ~2 l kg^{-1}. Protein binding is minimal at 15–25%. Procainamide is metabolized to its active metabolite *N*-acetyl procainamide (NAPA). NAPA has a V_d of ~1.5 l kg^{-1} and is 10% protein bound. The elimination half-lives of procainamide and NAPA are ~3 and 6 h, respectively. Fifty per cent of procainamide is eliminated unchanged in the urine.

Mechanism of Toxicity

Cardiac voltage-gated sodium channels reside in the cardiac cell membrane and open in response to depolarization of the cell. Procainamide binds to the transmembrane Na^+ channels and decreases the number available for depolarization. This creates a delay in the entry of Na^+ into the cardiac myocyte during phase zero of depolarization. As a result, the upslope of depolarization is slowed and the electrocardiogram (EKG) QRS complex widens. Procainamide may also affect phase three of the action potential, resulting in prolongation of repolarization and subsequent QTc prolongation on the ECG. Vasodilation associated with procainamide toxicity is due to interference with ganglionic transmission of catecholamine neurotransmitters. A reflex tachycardia may occur in response to this vasodilation. Procainamide may also have weak anticholinergic effects that produce tachycardia. Negative inotropic effects may occur in toxicity. The NAPA metabolite of procainamide has pharmacologic and toxicologic effects similar to those of the parent compound.

Acute and Short-Term Toxicity (or Exposure)

Animal

Rapid intravenous infusion of large doses of procainamide can produce central nervous system and respiratory depression as well as arrythmias.

Human

The primary toxicities observed with procainamide are cardiovascular in nature. Initially, a tachycardia may occur due to procainamide's anticholinergic properties or as a reflex response to vasodilation. Cardiac conduction disturbances may occur. On the ECG, these may be displayed as prolongation of the QRS and/or QTc duration. Heart block, bradycardia, and asystole have been reported. Procainamide can also cause ventricular tachycardia, ventricular fibrillation, and Torsades de Pointes. Severe hypotension due to decreases in cardiac output and/or vasodilation may be seen. Altered mental status and seizure activity can occur in procainamide toxicity.

Chronic Toxicity (or Exposure)

Animal

Procainamide is used in veterinary practice as an antiarrhythmic. Clinical effects seen in animals are similar to those seen in humans and include arrythmias, gastrointestinal complaints, and systemic lupus erythematosus-like syndrome.

Human

Procainamide may induce a syndrome similar to systemic lupus erythematosus. This syndrome consists of arthralgias, myalgias, pleurisy, rash, fever, and elevated nuclear antibodies. Patients who are slow acetylators are at increased risk for developing this syndrome. While some studies have reported that less than one in 500 on chronic procainamide therapy developed this syndrome, others have reported this syndrome in up to 30% of patients on long-term therapy. Other side effects with chronic use include development of neutropenia, thrombocytopenia, hemolytic anemia, agranulocytosis, liver failure, a myasthenia-like syndrome, and psychosis with hallucinations.

In Vitro Toxicity Data

Mutagenicity studies in rat and human hepatocytes as well as Chinese hamster ovaries have been negative.

Clinical Management

All patients presenting with toxicity or potential toxicity following ingestion of procainamide should be aggressively managed and monitored. Gastrointestinal decontamination should be considered only after initial supportive care has been provided and airway control has been assured. Activated charcoal ($1\,g\,kg^{-1}$) may be administered. Because procainamide sustained-release preparations exist, multidose charcoal administration ($1\,g\,kg^{-1}$ first dose and then $1/2\,g\,kg^{-1}$ q 4 h) may be considered along with whole bowel irrigation (polyethylene glycol–electrolyte solutions at $500\,ml\,h^{-1}$ for children and $2\,l\,h^{-1}$ for adults). Gastric lavage has questionable efficacy, especially in late presenters, and can induce an unwanted vagal response.

The management of the Na^+ channel blocking activity of procainamide consists of administration of sodium and/or alkalosis. Infusion of sodium bicarbonate by either intermittent bolus or by continuous infusion has been advocated for symptomatic patients. Lidocaine has been suggested in the treatment of ventricular dysrhythmias, although clear evidence is lacking. Other class IA and IC antiarrhythmics should be avoided due to their ability to block cardiac sodium channels.

Hypotension not responsive to intravenous fluids should be managed with vasopressors, such as dopamine, norepinephrine, epinephrine, and/or phenylephrine. If seizures occur, benzodiazepines should be administered. Due to their pharmacokinetic characteristics, moderate volume of distribution, and low protein binding, procainamide and NAPA may be removed via hemodialysis and hemoperfusion. Both procainamide and NAPA serum concentrations should be obtained. Normal therapeutic ranges are: procainamide, $3–14\,\mu g\,ml^{-1}$; NAPA, $12–35\,\mu g\,ml^{-1}$. Measurement of electrolytes, renal function tests, and arterial blood gases should be considered.

See also: Benzodiazepines; Charcoal; Gastrointestinal System; Polyethylene Glycol.

Further Reading

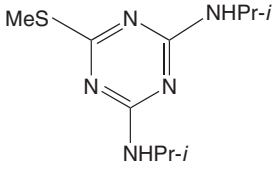
Smith WM and Gallagher JJ (1980) 'Les torsades de pointes': An unusual ventricular arrhythmia. *Annals of Internal Medicine* 93: 578–584.

White SR, Dy GL, and Wilson JM (2002) The case of the slandered Halloween cupcake: Survival after massive pediatric procainamide overdose. *Pediatric Emergency Care* 18: 185–188.

Prometryn

Larry J Dziuk

- Chemical Abstracts Service Registry Number: CAS 7287-19-6
- Synonyms: Prometryn(e) (preferred name); 2-Methylthio-4,6-bis(isopropylamino)-*s*-triazine; G 34161. Trade names include Caparol, Gesagard, Prometrex, Primatol Q, and Mercasin
- Chemical/Pharmaceutical/Other Class: Sulfur-substituted triazine pesticide
- Chemical Formula: $C_{10}H_{19}N_5S$
- Chemical Structure:



Uses

Prometryn is used as an agricultural herbicide.

Exposure Routes and Pathways

Ingestion is a possible route of exposure. Prometryn mixers, loaders, and applicators and field workers receive the most exposure by way of skin and eye contact, as well as from inhalation. The product is not available for use by the general public, so exposure to persons other than mixers, loaders, and applicators is limited to exposure by way of ingestion of food crops. However, risk due to ingestion of food is low because allowable residue limits on food crops are low.

Toxicokinetics

Triazine compounds are generally well absorbed in the gastrointestinal system. When administered by the oral route, the greatest concentrations of prometryn are found in the blood, spleen, and lungs. Dermal absorption is relatively high, with 7–15% of the material applied to skin being absorbed. Prometryn is excreted in urine or feces within 72 h. It is extensively metabolized, with less than 2% of the parent material appearing in the urine or feces.

Mechanism of Toxicity

On ingestion prometryn metabolizes, producing amine dealkylation and side chain oxidation. It affects the tricarboxylic acid cycle.

Acute and Short-Term Toxicity (or Exposure)

Animal

In toxicity studies evaluated in support of registration of the material for use as a pesticide, prometryn was regarded as being slightly to practically nontoxic. The rat oral LD_{50} is $1800\,mg\,kg^{-1}$ for male rats and $2076\,mg\,kg^{-1}$ for female rats. The dermal LD_{50} in the rat is greater than $3170\,mg\,kg^{-1}$. The 4 h inhalation LC_{50} value for rats was $4.96\,mg\,l^{-1}$.

Prometryn was not a sensitizer when applied to the skin of guinea pigs. It was determined to be a mild eye irritant to rabbits and produced only slight irritation when applied to the skin of rabbits.

Human

Prometryn has low acute toxicity. Symptoms include nausea or sore throat if swallowed.

Chronic Toxicity (or Exposure)

Animal

The US Environmental Protection Agency (EPA) has tested prometryn for carcinogenic potential and has classified the material as a group E constituent; that is, no evidence of human carcinogenic potential. In a 102 week feeding study with mice, chronic doses up to $429\,mg\,kg^{-1}$ were not associated with the production of cancer. There was no significant effect of dosing on clinical signs, mortality, gross pathology, or histopathology. Rats fed prometryn in the diet for 104 weeks at doses up to $80\,mg\,kg^{-1}$ developed concretions in the kidneys at the high dose. No evidence of carcinogenicity was found. Beagle dogs fed at dose equivalents of up to $37.5\,mg\,kg^{-1}$ over a 106 week period developed kidney effects at the high dose but no carcinogenicity was noted.

There was evidence of developmental toxicity in rats administered prometryn by gavage at a dose of $250\,mg\,kg^{-1}$ during gestational days 6–15. No developmental effects were noted in rats receiving a dose of $50\,mg\,kg^{-1}$. Rabbits receiving prometryn by gavage during gestational days 6–19 at a maximum dose of $72\,mg\,kg^{-1}$ experienced a slight but nonsignificant increase in abortions. In a two-generation reproductive toxicity study with rats, statistically significant decreases in body weight of the pups were noted in both generations at a dose of $\sim 50\,mg\,kg^{-1}$.

Human

No information was found relating to chronic toxicity in humans. According to the US EPA, systemic

toxicity of prometryn and other triazine herbicides is unlikely unless large doses are swallowed and acute toxicity develops.

In Vitro Toxicity Data

Using the Ames *Salmonella* test, prometryn was not mutagenic when tested up to the cytotoxic solubility limits. Prometryn was negative for bacterial DNA repair and gene mutation in an unscheduled DNA synthesis test using rat hepatocytes.

Clinical Management

Lavage and catharsis are recommended for ingestion. Oxygen therapy should be provided if needed. There is no specific antidote for prometryn.

Environmental Fate

Prometryn binds readily to organic matter in soil and tends to remain in the top 12 in. of soil after application. Degradation by soil microorganisms occurs in 1–3 months; the soil half-life is 60 days. In water, no hydrolysis occurred over a 28 day period.

Ecotoxicology

When tested in support of registration as an agricultural herbicide, prometryn was determined to be slightly toxic to amphibians, moderately toxic to fish, and slightly toxic to zooplankton. The 96 h LC_{50} to rainbow trout is 5.46 mg l^{-1}. The 96 h LC_{50} to bluegill sunfish is 7.95 mg l^{-1}. It is practically nontoxic to birds. The 8 day bobwhite quail and mallard duck dietary LC_{50} values are greater than 10 000 ppm. Prometryn is nontoxic to bees and earthworms.

Other Hazards

Thermal decomposition products may include oxides of carbon, nitrogen, and sulfur.

Exposure Standards and Guidelines

No specific occupational exposure limit has been established by Occupational Safety and Health Administration. Tolerance levels ranging from 0.1 to 0.5 ppm have been established for the presence of propachlor in seven crops.

See also: Pesticides.

Relevant Websites

http://www.elsevier-ecotox.com – Elsevier Science. *ECOTOX. Ecological Modelling and Ecotoxicology.* An Electronic Publication (7-150).

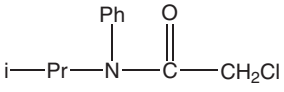
http://pmep.cce.cornell.edu – Pesticide Information Profile on Prometryn from EXTOXNET.

Propachlor

Larry J Dziuk

This article is a revision of the previous print edition article by Janye E Ash and Shayne C Gad, volume 2, pp. 588–589, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 1918-16-7
- Synonyms: 2-Chloro-*N*-isopropylacetanilide; 2-Chloro-*N*-(1-methylethyl)-*N*-phenylacetamide; Bexton; Bexton 4L; Kartex A; Niticid; Propachlore; Ramrod; Satecid; CP 31393; *N*-Isopropyl-α-chloroacetanilide
- Chemical/Pharmaceutical/Other Class: Halogenated hydrocarbon pesticide. Specifically, a chloroacetanilide herbicide
- Chemical Formula: $C_{11}H_{14}ClNO$
- Chemical Structure:



Uses

Propachlor is used as an herbicide; pre-emergence herbicide.

Exposure Routes and Pathways

Nondietary exposure to propachlor by a farmer as an applicator during mixing, loading, spraying, and flagging is probable. Dermal contact, ocular contact, and ingestion are possible exposure routes. Inhalation of spray or mist is another possible route of exposure.

Exposure of humans to propachlor through contamination of groundwater and runoff contamination of surface water after heavy precipitation is probable.

The dietary exposure (milligram per kilogram per day) to propachlor by the US population from treated food crops is possible. Residual amounts ($0.04\,mg\,kg^{-1}$) of propachlor remained in tomatoes up to 85 days after application of $6\,kg\,ha^{-1}$.

Toxicokinetics

Propachlor is absorbed through the gastrointestinal tract, through intact skin, and through the respiratory system after inhalation of dust or spray mist. It is metabolized via the mercapturic acid pathway. The major fecal metabolite is a cysteine conjugate. Rats administered ^{14}C propachlor orally excreted 98.6% of the dose in the urine and feces within 48 h. Approximately 50% is excreted as metabolites through urine or feces within 24 h.

Mechanism of Toxicity

Propachlor inhibits production of cytochrome oxidase (brain and kidneys) and cholinesterase in the liver.

Acute and Short-Term Toxicity (or Exposure)

Animal

In rats, the oral LD_{50} is $710\,mg\,kg^{-1}$ and the dermal LD_{50} is $2000\,mg\,kg^{-1}$. In mice, the oral LD_{50} is $392\,mg\,kg^{-1}$ and the dermal LD_{50} is $380\,mg\,kg^{-1}$. In ducks, the oral LD_{50} is $512\,mg\,kg^{-1}$. The main symptoms in acute poisoning relate to the central nervous system and include a state of excitement, trembling, and light convulsions. Inhalation of dust for 4 h caused inflammation of tracheal mucosa, 25% mortality, and hemorrhagic secretions in lungs and bronchi of rats. The effective threshold concentration was 136–456 mg m^{-3}. Propachlor severe dermatitis, ulceration, and necrosis of the skin of rabbits and mice. Four- and six-month studies with rats at 1/20, 1/100, and 1/200 of the LD_{50} showed inhibition of spermatopoiesis at the phase of spermatid formation and histomorphologic changes in the spermatopoietic epithelium.

In subchronic toxicity studies with mice, a dose-related decrease in white blood cells was noted as well as an increase in the incidence of centrilobular hepatocellular hypertrophy in mice receiving up to $5000\,mg\,kg^{-1}$ in the diet.

Human

The probable oral lethal dose 5–15 $g\,kg^{-1}$. Exposure to propachlor for 8 days caused erythematopapular contact eczema on hands and forearms of workers. The substance is considered a skin irritant. There have been no published reports of poisoning. There have been no reports of symptoms or diseases among occupationally exposed workers. Propachlor has been produced since 1965.

Chronic Toxicity (or Exposure)

Animal

Propachlor causes dystrophic changes in the liver of rats accompanied by decreased enzyme activities. In a 2 year study with rats, propachlor administered at doses up to $27\,mg\,kg^{-1}$ developed changes in the liver such as centrilobular hypertrophy, clear cell cytoplasmic alteration, and eosinophilic cytoplasmic alteration. There were no other changes noted. Mice receiving doses up to $105\,mg\,kg^{-1}$ over a period of 18 months developed an increase in the ratio of liver weight to body weight and a decrease in the ratio of kidney weight to body weight. However, no histological changes were noted.

Propachlor was not teratogenic when administered orally to rats at doses up to $200\,mg\,kg^{-1}$ over days 9–15 of gestation. Mice administered up to $270\,mg\,kg^{-1}$ during days 1–21 of gestation developed offspring with a statistically significant increase in hydrocephaly.

Human

Propachlor is only slightly hazardous with normal handling. No reports of poisoning of the general population or of workers are available other than for sensitization studies. Of 79 workers engaged in manufacturing a formulation of propachlor, 19% showed evidence of contact dermatitis. However, 108 workers engaged in the same manufacturing process tested 3 years later exhibited no evidence of sensitization.

In Vitro Toxicity Data

Propachlor caused increased aberrant metaphases in mouse bone marrow cells. Propachlor did not produce evidence of mutagenicity in the Ames spot test with or without microsomal fortification. Results of an *in vivo/in vitro* hepatocyte DNA repair assay were negative with rats administered up to $1000\,mg\,kg^{-1}$.

Clinical Management

Symptoms of poisoning include irritation and inflammation of the skin, eyes, and mucous membranes. Except for dermatitis, no clinical or laboratory signs of toxicity to man are known. If a small amount has

been ingested, an emetic (i.e., ipecac) should be given within the first hour. If the propachlor has been combined with a hydrocarbon, ipecac should not be used. Gastric lavage should be provided if a large amount has been ingested. If ingested more than 1 h prior, activated charcoal or magnesium sulfate should be used. If the substance has entered the eyes, an isotonic saline or water should be used. Exposed eyes should be flushed with running water for 15 min. Exposed skin should be washed with soap and water.

There is no specific antidote for poisoning. If the acute toxic effect is survived, recovery will be uneventful.

Environmental Fate

Rapidly degraded in the environment under most conditions. Does not bioconcentrate or biomagnify. Microbial degradation is the primary means of breakdown in soil. Soil half-lives of up to 3 weeks have been reported. Water solubility is $700\,mg\,l^{-1}$, which suggests mobility in aquatic systems. The vapor pressure is very low 2.5×10^{-4} mmHg at 25°C, suggesting no appreciable volatility to air.

Ecotoxicology

Ecotoxicity is somewhat variable. The material has a low to moderate toxicity to birds, a high toxicity to many aquatic organisms, and a low toxicity potential to bees. Oral LD_{50} pheasants $735\,mg\,kg^{-1}$; bobwhite quail oral LD_{50} $91\,mg\,kg^{-1}$; mallard duck LD_{50} (8 days study) $>5000\,mg\,kg^{-1}$; 96 h threshold limit median (TLM) for bluegill fingerlings $30\,mg\,l^{-1}$. The LC_{50} for rainbow trout is $0.17\,mg\,l^{-1}$. Propachlor poses a low hazard to earthworms and honey bees.

Other Hazards

When heated to thermal decomposition, irritant and corrosive fumes may be present. Products of thermal decomposition are oxides of nitrogen, hydrogen chloride, and carbon monoxide.

Exposure Standards and Guidelines

No specific occupational exposure limit has been established for propachlor. Tolerance levels ranging from 0.02 to 5 ppm have been established for the presence of propachlor in 50 foods and food by-products.

See also: Pesticides.

Relevant Websites

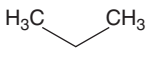
http://www.inchem.org – Environmental Health Criteria 147. Propachlor. International Programme on Chemical Safety. World Health Organization.

http://www.elsevier-ecotox.com – Elsevier Science. *ECOTOX. Ecological Modelling and Ecotoxicology.* An Electronic Publication (7-154).

Propane

Stephen R Clough

- Chemical Abstracts Service Registry Number: CAS 74-98-6
- Synonyms: Dimethylmethane; Propyl hydride (UN1978, DOT); Bottled gas; LP-gas
- Chemical/Pharmaceutical/Other Class: Aliphatic hydrocarbon
- Chemical Formula: C_3H_8
- Chemical Structure:



Uses

Propane is used principally as a fuel source for homes (including indoor and outdoor cooking) and industries and as an aerosol propellant. It is also used in the synthesis of organic chemicals, in the manufacture of ethylene, as a refrigerant, and as an extractant.

Exposure Routes and Pathways

Because propane exists as a gas at normal temperature and pressure, exposure generally occurs by inhalation (trace amounts of propane have been measured in air expired by humans). Typical background concentrations detected at ground level in major US cities range from 0.050 to 0.4 ppm. It is possible to spill liquid propane from a pressurized tank, causing frostbite on skin contact due to rapid evaporation and loss of heat. Propane has also been detected in cigarette smoke (~0.83 mg per cigarette).

Mechanism of Toxicity

Some sources classify propane as a simple anesthetic, although it can principally be classified as a simple asphyxiant; and concentrations that are high enough to displace oxygen would be expected to cause lightheadedness, loss of consciousness, and possibly death from asphyxiation.

Acute and Short-Term Toxicity (or Exposure)

Animal

Propane has been shown to have adverse effects on the cardiovascular system in the primate, dog, cat, and mouse. Guinea pigs exposed to 2.2–5.5% of the gas showed sniffing and chewing movements. In dogs, 1% caused hemodynamic changes, whereas 3.3% produced decreases in aortic pressure, stroke volume, and cardiac output and an increase in pulmonary resistance. Ten percent propane in the mouse and 15% in the dog did not produce arrhythmia but did produce weak cardiac sensitization.

Human

Propane is not considered to be inherently toxic to humans. Air concentrations up to 10 000 ppm (10%) for a few minutes will only produce slight dizziness in humans. At high concentrations, it may have a narcotic effect; but at concentrations below 100 ppm, propane causes no physiological effects in humans. However, it will cause chemical suffocation at concentrations that are high enough to displace oxygen.

Chronic Toxicity (or Exposure)

No information could be found on chronic toxicity of propane.

Clinical Management

Persons exposed to high concentrations of propane should vacate or be removed from the source of the gas and seek fresh air.

Ecotoxicology

There are no data in the US Environmental Protection Agency's ECOTOX database on propane. This is probably because highly volatile compounds such as propane, which exist as gases at normal environmental temperatures (e.g., $>0^{\circ}C$), would be expected to be found in air and not water. Some microbes can utilize propane as an energy source, whereas others are inhibited by its presence.

Other Hazards

Propane gas which is heavier than air is both an explosion and a fire hazard (the upper and lower explosive limits are 2.4% and 9.5% by volume, respectively). Extreme care must be taken to keep areas of expected high concentration free from ignition sources, such as sparks from static electricity. Explosion-proof equipment should be used in these areas.

Exposure Standards and Guidelines

The US Food and Drug Administration classifies propane as generally recognized as safe (GRAS). The Occupational Safety and Health Administration permissible exposure limit (time-weighted average) is 1000 ppm and the American Conference of Governmental Industrial Hygienists immediately dangerous to life and health level is 2100 ppm.

Miscellaneous

Propane is a colorless, highly flammable/explosive gas that is heavier than air. It occurs in natural gas at concentrations from 3% to 18%. It is emitted into the atmosphere from furnaces, automobile exhausts and sources of natural gas. With sufficient oxygen, it is combusted to carbon dioxide and water but carbon monoxide, a deadly gas, will be generated under leaner conditions. Some references state that propane is odorless while others provide an odor threshold of 22 000–36 000 mg m^{-3} (odor index = 425 at 20°C). In air, 1 ppm propane = 1.83 mg m^{-3}.

See also: Neurotoxicity.

Further Reading

Gosselin RE, Smith RP, and Hodge HC (1984) *Clinical Toxicology of Commercial Products*, 5th edn. Baltimore, MD: Williams and Wilkins.

Relevant Website

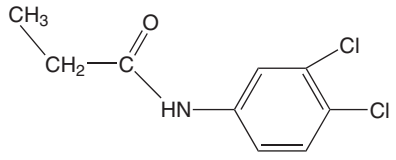
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Propane.

Propanil

Marcia D Howard

This article is a revision of the previous print edition article by Thuc Pham, volume 2, pp. 590–591, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 709-98-8
- Synonyms: *N*-(3,4-Dichlorophenyl)propionamide; 3,4-Dichloropropionanilide; Dipram; DCPA; Propanide; Grascide; Chem-Rice; Stampede; Stam
- Chemical/Pharmaceutical/Other Class: Herbicidal amide; Acetanilide
- Chemical Formula: $C_9H_9Cl_2NO$
- Chemical Structure:



Uses

Propanil is a postemergence herbicide used to control weeds in rice and potato crops.

Exposure Routes and Pathways

The general population may be exposed to propanil by inhalation and dermal contact from spraying or orally by consumption of contaminated food. Workers may also be exposed by dermal, ocular, or inhalation exposure. Exposures to crop workers may occur during application, contact with treated foliage, or pesticide-contaminated materials.

Toxicokinetics

Propanil is readily absorbed by ingestion, dermal, or inhalation exposure. Ocular exposure may also occur. Propanil is hydrolyzed by hepatic acylamidase forming 3,4-dichloroaniline and propionic acid. The major microsomal metabolites of 3,4-dichloroaniline are 6-hydroxy-3,4-dichloroaniline and *N*-hydroxy-3,4-dichloroaniline. Peak blood levels in rats occur after 1 h with acute oral exposure. Within 5 min of a single oral administration (650 and 1000 mg kg^{-1}), the compound is detected in blood and all tissues of the rat with maximum accumulation occurring within 1–6 h of treatment. Blood concentrations in rats are maintained 24 h after oral administration (1000 mg kg^{-1}, p.o.) but are undetectable 48–72 h later. When fed to cows for 4 days, 1.4% of the total dose of propanil was recovered in feces but was undetected in urine or milk.

Mechanism of Toxicity

In plants, propanil is toxic through inhibition of photosynthesis at the level of photosystem II. In animals, propanil induces methemoglobinemia resulting in tissue hypoxia.

Acute and Short-Term Toxicity (or Exposure)

Animal

Propanil has relatively low toxicity in mammals. Acute oral LD_{50} values are 0.360 g kg^{-1} (mice), 0.367–2.5 g kg^{-1} (rats), and 1.3 g kg^{-1} (dogs). In both rats and dogs, death was characterized by central nervous system (CNS) depression occurring over a 3 day period. For dermal exposure, the LD_{50} for rabbits is >5000 mg kg^{-1}.

Human

Acute exposure results in CNS depression and methemoglobinemia. Chloracne has been reported in production facility workers following dermal exposure. Death may occur due to respiratory failure. Ingestion results in a burning sensation in the mouth, esophagus, and stomach, nausea and vomiting, fever, dizziness, and drowsiness. Inhalation may cause nose and throat irritation. Prolonged or repeated dermal contact may result in slight skin irritation. Repeated or excessive exposure by any route may result in cyanosis.

Chronic Toxicity (or Exposure)

Animal

A 2 year feeding study resulted in a no-observed-adverse-effect level (NOAEL) of 600 ppm (15 mg kg^{-1} day^{-1}) in dogs. For rats, the NOAEL was 300 ppm (15 mg kg^{-1} day^{-1}) in a three-generation reproductive study. Teratology studies in rats established an NOAEL of 20 mg kg^{-1} day^{-1} (decreased pup size, delayed ossification at 100 mgkg^{-1} day^{-1}). Reproductive effects were observed only in exaggerated doses that were fatal to the mothers. Chronic effects from propanil exposure include centilobular enlargement of the liver, methemoglobinemia, decreased hemoglobin, and cyanosis although the dose levels producing these effects were many times greater than those expected from normal usage or exposure to

the compound. Long-term exposure may result in kidney and liver damage. There is no evidence of carcinogenicity in mice and rats.

Humans

Little is known regarding long-term effects of propanil. Methemoglobinemia would be expected.

Clinical Management

For oral exposure, induced vomiting is not recommended. Activated charcoal can be administered or gastric lavage (within 1 h of exposure) can be performed. Seizures should be controlled first. Oxygen should be administered to symptomatic patients. Intravenous methylene blue can be administered to patients suffering methemoglobinemia. Inhalation exposure can be treated by moving the patient to fresh air. Medical attention should be sought if breathing difficulty persists. Oxygen should be administered and assisted ventilation provided as required. For dermal exposure, contaminated clothing should be removed and the affected areas washed with soap and water. Eyes should be flushed with copious amounts of fresh water for 15 min.

Environmental Fate

Propanil is rapidly metabolized under anaerobic and aerobic conditions in a water and soil matrix. It is unlikely to be persistent for a sufficient amount of time to leach into groundwater in measurable quantities. Propanil is rapidly metabolized in soil with a half-life of 1–3 days. It is stable to photodegradation and chemical degradative processes but susceptible to biodegradation.

Ecotoxicology

Propanil is toxic to aquatic invertebrates (e.g., crayfish, worms, snails) and to fish. It is moderately toxic to birds.

Exposure Standards and Guidelines

The reference dose for propanil is 0.03 mg kg^{-1} day^{-1} and the chronic population adjusted dose is 0.003 mg kg^{-1} day^{-1}.

See also: Blood; Pesticides.

Relevant Websites

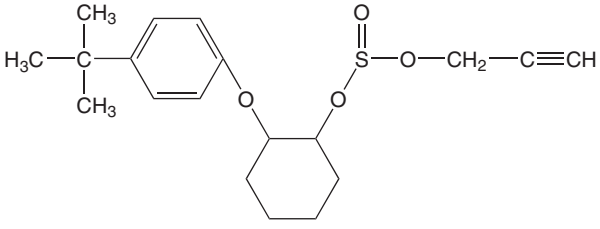
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Propanil.

http://www.intox.org – International Programme on Chemical Safety.

Propargite

Jing Liu

- Chemical Abstracts Service Registry Number: CAS 2312-35-8
- Synonyms: 2-[4-(1,1-Dimethylethyl)phenoxy] cyclohexyl-2-propynyl-sulfite; BPPS; Omite; Comite
- Chemical/Pharmaceutical/Other Class: Organic sulfite acaricide
- Chemical Formula: $C_{19}H_{26}O_4S$
- Chemical Structure:



Uses

Propargite is primarily used to control motile forms of mites.

Exposure Routes and Pathways

Dermal, oral, inhalation, and ocular exposures are all possible.

Toxicokinetics

Propargite can be absorbed from the gastrointestinal tract or skin after oral or dermal administration. It undergoes hydrolysis at the sulfite ester followed by oxidation/hydroxylation. Metabolites of propargite are eliminated in both urine and feces.

Mechanism of Toxicity

The mechanism of toxicity of propargite is unclear. Propargite is, however, an inhibitor of monoamine

oxidase and therefore can alter the metabolism of monoamines.

Acute and Short-Term Toxicity (or Exposure)

Animal

Propargite exhibits low acute toxicity via oral and dermal exposures. The oral LD_{50} value in rats is about 1–4 g kg^{-1}. With dermal exposure, the LD_{50} values are 250 mg kg^{-1} (male) and 680 mg kg^{-1} (female) in rats and >3 g kg^{-1} in rabbits. Propargite is, however, a strong eye and skin irritant that causes erythema, edema, and eschar formation in rabbits.

Human

Dermatitis is the major form of toxicity following dermal propargite exposure. Signs include erythema, burning, itching, exfoliation, and hyperpigmentation. Ocular exposure produces irritation. Changes in the chemical formulation have alleviated many of the acute irritant effects associated with propargite use.

Chronic Toxicity (or Exposure)

Animal

Both sexes of Crl:CD BR rats maintained on diets containing 50 or 100 mg kg^{-1} day^{-1} propargite for 13 weeks showed significantly lower body weights throughout the study. Various hematological and clinical chemical parameters such as erythrocyte count and hemoglobin level, urea nitrogen, and total protein were altered. Propargite was not genotoxic, mutagenic, or teratogenic but showed some carcinogenicity (jejunal sarcomas) in a species- and strain-specific manner in animal studies. Propargite caused increased incidence of intestinal tumors in CD (Crl:CDBR) and Sprague–Dawley rats but not in CD-1 mice or Wistar rats. No carcinogenicity was found in beagle dogs based on a 2 year feeding study.

Human

The US Environmental Protection Agency has listed propargite as a probable human carcinogen based on the appearance of intestinal tumors in animals.

Clinical Management

Treatment is symptomatic.

Environmental Fate

Propargite degrades rapidly under alkaline conditions in moist environments. It is, however, 'moderately persistent' to 'persistent' under neutral and acid conditions. Propargite has high affinity for soil and sediments and therefore has the potential of moving off the site of application.

Ecotoxicology

Propargite is highly to very highly toxic to freshwater aquatic organisms, fish, and invertebrates, with LC_{50} or EC_{50} values below 167 µg l^{-1}. Propargite is very highly toxic to estuarine/marine organisms, with LC_{50} values <100 µg l^{-1}. Propargite is expected to be highly toxic to amphibians, in particular early life stages. Propargite may pose a reproduction risk for avian species.

Exposure Standards and Guidelines

The reference dose for propargite is 0.04 mg kg^{-1} day^{-1} and the acceptable daily intake is 0.01 mg kg^{-1} day^{-1}.

See also: Pesticides.

Further Reading

Knowles CO (1991) Miscellaneous pesticides. In: Hayes WJ Jr. and Laws ER Jr. (eds.) *Handbook of Pesticide Toxicology*, ch. 22, p. 1473. San Diego, CA: Academic Press.

Relevant Websites

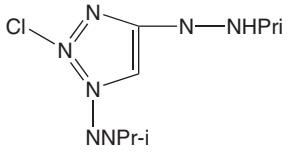
http://www.alternatives2toxics.org – Californians for Alternatives to Toxics.
http://www.inchem.org – FAO and WHO (1999). Pesticide Residues in Food (prepared by E. Bosshard).
http://www/agrimor.com – Propargite.
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Propargite.

Propazine

Raju Kacham

This article is a revision of the previous print edition article by Jayne E Ash and Shayne C Gad, volume 2, pp. 591–592, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 139-40-2
- Synonyms: 2,4-Bis(isopropylamino)-6-chloro-*s*-triazine; AI3-60348; BRN 0747081; CCRIS 1026; EINECS 205-359-9; G-30028; Geigy 30028; Gesamil; HSDB 1400; Maxx 90; Milocep; Milogard; Milo-pro; NSC 26002; Plantulin; Primatol P; Propasin; Propazin; Propinex; Prozinex
- Chemical/Pharmaceutical/Other Class: Triazine herbicide
- Chemical Formula: $C_9H_{16}N_5Cl$
- Chemical Structure:



Uses

Propazine is a selective preemergent herbicide for controlling broadleaf weeds and annual grasses (primarily pigweed) in sorghum. It is applied at the time of planting or immediately after. It is also used as a postemergence herbicide on carrots, celery, and fennel and is registered for use in greenhouses.

Background Information

The US Environmental Protection Agency (EPA) has determined that propazine belongs to a group of other triazines (including atrazine, simazine, and some related metabolites) that act through a common mechanism of toxicity based on ability to suppress the pituitary luteinizing hormone (LH) surge and elicit effects on reproductive function and development.

Exposure Routes and Pathways

Dermal and eye contact, inhalation of dust, and ingestion are possible routes of exposure.

Toxicokinetics

Propazine is readily absorbed and metabolized by amine-dealkylation and side-chain oxidation.

Seventy-two hours after oral administration of single dose of radiolabeled propazine to rats, 66% of the dose was excreted in the urine and 23% was excreted in feces. It may also accumulate in animal and human fatty tissues. Propazine and its metabolites were detected in lungs, spleen, heart, kidneys, and brain tissues of rat 8 days after dosing.

Mechanism of Toxicity

Propazine and its primary metabolite, diaminochlorotriazine, can attenuate the pituitary LH surge, leading to disruption of estrous cycle and certain reproductive and developmental processes. Propazine causes fatty degeneration. It also blocks metabolism of sugars and carbohydrates. It may also disturb the metabolism of some of the B vitamins (thiamine and riboflavin).

Acute and Short-Term Toxicity (or Exposure)

Animal

Propazine is slightly toxic by ingestion, inhalation, and by dermal contact. Oral LD_{50} values of propazine are 3850–7000 mg kg^{-1} in rats, 3180 mg kg^{-1} in mice, and 1200 mg kg^{-1} in guinea pigs.

Human

Symptoms of propazine exposure can include dizziness, dyspnea, muscle spasms, ataxia, anorexia, emaciation, diarrhea, coma, convulsions, and liver and kidney damage. It can also cause mild irritation to the skin, eyes, and upper respiratory tract.

Chronic Toxicity (or Exposure)

Animal

Similar to some other triazine herbicides, propazine induces mammary gland tumors in female (but not male) rats, likely through pituitary neuroendocrine disruption. No such tumors are noted in either sex of mice, however. Propazine delayed vaginal opening and affected testes weights in rat pups from exposed dams. After administering 500 mg kg^{-1} for 1–4 months, rabbits developed a type of anemia.

Human

Repeated exposure can lead to dermatitis. The US EPA considers propazine as a potential human carcinogen.

Clinical Management

Affected areas should be flushed with plenty of water for 15 min. Contaminated clothing should be

removed. In case of inhalation exposure, fresh air should be provided.

Environmental Fate

Propazine is highly persistent in soil and resistant to degradation by hydrolysis. The main degradation comes from microbial action. There is a possibility for contamination of ground water.

Ecotoxicology

It is practically nontoxic to slightly toxic to birds, and slightly toxic to fish.

Other Hazards

Propazine is combustible in open flames.

Exposure Standards and Guidelines

- Reference dose is $0.02\,mg\,kg^{-1}\,day^{-1}$.
- Acceptable daily intake of propazine is $0.0464\,mg\,kg^{-1}\,day^{-1}$.

The US EPA has established a Lifetime Health Advisory level of $10\,\mu g\,l^{-1}$ for propazine in drinking water.

See also: Common Mechanism of Toxicity; Pesticides; Pollution, Water.

Relevant Websites

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.
http://www.epa.gov – US Environmental Protection Agency.

Propene

Patricia J Beattie

- Chemical Abstracts Service Registry Number: CAS 115-07-1
- Synonyms: Propylene; 1-Propylene; Methylethylene; Liquid petroleum gas
- Chemical/Pharmaceutical/Other Class: Aliphatic alkene
- Chemical Formula: C_3H_6
- Chemical Structure: $H_2C=CH–CH_3$

Uses

Propene is used as a chemical intermediate in the production of polypropylene, acrylonitrile, propylene oxide, isopropanol, and cumene. Refineries use much of their production of propene internally as a refinery heating gas, to produce alkylates in gasoline, and to produce liquefied petroleum gas.

Exposure Routes and Pathways

Because propene is a gas, inhalation exposure is the primary route of entry.

Toxicokinetics

The toxicokinetics of propene have been studied in laboratory animals. In Sprague–Dawley rats, at steady state, 42% of inhaled propene is exhaled unchanged and is not absorbed into the bloodstream, with the remainder being metabolized and eliminated. Propene is metabolized to propene oxide, which reacts to form hemoglobin complexes at cysteine, histidine, and *N*-terminal valine. It is then further reacted to an alcohol and excreted.

Mechanism of Toxicity

Propene is classified as a simple asphyxiant and its toxicity is associated with the central nervous system effects associated with oxygen deprivation.

Acute and Short-Term Toxicity (or Exposure)

Animal

Inhalation of high concentrations of propene by experimental animals results in anesthetic effects similar to those seen in humans. Anesthesia has been induced in cats after exposure to concentrations of propene of 20–31% without causing other signs of toxicity. At higher concentrations, from 40% to 80%, blood pressure decreased, pulse increased, and an unusual heartbeat was reported. Cardiac sensitization was reported following propene exposure in dogs.

Human

Propene is relatively nontoxic to humans and has been investigated for use as an anesthetic. Its flammability and explosivity, however, indicate this application is

inappropriate. Exposure to a concentration of 6.4% for 2.25 min resulted in mild intoxication, a sensation of numbness, and an inability to concentrate. At 12.8% for 1 min, these same symptoms were more pronounced, with 24% and 33% for 3 min resulting in unconsciousness. Exposures from 40% to 75% for a few minutes caused reddening of the eyelids, flushing of the face, tearing, and coughing. This is consistent with the fact that liquefied propene may cause skin burns on direct contact. As with any asphyxiant, high exposures for sufficient time, resulting in oxygen deprivation, can result in death.

Chronic Toxicity (or Exposure)

Animal

Subchronic propene exposure to rats and mice for 2–14 weeks at concentrations ranging from 625 to 10 000 ppm resulted in no reported toxicity. Male and female Sprague–Dawley rats were exposed to propene at concentrations of 200, 1000, and 5000 ppm for 7 h day^{-1}, 5 days week^{-1} for 104 weeks, and male and female Swiss mice were exposed for 78 weeks. The mortality rate of the male rats increased slightly after exposures of 1000 and 5000 ppm and that of male mice after exposure to the highest dose. No evidence of other toxicity was observed. In another long-term study with exposures up to 10 000 ppm, 6 h day^{-1}, 5 days week^{-1} for 103 weeks in rats and mice, nontumorigenic lesions were reported in the nasal cavity of male rats. It was concluded that these effects were due to inflammatory changes from local irritation. No exposure-related changes in tumor incidence were reported.

In Vitro Toxicity Data

Propene was tested for mutagenic potential in L5178Y mouse lymphoma cells at concentrations up to 30% for 4 h in the presence or absence of liver S9 mix. Propene was not cytotoxic or mutagenic in the absence of S9. Inconsistent, nonreproducible mutagenic responses occurred in the presence of S9 mix. Propene was not mutagenic when tested in *Escherichia coli*.

Clinical Management

Overexposure to propene is treated by simply moving the victim to fresh air. If skin or eye irritation has occurred, affected areas should be flushed with water for at least 15 min. Recovery is usually rapid and complete.

Environmental Fate

Propene degrades in the atmosphere by reaction with photochemically produced hydroxyl radicals with a half-life of 14.6 h. It also reacts in air with ozone and nitrate radicals with half-lives of 1 and 4 days, respectively. In soil, volatilization is expected to be the primary fate due to propene's high vapor pressure. Volatilization also occurs from water, while remaining propene is readily degraded by microorganisms. This results in propene being unlikely to bioaccumulate or bioconcentrate in soil or aquatic organisms.

Exposure Standards and Guidelines

American Conference of Governmental Industrial threshold limit value is 200 ppm (8 h time-weighted average).

See also: Ethane; Propylene Oxide.

Further Reading

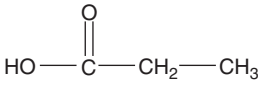
Clayton GD and Clayton FE (eds.) (1981–1982) *Patty's Industrial Hygiene and Toxicology: Volumes 2A, 2B, 2C: Toxicology*, 3rd edn., p. 3200. New York: Wiley.

Propionic Acid

Shayne C Gad and Samantha E Gad

This article is a revision of the previous print edition article by Jayne E Ash and Shayne C Gad, volume 2, pp. 593–594, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 79-09-4
- Synonyms: Ethanecarboxylic acid; Ethylformic acid; Carboxyethane; Methylacetic acid
- Chemical/Pharmaceutical/Other Class: Antifungal
- Chemical Structure:



Uses

Propionic acid is used as a feed and corn preservative, and as a chemical intermediate. It is also used to control fungi and bacteria in drinking water for livestock. Propionic acid has been qualitatively detected as a volatile component of baked potatoes and cooked meats. It has also been detected in other foods and beverages, including dairy products. Propionic acid is a major constituent (100–300 μg per cigarette) of the gas phase of the mainstream smoke of unfiltered cigarettes.

Exposure Routes and Pathways

Occupational exposure to propionic acid may occur through inhalation and dermal contact. The general population may be exposed to propionic acid via inhalation of ambient air, ingestion of food and drinking water, and dermal contact with this compound and other consumer products containing propionic acid.

Toxicokinetics

Propionic acid is rapidly absorbed through the skin. It is excreted primarily through expired air (77%) and urine and feces (7%). It is carried by blood to the liver, where it is metabolized and removed.

Mechanism of Toxicity

If sodium propionate is ingested or applied topically in an acid media, it becomes propionic acid. It oxidizes fatty acids, lowers pH values, and facilitates the citric acid cycle through interaction with coenzyme A. There has been evidence of heightened production of insulin in cows and sheep; the insulin later settles to an overall lower level.

Propionic acid inhibits $^{14}CO_2$ production from palmitate in both control and methylmalonic fibroblasts; propionic acid also inhibited ureagenesis in rat liver slices. These findings may explain the fatty degeneration of the liver and hyperammonemia in propionic and methylmalonic acidemia. Propionic acidemia is an autosomal recessive disorder caused by a defect of propionyl–coenzyme A carboxylase. The main clinical findings are vomiting, lethargy, hypotonia, and metabolic ketoacidosis, and early clinical onset occurs during the neonatal period in ~80% of the patients.

Acute and Short-Term Toxicity (or Exposure)

Animal

In animals, the symptoms include polytropism. It also produces central nervous system, cardiovascular, respiratory, and blood effects. It has also proven to be a severe skin irritant and severe eye irritant. In a rabbit skin irritation test, tissue necrosis was observed after application of 10 mg of undiluted propionic acid for 24 h. Propionic acid is corrosive to the gastric lining and, upon oral intubation, results in desquamation and hemorrhage. The intravenous LD_{50} is 625 mg kg^{-1} in mice. The oral LD_{50} in rats and mouse is 2.60–5.16 g kg^{-1} and 5.10 g kg^{-1}, respectively. Propionic acid was tested using micronucleus test *in vivo*, with no evidence of genotoxicity.

Human

Propionic acid produces burning or inflammation from contact with skin, eye, and mucous membranes. It is less toxic when ingested or inhaled than it is when dermal or ocular exposure occurs.

Chronic Toxicity (or Exposure)

Animal

Rats, mice, and hamsters administered 4% propionic acid in the diet for 7 days showed evidence of damage and cellular proliferation in the epithelium of the forestomach, and a five- to sixfold increase in cell proliferation in the mid-region of the rat forestomach after 27 days of treatment.

In Vitro Toxicity Data

Propionic acid has been tested using the *Escherichia coli* DNA repair assay, the SOS chromotest, the *Salmonella*/microsome mutagenicity test, and the sister chromatid exchange test *in vitro*. All tests except the DNA repair assay with *E. coli* yielded negative results. These data support other evidence that propionic acid is not mutagenic and that genotoxic events are unlikely to be the cause of forestomach lesions in rats fed propionic acid in the diet.

Clinical Management

The victim should be removed from exposure. Treatment is symptomatic. If exposure is dermal or ocular the area should be flushed with excess amounts of water. If ingested vomiting should not be induced.

Environmental Fate

Propionic acid has been detected in wastewater from olive oil production as a result of breakdown and oxidation of fatty acids, and data suggest that propionic acid is produced by photooxidation of anthropogenic compounds during long-range transport. Propionic acid is released to the environment via

effluents from the manufacture and use of coal-derived and shale oil liquid fuels, and from the disposal of coal liquefaction and gasification waste by-products, and wood preserving chemical waste by-products. Propionic acid may also be released to the aquatic environment in wastewater discharges from textile mills and sewage treatment facilities. Municipal and industrial landfills and hazardous waste sites via leachates can release propionic acid to groundwater supplies. Propionic acid can be emitted to air as a component of exhaust from gasoline and diesel fueled engines, and has been identified as an organic degradation and emission product from shop primers, primers, and finishing paints used on steel ships.

Propionic acid should exist as a vapor in the ambient atmosphere, and will be degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 11 days. Photolysis of propionic acid is not expected to be important. Biodegradation is likely to be the most important removal mechanism of propionic acid from water and soil. Hydrolysis is not expected to occur due to the lack of hydrolyzable functional groups.

Exposure Standards and Guidelines

Joint Expert Committee on Food Additives does not give an acceptable daily intake level. The American Conference of Governmental Industrial Hygienists threshold limit value, 8 h time-weighted average is 10 ppm.

See also: Acids.

Further Reading

Glasgow AM and Chase HP (1976) Effect of propionic acid on fatty acid oxidation and ureagenesis. *Pediatric Research* 10: 683–686.

Harrison PT (1992) Propionic acid and the phenomenon of rodent forestomach tumorigenesis: A review. *Food and Chemical Toxicology* 30: 333–340.

Henschel R, Agathos M, and Breit R (1999) Acute irritant contact dermatitis from propionic acid used in animal feed preservation. *Contact Dermatitis* 40: 328.

Lucke T, Perez-Cerda C, Baumgartner M, *et al.* (2004) Propionic acidemia: Unusual course with late onset and fatal outcome. *Metabolism* 53: 809–810.

Proposition 65, California

Samantha E Gad

This article is a revision of the previous print edition article by Shayne C Gad, volume 2, p. 594, © 1998, Elsevier Inc.

- Title: Safe Drinking Water and Toxic Enforcement Act
- Agency: State of California
- Year Passed: 1986
- Groups Regulated: Those doing business in California (except as preempted by Federal law)

Introduction

California voters in 1986 approved an initiative that reflected concerns about exposures to toxic chemicals via the environment, residence, and workplace. That initiative became the Safe Drinking Water and Toxic Enforcement Act of 1986, and is better known by its original name of Proposition 65. Proposition 65 requires the State to publish a list of chemicals known to cause cancer or birth defects or other reproductive harm. The list must be updated at least once a year, and includes over 700 chemicals.

Proposition 65 requires businesses to notify Californians about significant amounts of chemicals in the products they purchase, in their homes or workplaces, or that are released into the environment. The goal is that this information allows Californians to make informed decisions about protecting themselves from exposure to these chemicals. In addition, Proposition 65 prohibits California businesses from knowingly discharging significant amounts of listed chemicals into sources of drinking water. A business also includes any company outside of California that sells their products in California.

California Environmental Protection Agency's (Cal/EPA) Office of Environmental Health Hazard Assessment (OEHHA) administers the Proposition 65 program. OEHHA also evaluates all currently available scientific information on substances considered for placement on the Proposition 65 list.

What Types of Chemicals are on the Proposition 65 List?

Proposition 65 contains a wide range of naturally occurring and synthetic chemicals that are known to cause cancer or birth defects or other reproductive

harm. These chemicals include additives or ingredients in pesticides, common household products, food, drugs, dyes, or solvents. Listed chemicals may also be used in manufacturing and construction, or they may be by-products of chemical processes, such as motor vehicle exhaust.

How is a Chemical Added to the List?

There are three principal ways for a chemical to be added to the Proposition 65 list. A chemical can be listed if either of two independent committees of scientists and health professionals finds that the chemical has been clearly shown to cause cancer or birth defects or other reproductive harm. These two committees are the Carcinogen Identification Committee (CIC) and the Developmental and Reproductive Toxicant (DART) Identification Committee, and both are part of OEHHA's Science Advisory Board. The committee members are appointed by the Governor and are designated as the 'State's Qualified Experts' for evaluating chemicals under Proposition 65. When determining whether a chemical should be placed on the list, the committees base their decisions on the most current scientific information available. OEHHA staff scientists compile all relevant scientific evidence on various chemicals for the committees to review. The committees also consider comments from the public before making their decisions.

A second way for a chemical to be listed is if an organization designated as an 'authoritative body' by the CIC or DART Identification Committee has identified it as causing cancer or birth defects or other reproductive harm. The following organizations have been designated as authoritative bodies: the US Environmental Protection Agency, the US Food and Drug Administration (US FDA), the National Institute for Occupational Safety and Health, the National Toxicology Program, and the International Agency for Research on Cancer.

A third way for a chemical to be listed is if an agency of the state or federal government requires that it be labeled or identified as causing cancer or birth defects or other reproductive harm. Most chemicals listed in this manner are prescription drugs that are required by the US FDA to contain warnings relating to cancer or birth defects or other reproductive harm.

In addition to these three listing procedures, Proposition 65 also requires the listing of chemicals meeting certain scientific criteria and identified in the California Labor Code as causing cancer or birth defects or other reproductive harm. This method was used to establish the initial chemical list following voter approval of Proposition 65 in 1986.

What Requirements Does Proposition 65 Place on Companies Doing Business in California?

Businesses are required to provide a 'clear and reasonable' warning before knowingly and intentionally exposing anyone to a listed chemical. This warning can be given by a variety of means, such as by labeling a consumer product, posting signs at the workplace, distributing notices at a rental housing complex, or publishing notices in a newspaper. Once a chemical is listed, businesses have 12 months to comply with warning requirements.

Proposition 65 also prohibits companies that do business within California from knowingly discharging listed chemicals into sources of drinking water. Once a chemical is listed, businesses have 20 months to comply with the discharge prohibition.

Businesses with less than 10 employees and government agencies are exempt from Proposition 65's warning requirements and prohibition on discharges into drinking water sources. Businesses are also exempt from the warning requirement and discharge prohibition if the exposures they cause are so low as to create no significant risk of cancer or birth defects or other reproductive harm. Health risks are explained in more detail below.

What Does a Warning Mean?

If a warning is placed on a product label or posted or distributed at the workplace, a business, or in rental housing, the business issuing the warning is aware or believes that one or more listed chemicals is present. By law, a warning must be given for listed chemicals unless exposure is low enough to pose no significant risk of cancer or is significantly below levels observed to cause birth defects or other reproductive harm.

For a chemical that causes cancer, the 'no significant risk level' is defined as the level of exposure that would result in not more than one excess case of cancer in 100 000 individuals exposed to the chemical over a 70 year lifetime. In other words, a person exposed to the chemical at the 'no significant risk level' for 70 years would not have more than a 'one in 100 000' chance of developing cancer as a result of that exposure.

For chemicals that are listed as causing birth defects or reproductive harm, the 'no-observed-effect level' is determined by identifying the level of exposure that has been shown to not pose any harm to humans or laboratory animals. Proposition 65 then requires this 'no-observed-effect level' to be divided by 1000 in order to provide an ample margin of

safety. Businesses subject to Proposition 65 are required to provide a warning if they cause exposures to chemicals listed as causing birth defects or reproductive harm that exceed 1/1000th of the 'no-observed-effect level'.

To further assist businesses, OEHHA develops numerical guidance levels, known as 'safe harbor numbers' (described below) for determining whether a warning is necessary or whether discharges of a chemical into drinking water sources are prohibited. However, a business may choose to provide a warning simply based on its knowledge, or assumption, about the presence of a listed chemical without attempting to evaluate the levels of exposure. Because businesses do not file reports with OEHHA regarding what warnings they have issued and why, OEHHA is not able to provide further information about any particular warning. The business issuing the warning should be contacted for specific information, such as what chemicals are present, and at what levels, as well as how exposure to them may occur.

Safe Harbor Numbers

OEHHA has developed safe harbor numbers to guide businesses in determining whether a warning is necessary or whether discharges of a chemical into drinking water sources are prohibited. A business has 'safe harbor' from Proposition 65 warning requirements or discharge prohibitions if exposure to a chemical occurs at or below these levels. These safe harbor numbers consist of no significant risk levels for chemicals listed as causing cancer and maximum allowable dose levels for chemicals listed as causing birth defects or other reproductive harm. OEHHA has established safe harbor numbers for over 200 chemicals and is continuing to develop safe harbor numbers for listed chemicals.

Enforcement of Proposition 65

The California Attorney General's Office enforces Proposition 65. Any district attorney or city attorney (for cities whose population exceeds 750 000) may also enforce Proposition 65. In addition, any individual acting in the public interest may enforce Proposition 65 by filing a lawsuit against a business alleged to be in violation of this law. Lawsuits have been filed by the Attorney General's Office, district attorneys, consumer advocacy groups, and private citizens and law firms. Penalties for violating Proposition 65 by failing to provide notices can be as high as $2500 per violation per day.

How is Proposition 65 Meeting Its Goal of Reducing Exposure to Hazardous Chemicals in California?

Since it was passed in 1986, Proposition 65 has provided Californians with information they can use to reduce their exposures to listed chemicals that may not have been adequately controlled under other State or Federal laws. This law has also increased public awareness about the adverse effects of exposures to listed chemicals. For example, Proposition 65 has resulted in greater awareness of the dangers of alcoholic beverage consumption during pregnancy. Alcohol consumption warnings are perhaps the most visible health warnings issued as a result of Proposition 65.

Proposition 65's warning requirement has provided an incentive for manufacturers to remove listed chemicals from their products. For example, trichloroethylene, which causes cancer, is no longer used in most correction fluids; reformulated paint strippers do not contain the carcinogen methylene chloride; and toluene, which causes birth defects or other reproductive harm, has been removed from many nail care products. In addition, a Proposition 65 enforcement action prompted manufacturers to decrease the lead content in ceramic tableware and wineries to eliminate the use of lead-containing foil caps on wine bottles.

Proposition 65 has also succeeded in spurring significant reductions in California of air emissions of listed chemicals, such as ethylene oxide, hexavalent chromium, and chloroform.

Although Proposition 65 has benefited Californians, it has come at a cost for companies doing business in the state. They have incurred expenses to test products, develop alternatives to listed chemicals, reduce discharges, provide warnings, and otherwise comply with this law. Recognizing that compliance with Proposition 65 comes at a price, OEHHA is working to make the law's regulatory requirements as clear as possible and ensure that chemicals are listed in accordance with rigorous science in an open public process.

See also: Developmental Toxicology; Toxicity Testing, Reproductive.

Further Reading

Curry KK, Brookman DJ, Whitmyre GK, *et al.* (1994) Personal exposures to toluene during use of nail lacquers in residences: Description of the results of a preliminary study. *Journal of Exposure Analysis and Environmental Epidemiology* 4: 443–456.

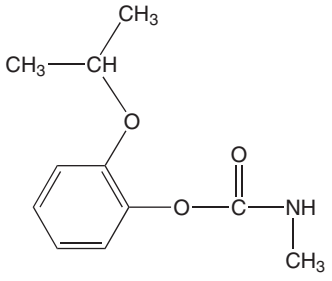
Relevant Website

http://www.oehha.ca.gov – State of California Environmental Protection Agency (Cal/EPA), Office of Environmental Health Hazard Assessment (OEHHA) Proposition 65. State of California's website for Proposition 65.

Propoxur

Paul R Harp

- Chemical Abstracts Service Registry Number: CAS 114-26-1
- Synonyms: 2-Isopropoxyphenyl-*N*-methylcarbamate; Baygon; Blattanex; IMPC; Invisi-Gard; IPMC; Propogon; Sendra; Sendran; Suncide; Tendex; Unden; Undene; BAY 39007; BAY 9010; BO 58 12315; ENT 25671; OMS 33; SHA 047802
- Chemical/Pharmaceutical/Other Class: *N*-Methylcarbamate insecticide
- Chemical Structure:



Uses

The main uses of propoxur include control of residential and commercial insect pests, domestic animal pests, and mosquitoes. It is available in a wide variety of formulations including aerosols, baits, dusts, emulsifiable concentrates, pest strips, pet flea collars, powders, ready-to-use solutions, and wettable powders.

Exposure Routes and Pathways

For both occupational and nonoccupational environments, dermal contact is the most common pathway but exposure has also occurred through ingestion and inhalation. The relatively high vapor pressure of propoxur (which is advantageous for mosquito control) has been implicated in several cases of inhalation exposure after recommended safety precautions were not observed. The rate of revolatization is influenced by temperature with applications in higher temperature, higher humidity environments presenting a greater risk of inhalation.

Toxicokinetics

Dermal absorption of propoxur in humans has been estimated to be ~ 16%; estimated absorption from the gastrointestinal tract in experimental studies with humans has been complicated due to propoxur-induced emesis. A biological half-life of 3.1 h has been determined and 2-isopropoxyphenol is the major metabolite. The majority of the dose undergoes urinary excretion within 48 h of exposure. In rats, both the parent compound and 2-isopropoxyphenol appear to be eliminated primarily in the urine as sulfate conjugates.

Mechanism of Toxicity

Propoxur binds and inhibits acetylcholinesterase, the enzyme responsible for metabolizing the neurotransmitter acetylcholine and terminating its action at cholinergic synapses. Exposure to propoxur results in synaptic accumulation of acetylcholine in both the central and peripheral nervous systems and hyperstimulation of muscarinic and nicotinic receptors leading to 'cholinergic crisis'. In contrast to the organophosphate anti-cholinesterases, acetylcholinesterase inhibition by the *N*-methylcarbamates is reversible with fairly rapid reactivation occurring through spontaneous decarbamoylation or via hydrolysis of the carbamate.

Acute and Short-Term Toxicity (or Exposure)

Animal

Signs of acute exposure in laboratory animals are similar to those described for humans and recovery from nonlethal exposures occurs rapidly. LD_{50} values (or ranges) reported for acute exposure in rats are 80–191 mg kg^{-1} (oral), from 1000 to >2400 mg kg^{-1} (dermal), and >1.44 mg l^{-1} (inhalation).

Human

The acute effects of exposure are due to cholinergic overstimulation and may include the SLUDGE (salivation, lacrimation, urination, diarrhea,

gastrointestinal cramping, and emesis) syndrome, respiratory depression, bronchospasms, increased bronchial secretions, pulmonary edema, blurred vision, miosis, headache, tremors, muscle fasciculations, convulsions, mental confusion, coma, and death (due to respiratory failure). Symptomatic recovery from nonlethal exposures occurs very rapidly (usually within a few hours).

Chronic Toxicity (or Exposure)

Currently, insufficient evidence exists to indicate any significant long-term health risk associated with propoxur exposure.

Clinical Management

Persons providing medical assistance should avoid contact with contaminated clothing. Contaminated clothing should be removed and either laundered or discarded. Contaminated leather garments such as shoes or gloves should be discarded. Exposed dermal areas should be cleaned thoroughly with soap and water. Exposed eyes should be flushed with generous amounts of clean water for at least 15 min. If necessary, an endotracheal tube should be used to maintain a clear airway, aspirate any secretions, and provide oxygen via mechanical ventilation.

For ingestion, if the patient is asymptomatic and can be treated soon after exposure, activated charcoal may be used to reduce absorption of the carbamate. If potentially life-threatening quantities have been ingested, gastric lavage should be considered if it can be conducted within $\sim$1 h of exposure. Charcoal and/or catharsis are contraindicated in presence of severe vomiting or diarrhea. Muscarinic effects (i.e., SLUDGE) may be reduced by intravenous or intramuscular administration of atropine. Seizures can be treated with intravenous benzodiazepines (diazepam or lorazepam); phenobarbital may be helpful for recurrent seizures. Pralidoxime is indicated in cases of mixed exposure to both carbamates and organophosphorus compounds but is contraindicated in cases of carbamate-only exposure. Furosemide may be useful for pulmonary edema that continues after full atropinization. Metabolite analysis of a urine sample may allow confirmation of the intoxicating agent.

Environmental Fate

Propoxur is of moderate to low persistence in the soil (half-life of 14–50 days). It does not bind with high affinity to soil and therefore tends to be mobile. It is highly water soluble and thus has potential for leaching into groundwater. Propoxur is very mobile in sandy loam, silt loam and silty clay soils. Propoxur degrades in water at a rate of $\sim$1.5% per day at neutral pH. Propoxur is well absorbed into plant tissues and can thereby be active against insects for up to 1 month.

Ecotoxicology

Propoxur is very highly toxic to many birds. The LD_{50} in quail, mourning doves, and finches was 25.9, 4, and 4 mg kg^{-1}, respectively. LD_{50} values were from 6 to 120 mg kg^{-1} in other bird species. Acute signs of toxicity in birds included tearing, salivation, muscle incoordination, diarrhea, and tremors. Death generally occurred rapidly (5–45 min) with severe acute poisoning and recovery was also rapid. Propoxur is moderately to slightly toxic to aquatic species. LC_{50} values (96 h) were 3.7–6.6 mg l^{-1} in trout and in bluegill. Propoxur does not markedly bioaccumulate. Propoxur is highly toxic to honeybees.

Exposure Standards and Guidelines

The reference dose for propoxur is 0.005 mg kg^{-1} day^{-1}. The acceptable daily intake is 0.02 mg kg^{-1} day^{-1}.

See also: Carbamate Pesticides; Cholinesterase Inhibition; Pesticides.

Further Reading

Ecobichon DJ (2000) Carbamates. In: Spencer PS and Schaumburg HH (eds.) *Experimental and Clinical Neurotoxicology*, 2nd edn., pp. 289–298. New York: Oxford University Press.

Relevant Websites

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.
http://www.epa.gov – US Environmental Protection Agency.

Propoxyphene

Christopher P Holstege

This article is a revision of the previous print edition article by Regina M Rogowski, volume 2, pp. 595–596, © 1998, Elsevier Inc.

- Chemical Name: Propoxyphene
- Chemical Abstracts Service Registry Number: CAS 469-62-5
- Synonyms: Darvocet-N; Darvon; Darvon-N; Darvon pulvules; Propoxyphene hydrochloride; Propoxyphene napsylate; Wygesic
- Chemical/Pharmaceutical/Other Class: Synthetic opioid

Uses

Propoxyphene is used as an analgesic. It is also used as a drug of abuse.

Exposure Routes and Pathways

All propoxyphene preparations are for oral use; ingestion is the most common route of exposure. Propoxyphene-containing pharmaceuticals are also solubilized and injected for purposes of abuse.

Toxicokinetics

Propoxyphene is readily absorbed from the gastrointestinal tract with measurable levels within 5 min and peak plasma levels within 1–2 h following therapeutic dosing. Propoxyphene undergoes extensive first pass metabolism and is primarily metabolized in the liver by *N*-demethylation to norpropoxyphene. Propoxyphene and norpropoxyphene are widely distributed throughout the body. Concentrations in the tissues are reportedly 10–40 times greater than in the blood. Peak plasma levels of propoxyphene and norpropoxyphene occur within 1–2 h and 2–4 h, respectively. Propoxyphene and norpropoxyphene are highly protein bound (80%). The volume of distribution is 10–26 l kg^{-1}. Norpropoxyphene is a pharmacologically active metabolite. Serum norpropoxyphene levels are reportedly higher than propoxyphene due to norpropoxyphene's slower metabolism. Norpropoxyphene is further metabolized in the liver to a variety of inactive metabolites that include: *p*-hydroxypropoxyphene, norpropoxyphene carbinol, *p*-hydroxynorpropoxyphene, dinorpropoxyphene, cyclic dinorpropoxyphene, and dinorpropoxyphene carbinol. Propoxyphene and its metabolites are excreted in the urine, with less than 10% of a propoxyphene dose excreted unchanged in the urine. High concentrations of propoxyphene and norpropoxyphene have been found in the bile, suggesting that enterohepatic recirculation occurs. There is substantial individual variation in the elimination half-life of propoxyphene, with the elimination half-life of propoxyphene ranging from 8 to 46 h and norpropoxyphene from 6 to 54 h. The elimination half-life of propoxyphene is prolonged following over dosage, repetitive dosing, and shock.

Mechanism of Toxicity

Propoxyphene is an agonist of opioid μ receptors. It is this opioid effect that is responsible for the central nervous system and respiratory depression seen in overdose. Both propoxyphene and norpropoxyphene are potent blockers of myocardial sodium channels, an effect identical to type IA antidysrhythmic agents. This myocardial sodium channel blockade may result in prolongation of the electrocardiogram QRS complex, arrhythmias, and cardiovascular depression seen in propoxyphene poisoning.

Acute and Short-Term Toxicity (or Exposure)

Animal

Central nervous system and cardiovascular effects in animals are similar to those seen in humans.

Human

Acute toxicity with propoxyphene has been reported to cause nausea, vomiting, miosis, confusion, restlessness, somnolence, coma, apnea, seizures, hypotension, arrhythmias, pulmonary edema, and cardiac arrest.

Chronic Toxicity (or Exposure)

Animal

Pregnant rats fed up to 400 mg kg^{-1} day^{-1} demonstrated ~20% maternal mortality, decreased fertility, and some fetal death. No teratogenic effects were observed.

Human

Tolerance and dependence may occur with prolonged use. Abrupt cessation in patients utilizing propoxyphene chronically may result in an opioid withdrawal syndrome in both adults and neonates.

Clinical Management

In patients presenting with propoxyphene toxicity, the airway should be patent and adequate ventilation assured. If the patient has either inadequate ventilation or a poor gag reflex, then the patient may be at risk of subsequent CO_2 narcosis, worsening acidosis, and/or aspiration. If necessary, endotracheal tube intubation should be performed. The initial treatment of hypotension consists of intravenous fluids. There should be close monitoring of the patient's pulmonary parameters to ensure that pulmonary edema does not develop as fluids are infused. The patient should be placed on continuous cardiac monitoring with pulse oximetry. Frequent neurological checks should be made. Gastrointestinal decontamination should be considered only after initial supportive care has been provided and airway control has been assured. Activated charcoal ($1\,g\,kg^{-1}$) may be administered.

Naloxone may be of benefit in reversing the neurological and respiratory depressant effects of propoxyphene. Naloxone may also decrease the propensity for developing seizures after overdose. Naloxone has no effect on the potential myocardial sodium channel blocking properties of propoxyphene. The management of the sodium channel blocking effects of propoxyphene consists of administration of sodium and/or alkalosis. Infusion of sodium bicarbonate either by intermittent bolus or by continuous infusion has been advocated. The indications for sodium bicarbonate infusion include a QRS duration of >100 ms, persistent hypotension despite adequate hydration, and dysrhythmias. During infusions of sodium bicarbonate, close monitoring of electrolyte, pH, and fluid balance should be performed. Hyperventilation has also been shown to be effective in reversing myocardial sodium channel blocking activity due to the induced respiratory alkalosis. Lidocaine therapy has been suggested in the treatment of ventricular dysrhythmias, although clear evidence is lacking. Class IA and IC antiarrhythmics should be avoided due to their ability to block cardiac sodium channels.

See also: Charcoal; Gastrointestinal System; Poisoning Emergencies in Humans.

Further Reading

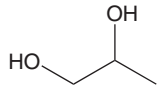
Hantson P, Evenepoel M, and Ziade D (1995) Adverse cardiac manifestations following dextropropoxyphene overdose: Can naloxone be helpful. *Annals of Emergency Medicine* 25: 263–266.

Sloth-Madsen PS, Strom J, and Reiz S (1984) Acute propoxyphene self-poisoning in 222 consecutive patients. *Acta Anaesthesiologica Scandinavica* 28: 661–665.

Propylene Glycol

Vijay M Vulava

- Chemical Abstracts Service Registry Number: CAS 57-55-6
- Synonyms: 1,2-Propanediol; α-Propylene glycol; Methyl glycol; Methylethyl glycol; Methylethylene glycol; Monopropylene glycol; PG 12; Sirlene; 1,2-Dihydroxypropane; 1,2-Propylene glycol; 2-Hydroxypropanol; 2,3-Propanediol; Propane-1,2-diol; Dowfrost; Propylene glycol usp; 1,2-Propylenglykol; Solar winter ban; Sentry propylene glycol; Isopropylene glycol; Ucar 35; Solargard P; Aliphatic alcohol; Chilisa FE; Ilexan P; Inhibited 1,2-propylene glycol; Prolugen; Propanediol; Trimethyl glycol
- Chemical/Pharmaceutical/Other Class: Glycols; 1,2-Diols
- Chemical Formula: $C_3H_8O_2$
- Chemical Structure:



Uses

Propylene glycol is one of the most commonly used humectants – substances that have a high affinity for water and have a stabilizing action on the water content of a material. Propylene glycol is used to maintain moisture within a narrow range in certain food products, such as coconut and marshmallows, in certain medicines and cosmetics as well as in tobacco, and is a solvent for food colors and flavors. The US Food and Drug Administration has classified propylene glycol as an additive that is 'generally recognized as safe' for use in food.

Propylene glycol is commonly used to make antifreeze and deicing solutions for cars, airplanes, and boats; to make polyester compounds; and as solvents in the paint and plastics industries. It is used as a substitute for ethylene glycol mono-alkyl ethers in all-purpose cleaners, coatings, inks, nail polish, lacquers, latex paints, and adhesives. It is also used to create artificial smoke or fog used in fire-fighting training and in theatrical productions.

The general population is exposed to propylene glycol by oral intake, dermal contact, and inhalation. The average daily intake of propylene glycol from food products in the United States has been estimated at 2400 mg day^{-1} (34 mg kg^{-1} body weight (bw) per day for a 70 kg person). Propylene glycol is an inert ingredient in some pharmaceutical preparations. Propylene glycol is also found in many pharmaceuticals that are administered intravenously, which represents a unique exposure route for certain subpopulations.

Exposure Routes and Pathways

As propylene glycol is ubiquitous in several foods, medical, and cosmetic products, humans are commonly exposed via several routes.

Toxicokinetics

The pharmacokinetics of propylene glycol is reasonably well understood in humans as well as animals. Propylene glycol is rapidly and extensively absorbed followed by rapid distribution into total body water. Dermal absorption studies in humans have shown that absorption of propylene glycol through intact skin is very limited. However, once the dermal layers are disturbed (such as with burns or irritation), dermal absorption can be a significant source of exposure. Except for the amount entering the nasopharynx and being swallowed, under normal exposure conditions propylene glycol exposure by inhalation is not toxicologically relevant due to its low vapor pressure (0.07 mmHg).

Total body clearance occurs by metabolic clearance and by renal excretion. The excretion of propylene glycol is species dependent. Humans clear ~45% of propylene glycol via kidney, and in dogs, up to 88%. In rats and rabbits, very little of the parent compound is excreted by the kidney until saturation of metabolism occurs. Inhibition of alcohol dehydrogenase by pyrazole increases urinary excretion of propylene glycol to 75% in rats.

The rate-determining step in the metabolic clearance of propylene glycol in humans and animals is NAD-dependent alcohol dehydrogenase. Humans clear propylene glycol similarly to rats and rabbits, but saturation of metabolic clearance occurs at lower doses (up to 8–10 times) in humans than in rats and rabbits. The lower dose required for saturation in humans is related to the expression of various isoforms of alcohol dehydrogenase in various species and in different tissues. By a NAD-dependent reaction, alcohol dehydrogenase converts propylene glycol to lactaldehyde, which is further metabolized to lactate. Since propylene glycol has a chiral center, technical grade propylene glycol results in the formation of 50/50 D,L-lactate. L-Lactate is indistinguishable from endogenous lactate, which is a good substrate for gluconeogenesis. D-Lactate is less readily converted to glucose than L-lactate, which prolongs its half-life leading, under conditions of prolonged exposure (e.g., intravenous infusion), to D-lactic acidosis. It is difficult to cause L-lactic acidosis even with very high doses of propylene glycol because of its efficient detoxification via gluconeogenesis.

The second reason for lack of development of L-lactic acidosis is the saturation of alcohol dehydrogenase, which results in a constant rate of lactate production. Due to removal of L-lactate by gluconeogenesis, a further increase in lactate levels is not possible after saturation of metabolism.

Mechanism of Toxicity

The absorption, distribution, metabolism, and excretion of propylene glycol have been studied in humans, cats, rats, mice, and rabbits. There are no identifiable differences between humans and animals in the toxicity of propylene glycol. Toxic effects of propylene glycol occur only at very high doses. Since propylene glycol has very low intrinsic toxicity, saturation of metabolism plays a protective role in its toxicity since the conversion of propylene glycol to the more toxic lactate (particularly D-lactate) is slowed. Because of low alcohol dehydrogenase activity in infants and children, this protective effect is more pronounced in infants and up to 5 years of age. High blood levels of propylene glycol during continuous therapeutic infusion in pediatric intensive care patients 15 months of age and younger were not associated with any acute toxicity. The knowledge that human metabolism of propylene glycol saturates at an 8–10 times lower dose than in rats or rabbits provides further confidence that human developmental or reproductive risks are of negligible concern.

Propylene glycol administered to mice in drinking water at up to 5% (w/v) had no effect on fertility of either males or females in either the first or second generation. Other data indicate that this compound is not a reproductive or developmental toxicant in mice, rats, hamsters, or rabbits. There are no major

differences in general toxicity between humans and most animals, and toxicity only occurs at very high doses (LD_{50} values of 8–46 g kg^{-1} in rats, and is estimated to be >15 g kg^{-1} in humans). Current estimated exposures to propylene glycol are of negligible concern for reproductive or developmental toxicity in humans.

No indications on mutagenicity or carcinogenicity have been found in laboratory animal studies. Subcutaneous injections in mice led to a small increase in fetal malformations, but experiments with oral exposure of mice over several generations did not show any effects of toxicity to reproduction.

Propylene glycol is mildly to moderately irritating to skin in concentrations above 10%. No irritation was seen in rabbit eyes. Several cases of allergy have been described, and concentrations above 10%, particularly if occluded, may give rise to allergic skin reactions. With skin affected by disease or damage the risk of irritation and allergic reaction is increased. Reactions have been described by 2% on eczematous skin. As propylene glycol is widely used, allergy cases are considered unusual. Propylene glycol may be absorbed through skin and increase the absorption of other substances.

Acute and Short-Term Toxicity (or Exposure)

There are sufficient data to characterize the acute and chronic toxicity of propylene glycol in laboratory animals, including nonhuman primates. In humans, information on toxicity is limited to medical case studies. However, because of the similarities in the toxicokinetic profile of propylene glycol across species, the toxicity data from the animal studies can be extrapolated to human exposures.

Propylene glycol has very low systemic toxicity in experimental animals and very high doses are required to determine a toxic level. Central nervous system (CNS), hematologic, hyperosmotic, and cardiovascular effects have been noted in humans and animals and high serum concentrations of propylene glycol may result in lactic acidosis and hyperosmotic changes in the blood.

Animal

Animals lethally intoxicated undergo CNS depression, narcosis, and respiratory arrest. Acute oral toxicity has been well characterized in the rat, mouse, rabbit, dog, and guinea pig with LD_{50} values, 8–46 g kg^{-1}, reported at very high oral doses. An average daily dose of 1.7 g kg^{-1} bw in male rats and 2.1 g kg^{-1} bw in female rats has been shown to have no adverse effect on body weight gain, mortality, hematology, urinary cell excretion, renal function, serum chemistry, or absolute and relative organ weights.

Human

In humans, a lethal oral dose has been estimated to be >15 g kg^{-1} for an adult. Mortality has occurred in hospitalized infants after repeated exposure to propylene glycol in medication.

Chronic Toxicity (or Exposure)

Animal

There are few studies that investigated chronic exposure of propylene glycol to animals. A propylene glycol diet at 2 and 5 g kg^{-1} bw per day fed to dogs for 2 years resulted in RBC destruction in dogs fed with higher amounts of propylene glycol while no effect was observed in dogs fed at lower concentration. In a continuous inhalation study chronic toxicity of propylene glycol (55–113 ppm) in Rhesus monkeys and rats were studied for up to 1 year. Both rats and monkeys inhaling propylene glycol gained more weight than the control group; no adverse effects were noted.

Results from animal studies indicate that intermediate and chronic exposure to propylene glycol may lead to changes in hematological parameters and hemolysis of RBCs. Cats exposed to oral administration of propylene glycol developed Heinz bodies in RBCs and decreased RBC survival. Doses as low as 0.424 g kg^{-1} bw per day have resulted in Heinz body formation in cat erythrocytes.

Human

Chronic occupational exposure to propylene glycol in humans may occur through dermal contact or through inhalation of airborne propylene glycol from heating or spraying processes. Propylene glycol occupational exposure data are limited to several small studies. An investigation measuring propylene glycol exposure in motor servicing workers did not detect normal urinary propylene glycol levels. Another study measured airborne propylene glycol exposure (geometric mean 350 μg m^{-3}, maximum 12 700 μg m^{-3}) among Swedish painters during indoor application of water-based paints. Elevated propylene glycol levels were measured in urine samples collected pre- and postshift from aircraft deicing workers (range: 0.72–13.44 mg l^{-1}; 0.41–10.58 mg g^{-1} creatinine); and in urine samples from a comparison group (range: 0.29–10.7 mg l^{-1}, 1.18 mg g^{-1} creatinine). In a National Institute for Occupational Safety and Health 'Health Hazard

Evaluation' of aircraft deicing workers, personal breathing zone air samples for propylene glycol over a 6 h period ranged from 10 to 21 mg m^{-3}, with a mean of 15 mg m^{-3}.

In Vitro Toxicity Data

Propylene glycol in a concentration of 0.5–1.0% has been shown to inhibit natural cytotoxicity and neutrophil chemiluminescence in human cells *in vitro* in one study.

In vitro studies of embryonic development suggest that propylene glycol alters the development of mouse zygotes. Treatment with propylene glycol caused cell membrane damage and altered pH, resulting in a decrease in embryonic development.

Clinical Management

Degree of injury must be considered when determining initial treatment. When large amounts of propylene glycol are ingested, copious amounts of water should be given to the patient to dilute stomach contents. In case of inhalation exposure, the patient should be removed to fresh air and be given supplemental oxygen in case breathing becomes difficult. In case of dermal exposure, the exposed skin should be immediately washed with copious water and soap. Upon exposure to propylene glycol, eyes should be flushed with copious water including areas under eye lids. In all cases, a physician should be consulted in case of serious injury.

Environmental Fate

Propylene glycol has a low vapor pressure (0.07 mmHg at 20°C) and is miscible with water. High solubility of propylene glycol in water ensures at least partial removal of the compound will occur by wet deposition when released to atmosphere as vapors. Relatively low Henry's law constant values for the compound suggest that releases to surface water will not partition to the atmosphere via volatilization. Adsorption to sediment or soil particulates is not significant due to low sorption partitioning coefficient (K_{oc}) value and hence propylene glycol can have a high mobility in soil and could leach into groundwater. Low octanol/water partition coefficient (K_{ow}) suggests that bioconcentration and biomagnification are also not likely to occur.

Propylene glycol released to the atmosphere is expected to undergo rapid photochemical oxidation via reaction with hydroxyl radicals. The half-life for the photochemical oxidation of propylene glycol has been estimated to be 20–32 h.

Biodegradation by a variety of acclimated and unacclimated microorganisms, under both aerobic and anaerobic conditions, is also the most important transformation process for propylene glycol in surface waters and soils. Half-lives for the biotransformation of propylene glycol generally range from 1 to 4 days under aerobic conditions and from 3 to 5 days under anaerobic conditions. The rates of biodegradation of propylene glycol in soils are significantly dependent on substrate concentrations, soil types, and ambient soil temperatures, but nutritional supplements had minimal effects. Propylene glycol rapidly disappears from culture flasks containing activated sludge microorganisms under both aerobic and anaerobic conditions.

Exposure Standards and Guidelines

Currently there are no published or recommended exposure standards for propylene glycol. However, the World Health Organization suggests an acceptable daily intake of 0–25 mg kg^{-1}.

See also: Food Additives.

Further Reading

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Relevant Websites

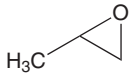
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Propylene Glycol.

http://cerhr.niehs.nih.gov – National Toxicology Program, Center for the Evaluation of Risks to Human Reproduction, US Department of Health and Human Services.

Propylene Oxide

Ada Kolman and Siv Osterman-Golkar

- Chemical Abstracts Service Registry Number: CAS 75-56-9
- Synonyms: 1,2-Propylene oxide; 1,2-Epoxypropane; Methyloxacyclopropane; Methyloxirane; Methyl ethylene Oxide
- Chemical/Pharmaceutical/Other Class: Epoxides
- Chemical Formula: C_3H_6O
- Chemical Structure:



Uses

Propylene oxide is widely used in the chemical industry as an intermediate in the production of a broad spectrum of materials, such as polyether polyols, propylene glycol, and propylene glycol ethers. These products are further used in the manufacture of polyurethane, lubricants, and detergents. Propylene oxide is used for the fumigation of dried fruit and various other foodstuffs. Propylene oxide is also used for embedding tissues for electron microscopy.

Exposure Routes and Pathways

Propylene oxide is a volatile colorless liquid with an ethereal odor. Occupational exposure by inhalation of contaminated air, as well as contact with eyes and skin, provide the significant routes of exposure. It is not known to occur as a natural product.

Toxicokinetics

Studies in experimental animals have demonstrated that propylene oxide is readily absorbed and effectively metabolized. Only a minor fraction of the compound is exhaled unchanged. The main metabolic pathways are enzyme-catalyzed reactions with glutathione and water.

The distribution of propylene oxide within the body has been studied in experimental animals by means of measurement of its alkylation products (adducts) with DNA in various tissues and with hemoglobin in red blood cells. In rats, exposed to the compound by inhalation, the highest DNA adduct levels were found in the nasal epithelia, followed by lung, lymphocytes, spleen, liver, and testis. The adduct level in the respiratory mucosa was ~30-fold higher than in the testis. The dose response for adduct formation was linear up to 500 ppm of propylene oxide in the air. Propylene oxide is ~5–10 times less efficient than the related compound ethylene oxide concerning its alkylation capacity *in vivo*.

Mechanism of Toxicity

The toxic effects of propylene oxide are related to its ability to react directly, without metabolic activation, with various components of cells, including DNA, RNA, and proteins.

Acute and Short-Term Toxicity (or Exposure)

Animal

The toxicity of propylene oxide has been studied in several animal species. Acute oral LD_{50} values of 380 mg kg^{-1} in the rat and 660 mg kg^{-1} in the guinea pig have been reported. The 4 h LC_{50} value for inhalation is 4000 ppm for the rat and 1740 ppm for the mouse. Propylene oxide neuropathy has been demonstrated in rats. In a 7 week study, animals exposed to propylene oxide at a concentration of 1500 ppm in air, developed ataxia in the hind legs.

Human

Contact with propylene oxide may cause severe skin and eye irritation. Cases of allergic contact dermatitis and hand eczema have been described. Inhalation of propylene oxide may result in spasm, inflammation, and edema of the larynx and bronchi, as well as pulmonary edema leading to pneumonia. Symptoms of exposure may include burning sensation, coughing, wheezing, headache, nausea, and vomiting. Propylene oxide may cause central nervous system depression and other neurological disorders.

Chronic Toxicity (or Exposure)

Animal

Chronic and subchronic exposure of rats to propylene oxide by inhalation induced respiratory cell hyperplasia, irritation and toxicity in the nasal epithelium, at a concentration of 300 ppm or higher. No adverse effects on reproduction were observed in rats or rabbits exposed to propylene oxide at up to 500 ppm. *In vivo* studies in rodents of dominant lethal mutations, sperm abnormalities, micronuclei, chromosomal aberrations, and sister chromatid exchanges

have given negative results. Neither chromosomal aberrations nor sister chromatid exchanges were induced in monkeys exposed to 300 ppm. Long-term carcinogenicity studies in rodents, administered propylene oxide by different routes, demonstrated increased incidences of tumors mainly at the site of contact. At oral administration, tumors of the forestomach, which were mainly squamous-cell carcinomas, were produced in rats. In rats of both sexes exposed by inhalation, papillary adenomas of the nasal cavity were observed, as well as thyroid adenomas and carcinomas in females. Increased incidences of mammary fibroadenomas and adenocarcinomas have been observed in females. In mice exposed by inhalation, propylene oxide produced hemangioma and hemangiosarcoma of the nasal cavity and a few malignant nasal epithelial tumors. Subcutaneous administration of propylene oxide to mice produced local sarcoma.

Human

Convincing epidemiological data on cancer in humans are lacking for propylene oxide. However, based on the body of data including positive responses in tests for toxicity and carcinogenicity in experimental animals, DNA adduct formation, and also propylene oxide genotoxicity in several *in vitro* tests in mammalian cells, the International Agency for Research on Cancer has classified propylene oxide as 'possibly carcinogenic to humans'.

In Vitro Toxicity Data

Propylene oxide is mutagenic in several microorganisms and in *Drosophila*. It induces sister chromatid exchanges and chromosomal aberrations, as well as DNA damage (single- and double-strand breaks) in human cells. Propylene oxide induces neoplastic cell transformation in mouse embryo cells.

Clinical Management

If propylene oxide is swallowed, the mouth should be washed out with water. Vomiting should not be induced. In case of inhalation of the compound, the person should be moved to fresh air. If breathing is difficult, oxygen should be given. If not breathing, artificial respiration should be given. In case of contact with eyes or skin, contaminated areas should immediately be flushed with plenty of water for at least 15 min. In all cases of extensive exposure, immediate medical advice should be sought.

Environmental Fate

Propylene oxide is not expected to bioaccumulate. When released into water, it is hydrolyzed with a half-life between 10 and 30 days. Degradation of propylene oxide in the air may occur by reaction with photochemically produced hydroxyl radicals.

Other Hazards

Propylene oxide is extremely flammable. Propylene oxide–air mixtures may be explosive by contact with heat or by ignition. Propylene oxide is incompatible with acids, bases, oxidizing agents, polymerization catalysts, epoxy resins, and high temperatures. It reacts violently with acetylide-forming metals such as copper or copper alloys.

Exposure Standards and Guidelines

Recommendations regarding limits for occupational exposure to propylene oxide differ markedly. The current 8 h time-weighted average (TWA) established by the Occupational Safety and Health Administration in the United States is 100 ppm. The American Conference of Governmental Industrial Hygienists recommends a threshold limit value of 2 ppm as an 8 h TWA. In European countries, the limits of exposure are in the range 1–20 ppm (8 h TWA).

See also: Epichlorohydrin; Ethylene Oxide.

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Prostaglandins

Samantha E Gad

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- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Unsaturated derivatives of arachidonic acid, a 20-carbon fatty acid

Uses

Prostaglandins are a family of naturally occurring compounds found mainly in animals, and are involved in numerous physical processes, both beneficial and pathological. These include inflammation (especially allergy related), cell growth regulation, and smooth-muscle contraction. They are hormone-like chemicals that are produced in the cell membranes of virtually every organ. Like all eicosanoids, they are derived from oxidized arachidonic acid, an essential polyunsaturated fatty acid. They are produced and metabolized in the kidney from essential fatty acids, and can be found in various tissues and body fluids. Perhaps the most commonly known therapeutic application related to prostaglandins is the use of aspirin to stop inflammation and pain. Aspirin works by inhibiting the synthesis of prostaglandins. Prostaglandins have a number of therapeutic uses, for example, preventing ulcers, reducing the potential size of myocardial infarctions, and to treat glaucoma. Another example is that prostaglandins are also used to promote cervical or uterine contractions in pregnant women, either as a labor inducer or as an abortifacient (i.e., RU486).

Exposure Routes and Pathways

Aside from endogenous production, prostaglandins can be absorbed into the body through ocular exposure, inhalation, ingestion, or injection.

Toxicokinetics

The prostaglandins as a group are known for having extremely short life span, sometimes as short as several minutes within an organism before enzymatic action breaks them down. They are produced on demand, and act in very small amounts to regulate the nervous, respiratory, cardiovascular, reproductive, endocrine, immunological, and renal systems.

In the case of glaucoma treatment, a prostaglandin derivative (S-1033) is rapidly absorbed through the eyes and into the plasma.

Drug Interactions

NSAIDs (nonsteroidal antiinflammatory drugs): Isolated cases of adverse neurological side effects have been seen with naproxen or phenylbutazone given with misoprostol. Misoprostol also increases the abdominal pain and other side effects of diclofenac and indometacin (indomethacin). Paracetamol (acetaminophen) intensifies pain if given with mifepristone and sulprostone used to induce abortion.

Digitalis glycosides: Epoprostenol caused a small predicted decrease in digoxin clearance in the short term; this may not be clinically significant.

Mechanism of Toxicity

Mast cell degranulation releases arachidonic acid, which is broken down by the enzyme cyclooxygenase to form prostaglandins, mediated by prostaglandin H synthase (PHS). PHS is a peroxidase involved in biotransformation, adding a peroxide oxygen to the xenobiotic. Interaction with some substances, such as benzo[*a*]pyrene or aflatoxin B1, may catalyze the release of teratogenic and tumorigenic metabolites. By oxidizing acetaminophen to *N*-acetyl-benzoquinoneimine, PHS may also contribute, along with cytochrome P450 and glutathione, to the nephrotoxic effects of the drug. PHS peroxidation may also suppress bone marrow by binding to proteins and DNA in the marrow. The role of PHS in biotransformation is mediated by the availability of archidonic acid, which may be the key to controlling its toxic effect.

Acute and Short-Term Toxicity (or Exposure)

Human

Some prostaglandins have vasoconstrictive and or bronchodilatory properties while others are vasodilatory and or bronchoconstrictive. Prostaglandins regulate smooth muscle function in the lungs, heart, and uterus. They contribute to changes in the oxygen flow to the heart causing rapid changes in coronary blood flow. Asthmatics exposed to prostaglandin F2a (or Dinoprost) may experience bronchospasm, arrhythmia, or hyperventilation. It may induce grand mat seizures in epileptics. Prostaglandins contribute to platelet aggregation of blood clots and NSAIDs such as aspirin or ibuprofen counteract that activity.

When used to induce labor, Dinoprost effects may include cervical laceration or rupture with retention of the placenta or hemorrhaging. It may affect the

alimentary tract as well, causing nausea, vomiting, and diarrhea. In two cases, women died of cardiovascular collapse following a 40 mg dose of Dinoprost. The TD_{Lo} is 20 $\mu g\,kg^{-1}$.

Chronic Toxicity (or Exposure)

Animal

PHS is suspected of contributing to bladder cancer in dogs by converting aromatic amines to reactive radicals through one-electron oxidation in the liver.

Human

PHS could be a human carcinogen, since dogs and humans have similar tumorigenic responses to aromatic amines.

Clinical Management

Since prostaglandins are rapidly metabolized in the body, discontinued use and supportive therapy are usually the recommended treatments for a toxic response. In cases of placental retention, blood transfusions may be necessary.

See also: Ethylene Glycol Monoethyl Ether.

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Zurier RB (2003) Prostaglandins: Then and now and next. *Seminars in Arthritis and Rheumatism* 33: 137–139.

Relevant Website

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Proteomics

Udayan M Apte and Harihara M Mehendale

Introduction

Proteomics is the study of 'proteome' or the protein expression in the cell on a global level. It is known that each cell in the body of a living organism has a complete set of genes but the cell type and functions performed by the cells are governed by stringently controlled processes of transcription (DNA to mRNA) and translation (mRNA to protein). The revolution in genomic technology has enabled us to study gene expression of thousands of genes simultaneously using high-density microarray. Although microarray technology has revolutionized the way one studies gene expression in the cell, it has some obvious drawbacks. It has been noted that there is very poor correlation between the microarray data and the protein expression data, largely due to post-transcriptional control of gene expression. Only the genes with high 'codon bias' exhibit high correlation in mRNA and protein expression in the cell. Thus, even though the microarray technology remains a very powerful tool, proteomic techniques to study global protein expression are becoming increasingly popular.

For toxicology the advantages of proteomics goes beyond the ability to compare protein expression differences. Proteomics allows a researcher to study protein modifications due to toxic treatment and more importantly allows identification of toxicant-protein adducts. Extraordinary advances in technology during the last decade have made proteomics a highly accurate, relatively inexpensive, and, therefore, a routinely used technique in biomedicine and toxicology. Proteomics is applied in toxicology mainly for three purposes: protein expression profiling, that is, assessment of changes in protein expression following toxicant exposure (control vs. treatment type comparisons); protein modification studies, that is, identification and characterization of protein adducts formed due to interaction of reactive metabolites with proteins,

changes in structure and chemistry of proteins following toxicant exposure; and finally for predictive toxicology where protein expression patterns of model toxicants are used to predict toxic effects of novel compounds.

Analytical Approaches in Proteomics

There are four main steps in any proteomic analysis (**Figure 1**):

1. Obtaining protein samples from cell cultures or tissues.
2. Breakdown of proteins to peptide mixture and further resolution of peptides.
3. Mass spectrometric analysis of peptides.
4. Identification of protein from mass spectrometric analysis.

Isolation of Proteins

Any proteomic study starts with the collection of proteins from biological samples such as cell culture media, cultured cells, serum, or any biological fluid, and a variety of animal tissues. The first step is to obtain a protein sample under conditions of least protein degradation. This involves use of various protease inhibitors that stop the protein degradation. The use of protease inhibitors depends on the type of sample and the analytical technique used in the subsequent analysis. The selection of protease inhibitors used is critical since many protease inhibitors and detergents used in the preparation of tissue homogenates can interfere with mass spectrometry (MS) procedures, which are an integral part of the proteomic analysis.

Breakdown of Proteins to Peptides and Further Resolution of Peptides

The second step of proteomic analysis involves breaking down of the proteins into smaller peptides (typically six amino acids long) and separating them depending upon molecular weight and/or charge to increase the resolution of proteomic analysis. The protein sample obtained from cells or tissue is further digested using various proteases with known properties to obtain a mixture of peptides. While various enzymes or enzyme combinations such as trypsin, chymotrypsin (their mixture), and Gluc-C are used, trypsin remains by far the most popular choice for protein breakdown into peptides. Most proteins are extremely large and complex molecules, and have posttranslational modifications such as phosphorylation and glycosylation, which renders them unsuitable for mass spectrometric analysis. Digesting proteins into peptides enables accurate detection of their mass by MS.

Breaking the proteins into smaller peptides using proteases produces a large and complex mixture of peptides. This peptide mixture needs further separation, mostly depending upon size (molecular weight) of the peptides, in order to achieve higher resolution and accuracy in MS analysis. Therefore, an integral part of the second step in proteomic is separation of individual peptides contained in the mixtures of peptide using one of the two methods: preparative isoelectric focusing or high-performance liquid chromatography (HPLC). The peptides separated, using

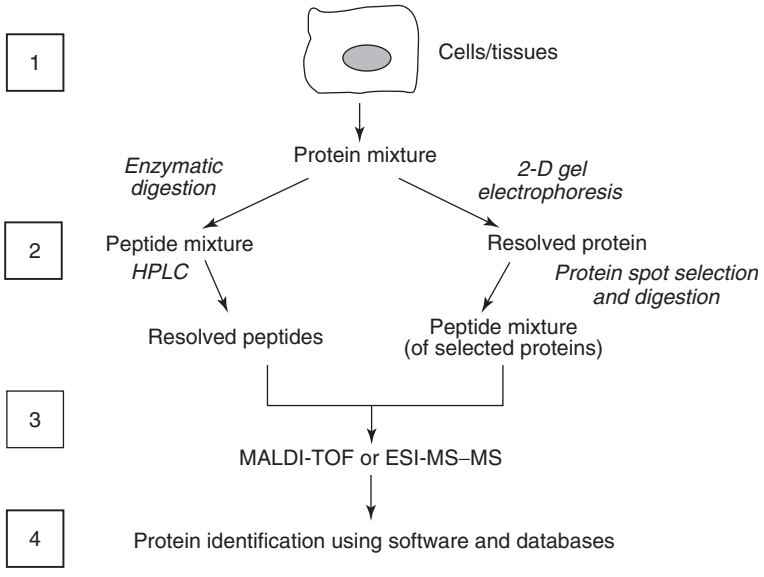

Figure 1 Schematic representation of the general analytical approach in a typical proteomic study.

one of these methods, are subjected to MS analysis in the final step of proteomic analysis.

Alternatively, one can separate the proteins before breaking them into peptides and then subject them to protein breakdown to obtain the peptide. This approach involves mainly the use of gel-based procedures such as one-dimensional or two-dimensional electrophoresis (2-DE) to resolve protein mixture into protein spots based on molecular weight and charge (isoelectric point or p*I*). Following separation, protein spots are cut out from the gel; protein is harvested from gel matrix, digested by peptides, and subjected for further mass spectrometric analysis.

Since both these approaches have their own advantages and disadvantages, the choice of method used is generally based on the type of analysis (expression analysis vs. adduct identification). Most of the proteomic studies use one of the two approaches that have evolved in recent years:

1. Proteins are resolved on 2-DE followed by protein spot excision from the gel and peptide digestion.
2. Enzymatic protein digestion to obtain peptide mixture, which is separated using HPLC.

The product of this peptide resolution is the subjected to matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF) in the case of 2-DE or electrospray ionization-mass spectrometry (ESI-MS) analysis in the case of HPLC separation.

Mass Spectrometric Analysis

MS is the heart of proteomic analysis and the success of proteomic experiments depends largely on the sensitivity and accuracy of MS equipment used to identify peptide sequences. MS machines have three main components (**Figure 2**): a source, which generates peptide ions, a mass analyzer, which separates peptide ions based on mass to charge ratio (*m/z*), and a detector that detects the ion resolved by the mass analyzer. All the modern MS machines are computer controlled and assisted by highly intelligent software.

There are two main types of MS equipment used in proteomic analysis, which function differently and provide different types of results. These are MALDI-TOF and ESI-MS–MS.

MALDI-TOF It stands for matrix-assisted laser desorption ionization-time-of-flight mass spectrometer. It is a very popular type of MS instrument used in proteomic analysis mainly because it is easy to use, robust, and has very high sensitivity. MALDI-TOF is generally used in combination with 2-DE based protein separation. Proteins are separated by 2-D gel and digested out of the gel to obtain a peptide mixture. This peptide mixture is then mixed with a chemical matrix (e.g., α-cyano-4-hydroxycinnamic acid), spotted on a chip, and allowed to evaporate in the air. This results in the formation of a crystal lattice containing the peptide mixture. This crystal lattice containing the sample peptides is then placed in the MALDI-TOF and excited using a laser. The chemical matrix is excited by absorbing photons from the laser; the extra energy is transmitted to the peptides converting the peptides into peptide ions. These peptide ions are then ejected into the gas phase from the crystal lattice. The matrix material aids in the formation of a crystal lattice and in turn in generation of peptide ions.

The peptide ions formed in the matrix-assisted source now enter the mass analyzer. The mass analyzer in MALDI-TOF is of time-of-flight (TOF) type. The peptide ions pass down the mass analyzer towards a detector, which is placed at the other end of the mass analyzer. The time of flight of each ion depends upon the *m/z* ratio of that particular ion. The general rule is that greater the *m/z* ratio, faster the movement of the ion. One of the problems with this process is that peptide ions with the same *m/z* value are poorly resolved. To overcome this problem, a device called as reflectron is used. The reflectron is placed at the end of the mass analyzer and focuses on ions with the same *m/z* ratio together and sends them to the detector. The addition of a reflectron has greatly increased the accuracy and sensitivity of the MALDI-TOF analysis.

ESI-MS–MS The electrospray ionization tandem MS (ESI-MS–MS) is generally used in combination with HPLC as method of separating digested peptide

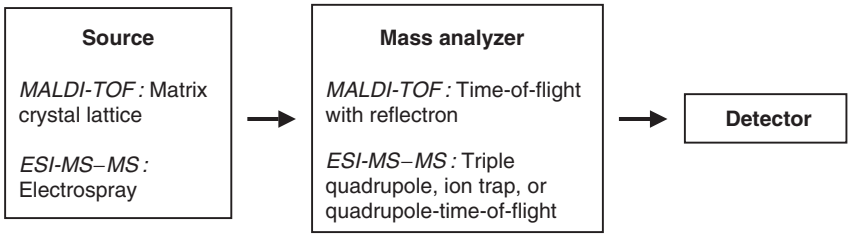

Figure 2 Schematic diagram depicting the main components of a mass spectrometry instrument.

mixtures rather than 2-DE. A major difference between MALDI-TOF-MS and ESI-MS–MS is that the peptide samples are in solution (liquid phase) in ESI-MS–MS as opposed to solid matrix in the case of MALDI-TOF. Proteins obtained from cells or tissues are digested using proteases and the resulting peptide mixture is resolved using tandem HPLC methods. Thus, separated peptides are in a solution of solvents (mostly the mobile phase chemicals such as acetonitrile) and water. The ESI source is a fine pointed needle of stainless steel maintained at very high voltage. The peptides separated from HPLC are directly introduced into the ESI source, which are sprayed inside the MS machine under extreme pressure and high voltage resulting in ionization of peptides. In the next step, called as desolvation, the HPLC solvents that enter MS instruments along with the peptides are removed and the remaining peptide ions are taken to a series of mass analyzers.

There are three main types of mass analyzers in ESI-MS–MS instruments: triple quadrupole, ion traps, and quadrupole-time-of-flight (Q-TOF). There are several differences between the mass analyzers in MALDI-TOF and in ESI-MS–MS. Unlike in MALDI-TOF-MS, in ESI-MS–MS two mass analyzers are used in tandem to increase the sensitivity of the technique. The peptide ions produced by the ESI sources are carried to the first mass analyzer and only peptides of a set *m/z* ratio are selected. The selected ions are then carried to a collision cell where they are subjected to additional fragmentation to produce smaller amino acid ions using a process called as collision induced dissociation (CID). The CID process employs inert gases such as argon for the dissociation of peptides. These smaller amino acid ions are then resolved in the second mass analyzer before sending to the detector. This process essentially enables highly sensitive detection of actual amino acid sequence of the peptides based on the *m/z* ratios of individual amino acids.

Protein Identification from MS Data

The final step in any proteomic study is the identification of proteins using the MS data. This is highly dependent on large databases of protein sequences and computer algorithms that can compare and interpret the MS spectra using these databases. Since the output of MALDI-TOF and ESI-MS–MS analyses are essentially different, they employ different types of computer software for the interpretation of MS spectra.

The databases used to analyze the MALDI-TOF MS data contain sequence information about thousands of proteins from a number of species. These sequences can be subjected to 'virtual digestion' using the same proteases used in the actual study (e.g., trypsin) to generate virtual peptide sequences. The algorithms can generate information about actual *m/z* values of these peptides. Then the peptide masses calculated from the MS spectra are compared with the virtual peptide masses to find accurate 'hits'. As one can imagine, the more the number of hits in such as query, higher the accuracy of the peptide identified. This process of identification of peptides based on masses is called as 'peptide mass fingerprinting' (PMF). The most popular database used for PMF is 'SWISS-PROT'. The software programs used to compare the MS data with the databases include PepSea, PeptIdent, MOWSE, ProFound, and Mascot.

In the case of the ESI-MS–MS data, actual amino acid sequence can be deduced. This is possible due to the CID processes, which breaks the peptides further into amino acid ions. Each amino acid ion has a specific mass and by calculating masses of specific amino acid from the MS spectra, the exact sequence of the peptide and in turn the protein can be deduced. The workhorse of such analysis is a program called 'Sequest'. Since the ESI-MS–MS analysis provides information about the actual amino acid sequence, it is also useful to obtain information about protein modifications (such as phosphorylation) and toxicant-induced protein adducts. This has become even easier with the advent of new software tools and highly intelligent algorithms such as 'SALSA'.

Advantages and Disadvantages of Proteomic Technologies

Extraordinary technical advancement in mass spectrometry in last 10 years has made proteomic analysis a routine technique used in toxicology in academic, government, and industry settings. The major advantage of proteomics is that it generates information about proteins in the cell, which are the functional entities in biology, as opposed to RNA expression, which has relatively poor correlation with physiological endpoints. Proteomics has also enabled identification of protein adducts induced by toxicant exposure in easily collectable human tissues such as serum. This will potentially introduce novel biomarkers of exposure and revolutionize the field of risk assessment. Since there are diverse proteomic approaches available, the choice of the method used depends upon the end result desired. The 2-DE combined with MALDI-TOF can be used for efficient, high throughput analysis of changes in protein expression between control and treatment groups, while the ESI-MS–MS analysis works better for identification of protein modifications and chemical nature of toxicant-induced protein adducts.

The highly technical nature of proteomic analysis is its major disadvantage. The success of a proteomic study is highly dependent upon various factors: the quality of protein samples, protein separation, enzymatic digestion to obtain peptide mixtures, and mainly the sensitivity and accuracy of MS instruments. Thus, proteomics demands implementation by highly skilled technicians and expensive instrumentation for generation of meaningful information. Secondly, protein expression in the cell is extremely dynamic and changes with physiological condition, nutritional status, and exposure to toxicants or drugs can change this dynamic process. Therefore, a proteomic study is generally a snapshot of protein expression in the cell at a given time under the given experimental conditions. Nevertheless, proteomic technologies have and are constantly maturing and have become an integral part of toxicological analysis.

See also: Microarray Analysis.

Further Reading

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Liebler DC (2002) *Introduction to Proteomics: Tools of the New Biology*. Totawa, NJ: Humana Press.

Marshall T and Williams KM (2002) Proteomics and its impact upon biomedical science. *British Journal of Biomedical Sciences* 59(1): 47–64.

Relevant Website

http://dir.niehs.nih.gov/proteomics – Proteomics Group, National Center for Toxicogenomics (of the National Institute of Environmental Health Sciences).

Prunus Species

Christopher P Holstege

- Chemical Name: *Prunus* species
- Synonyms: *Prunus armeniaca* (Apricot); *P. avium* (sweet cherry); *P. caroliniana* (cherry laurel); *P. cerasus* (sour cherry); *P. domestica* (common plum); *P. dulcis* (almond); *P. malus pumila* (common apple and crab apple); *P. persica* (peach); *P. serotina* (wild cherry); *P. virginiana* (chokecherry)
- Chemical/Pharmaceutical/Other Class: Cyanogenic glycosides

Background Information

The *Prunus* species includes a group of more than 400 trees and shrubs. They often find ornamental use because of their flowers, fruit, and nuts.

Exposure Routes and Pathways

Exposure is by ingestion of seeds, leaves, stems, roots, and fruit. Crushed seeds of some of the mentioned varieties are marketed as health foods. They are also marketed and sold surreptitiously as cancer remedies or vitamin supplements. Laetrile and Aprikern are some 'health' products consisting of crushed seeds from the *Prunus* species.

Toxicokinetics

Cyanogenic glycosides contain amygdalin. Amygdalin is erratically absorbed from most of the gastrointestinal tract but is effectively absorbed from the duodenum. Amygdalin is not toxic until it is metabolized by the enzyme emulsin to hydrocyanic acid. This metabolism may occur slowly and result in delayed clinical toxicity. Emulsin is found within the seeds of the *Prunus* species and in certain bacteria found within human intestinal flora. The presence of amygdalin in the seed kernels is not harmful unless the seed is crushed (masticated) and moistened, allowing release of emulsin. Amygdalin may result in cyanide toxicity in humans. Cyanide is converted to thiocyanate by an enzymatic reaction catalyzed by rhodanese. Thiocyanate is renally excreted.

Mechanism of Toxicity

Cyanide reversibly binds the ferric iron associated with the cytochrome oxidase system, thereby inhibiting the mitochondrial respiratory chain. This results in an inability to adequately utilize oxygen and causes 'internal asphyxia'. Cyanide combines with hemoglobin to form cyanhemoglobin, which does not transport oxygen. Cyanide also inhibits antioxidant enzymes such as catalase, superoxide dismutase, and glutathione peroxidase.

Acute and Short-Term Toxicity (or Exposure)

Animal

Ingested *Prunus* plant material has not been associated with toxicity in mammals.

Human

Cyanide poisoning due to the accidental ingestion of these plants is rare. Acute symptoms are the same as in cyanide poisoning and include difficulty in breathing, dyspnea, muscular twitching, headache, muscle spasms, ataxia, seizures, coma, and death. The onset of symptoms may be very rapid with few premonitory signs, or may be delayed.

Clinical Management

Supportive therapy should be provided for all patients with *Prunus* exposures. Activated charcoal may be effective if administered early. Oxygen (100%) should be administered in symptomatic patients. Administration of the cyanide antidote kit may be necessary in symptomatic patients with metabolic acidosis. The cyanide antidote kit contains amyl nitrite inhalant, sodium nitrite, and sodium thiosulfate. Hydroxycobalamin has also been utilized as an effective antidote for cyanide toxicity. Diazepam may be used to control seizures. Acidosis should be treated with sodium bicarbonate.

See also: Amyl Nitrite; Charcoal; Cyanide; Diazepam.

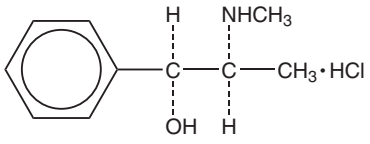
Further Reading

Hall AH and Rumack BH (1986) Clinical toxicology of cyanide. *Annals of Emergency Medicine* 15: 1067–1074.

Pseudoephedrine

Brenda Swanson-Biearman

- Chemical Abstracts Service Registry Number: CAS 670-40-6
- Synonyms: Pseudoephedrine hydrochloride; Pseudoephedrine sulfate; D-Isoephedrine; Isoephedrine; Sudafed®
- Chemical/Pharmaceutical/Other Class: Pseudoephedrine is a stereoisomer of ephedrine, in the drug class of sympathomimetics. It occurs naturally in plants of the genus *Ephedra*.
- Chemical Structure:



Uses

Pseudoephedrine is an orally active sympathomimetic amine exerting its decongestant action by acting directly on α-adrenergic receptors in the respiratory tract mucosa producing vasoconstriction resulting in shrinkage of swollen nasal mucous membranes, reduction of tissue hyperemia, edema, and nasal congestion, and an increase in nasal airway patency. Drainage of sinus secretions is increased and obstructed eustachian ostia may be opened. Relaxation of bronchial smooth muscle by stimulation of β-adrenergic receptors may also occur.

Exposure Routes and Pathways

Accidental and intentional exposures to pseudoephedrine occur most often by the oral route and involve either the pure form or multisymptom cold preparations containing pseudoephedrine in combination with antihistamines, analgesics, and antitussive agents.

Toxicokinetics

Pseudoephedrine is well absorbed from the gastrointestinal tract within 15–30 min, with peak effect between 30 and 60 min for prompt release dosage forms. Duration of action persists for 4–6 h for noncontrolled release formulations while extended release capsules may increase the duration of action to 12 h. Pseudoephedrine has been shown to have a mean elimination half-life of 4–6 h, which is dependent on urine pH. The elimination half-life is decreased at urine pH <6 and may be increased at urine pH >8, varying the half-life from 1.9 to 21 h. Approximately 55–75% of the parent drug is

excreted unchanged in the urine; the remainder is hepatically metabolized by *N*-demethylation to an inactive metabolite. A small amount is excreted as the active metabolite, norpseudoephedrine. Pseudoephedrine is 20% protein bound with a volume of distribution of 2.1–3.3 l kg^{-1}.

Mechanism of Toxicity

Pseudoephedrine is a weak base (p*K*, 9.4) that stimulates both α- and β-adrenergic receptors, as well as the release of neuronal norepinephrine. β1 stimulation produces increased heart rate and blood pressure. The α-adrenergic effects are believed to result from the reduced production of cyclic adenosine-3′,5′-monophosphate (cyclic 3′,5′-AMP) by inhibition of the enzyme adenyl cyclase, whereas β-adrenergic effects appear to be caused by the stimulation of adenyl cyclase activity.

Acute and Short-Term Toxicity (or Exposure)

Animal

Following the ingestion of a large dose of pseudoephedrine, dogs and cats may exhibit hyperactivity, mydriasis, depression, vomiting, hyperthermia, disorientation, bradycardia, and tachycardia. Therapy is directed at prevention of absorption and control of tachyarrhythmias with lidocaine (dogs only) or procainamide (dogs only). Diazepam may be used for symptoms associated with central nervous system (CNS) stimulation.

Human

Hypertension and tachycardia are the primary toxic manifestations of pseudoephedrine overdose. An amount of more than three or four times the maximum daily dosage for adults or children may produce symptoms of β-adrenergic stimulation. In severe poisonings, cardiac dysrhythmias and cerebral hemorrhage due to hypertensive crisis may occur. Anxiety, muscle tremor, and seizures may result from CNS stimulation. Hallucinations, drowsiness, and/or irritability are more common symptoms exhibited by children. Hypokalemia and hyperglycemia may be noted. Acute renal failure and rhabdomyolysis have occurred in rare instances with large overdoses.

Chronic Toxicity (or Exposure)

Animal

Pseudoephedrine has been used in a dog model of allergic nasal congestion.

Human

Pseudoephedrine is generally well tolerated in therapeutic doses. Common adverse effects include CNS stimulant effects (e.g., tremor, restlessness, nervousness, irritability) and gastrointestinal effects (e.g., nausea, vomiting, dysgeusia).

Clinical Management

Basic and advanced life-support measures should be instituted as necessary. Activated charcoal may be considered for substantial recent ingestions. The cardiac and hemodynamic status should be carefully monitored. Prolonged observation (at least 24 h) may be necessary in patients ingesting sustained-release formulations. β-Adrenergic blocking agents and antiarrhythmic agents may be necessary to treat cardiac complications. Hypertension is generally transient, requiring only observation. Antihypertensive agents may be necessary in rare instances. Administration of benzodiazepines may result in decreased blood pressure in hypertensive pseudoephedrine overdose. Symptoms of CNS stimulation usually respond to a calm environment and supportive measures. Benzodiazepines can be administered for seizures. Treatment of exposure to products in which pseudoephedrine is combined with antihistamines, antitussives, analgesics, and/or alcohol must include toxicologic management of the concurrent drugs involved. In symptomatic patients, laboratory evaluation should include electrolytes and blood glucose, and creatine phosphokinase in more severe overdoses.

See also: Benzodiazepines; Charcoal; Diazepam; Ephedra; Hypoglycemics, Oral.

Further Reading

Mariani PJ (1986) Pseudoephedrine-induced hypertensive emergency: Treatment with labetalol. *American Journal of Emergency Medicine* 4: 141–142.

Sauder KL, Brady WJ, and Hennes H (1997) Visual hallucinations in a toddler: Accidental ingestion of a sympathomimetic over-the-counter nasal decongestant. *American Journal of Emergency Medicine* 15: 521–526.

Psilocybin *See* Mushrooms, psilocybin.

Psoralen (P) and Long-Wave Ultraviolet Radiation (UVA) *See* PUVA.

Psychological Indices of Toxicity

Bernard Weiss

The Emergence of Psychological Measures

Psychology is the science that strives to understand, measure, and modify our behavior: what we do, what we say, what we think, and what we feel. At its most basic level, behavior describes how we manipulate and respond to our environment. It is the ultimate output of the nervous system. Its domain ranges across the entire universe of human activities from simple reflexes to the creation of cosmological theories. Toxicology, however, at least in the formal sense, recognized the crucial role of behavioral neuroscience only recently. Perhaps behavior seemed somewhat exotic compared to the study and traditional endpoints of death and tissue damage. However, step back from the brink that these endpoints represent and toxicology swiftly becomes a more complex and subtle enterprise.

Behavior began to insinuate itself into toxicology in the late 1960s and early 1970s. It was not a total novice, though. It came with an impressive technology molded by the discipline of behavioral pharmacology, which had begun to emerge in the 1950s with the discovery of the tranquilizing drugs. These drugs, offering the prospect of chemotherapy for psychological disorders, needed a scientific support structure. Behavioral pharmacology provided the consummate scientific basis for appraising and discovering drugs designed to alter behavior. Neurochemistry blossomed at the same time, but only the patient's behavior, measured either in a clinical or in a laboratory setting, could be the arbiter of a successful search. The same technology transferred effortlessly to the study and measurement of adverse behavioral effects, the theme of the discipline of behavioral toxicology.

Acceptance of the notion that behavioral measures could yield evidence of toxicity also benefited from the insistence of Soviet scientists that central nervous system (CNS) function and behavior offered more sensitive and appropriate measures of toxicity than the criteria prevailing in the West. Because of its own scientific history, especially the influence of Pavlov, and its political doctrines, Soviet toxicology elevated the CNS to a dominant role. Soviet scientists maintained that their exposure standards, generally much lower than those prevailing in the West, derived from their reliance on indices of CNS function rather than detectable tissue damage. Although some of these claims proved scientifically equivocal, perhaps because they needed to comply with political doctrines, they aroused the interest and attention of Western scientists who then began to apply and develop a more sound behavioral technology.

Behavioral criteria had also been adopted by industrial hygienists to set exposure standards for inhaled materials. The short-term exposure limit prescribed by American Conference of Governmental Industrial Hygienists (ACGIH) singled out performance criteria, such as reduced work efficiency and impairment of self-rescue, as indications of excessive exposure. The courts also played a role. They had begun to accept complaints of defective psychological functioning as legitimate grounds for suits alleging excessive workplace exposure. Finally, the environmentalist movement, changing its focus from tangible pollution, such as filthy waterways and mass kills of wildlife, began to recognize the possibility of subtle functional effects arising from prolonged low-level exposure to environmental chemicals. Reductions in IQ scores stemming from lead exposure prompted the elimination of lead from gasoline. Proposed links between environmental chemicals and neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease further aroused the public's interest.

Data from both the laboratory and the field began to converge once behavior became a source of questions about adverse effects. Both information conduits offered evidence of widespread behavioral consequences of chemical exposure generally measurable only with the appropriate application of psychological assessment methods. Workplace surveys, for example, showed many more complaints of symptoms such as sleep disturbances, excitability, depression, irritability, restlessness, apathy, and nervousness in workers exposed to neurotoxicants than in unexposed workers. Animal experiments revealed deficits in critical functions such as learning, even in superficially healthy subjects. As the literature grew, the rationale for psychological testing became more

solidly entrenched. No other approach seemed comparable in unmasking changes that typically would go undetected, nor did other indicators seem as responsive to early health effects.

Occupational Sources of Adverse Psychological Responses

Much of what we have learned about the detrimental psychological effects of exposure to chemicals has come from the workplace. One reason is that greater hazards were tolerated in the workplace than in the communal environment. Another is that research protocols could be more specific about the chemicals because they could be identified with designated industrial processes.

Metals

Heavy metals are acknowledged inducers of behavioral toxicity. Lead, mercury, and manganese, especially, are associated with unique syndromes. Other metals, such as aluminum, selenium, thallium, and tin, have also been implicated in adverse behavioral effects. **Table 1** lists some of the symptoms ascribed to metal toxicity. They range in severity from subjective complaints, such as fatigue and depression, to clear neurological deficits such as tremor. Manganese is especially intriguing. Most identified victims of manganese poisoning have been miners exposed by breathing dust containing the ore. The earliest indications of toxicity typically consist of psychological signs such as extreme emotional lability marked by abnormal laughter and crying. In the South American mining communities where manganese intoxication is endemic, the syndrome is known as 'locura manganica', or manganese madness. Later, more direct neurological signs begin to loom. Some of these, such as abnormal gait and slowness of movement, are reminiscent of Parkinson's disease.

Other sources of manganese exposure, such as ferromanganese processing and ore-crushing plants, expose workers to much lower levels of inhaled manganese. Even in these workers, who give no indication of clinical deficits when examined by neurologists, psychological tests reveal an elevated incidence of fatigue, tinnitus, finger tremor, and increased irritability. Behavioral testing of animal subjects also unmasks subtle deficits at exposure levels too low to induce overt neurological signs. In monkeys trained to pull against a weight with a rowing motion, manganese treatment elicits long pauses between responses such as those that might be expected under conditions of fatigue. Such findings make many scientists wary of proposals to introduce manganese compounds as additives for gasoline. They fear that dispersal of manganese into the environment may create the same intractable health problems that followed the introduction of leaded gasoline.

Table 1 Symptoms ascribed to metal toxicity

Ansomia	Incoordination
Appetite loss	Irritability
Depression	Paresthesias
Disorientation	Polyneuritis
Dizziness	Somnolence
Fatigue	Tremor
Headache	Visual disturbances
Insomnia	Weakness

Metallic (elemental) mercury, the mercury found in thermometers, also generates a constellation of both behavioral and neurological signs. Mercury is extremely volatile so that it enters the body through inhalation. Mercury vapor readily passes from the lung into the blood and then penetrates into the brain, where, at high enough levels, it produces neurotoxicity. Such effects have been recognized for centuries. Bernardo Ramazzini, often called the father of occupational hygiene because of his celebrated work published in the eighteenth century, was keenly aware of mercury poisoning and its manifestations. His descriptions of the sufferings of Venetian mirror gilders and of workers in other occupations who came in contact with mercury are exquisitely detailed and vivid. High exposures to mercury were also experienced by mercury miners; even today, in the famed mercury mines of Almaden in Spain, miners work only a few hours each week to preclude mercury poisoning.

The cardinal neurological marker of mercury vapor intoxication is tremor. Workers in the hat industry frequently suffered from mercury poisoning. Mercury compounds helped convert the stiff, straight animal fur into a limp, flexible mat that could be shaped into a hat. Vapor escaped during the process, and, inhaled day after day by the workers, sometimes evoked tremor so severe that some found it difficult even to walk without support. A survey of hat factories conducted by the US Public Health Service in 1940 found a clear relationship between workplace mercury levels and the severity and incidence of tremor. Eventually, the mercury compounds were replaced.

However, even in persons exposed to much lower ambient levels, abnormalities in the frequency components of the tremor can be detected with appropriate instrumentation and mathematical analysis. A technique introduced in 1973 involved having the worker insert a finger into a slot connected to a transducer that converted tremor into an electrical signal. The worker was instructed to maintain a

pressure within limits signaled by a pair of lights. The tremor signal, fed directly into a digital computer, was then broken down into its frequency components. Unlike normal tremor, the mercury-induced tremor showed two peaks, at different frequencies, rather than one. This study of women exposed to mercury vapor in the course of calibrating pipettes was able to trace their recovery, after removal from the factory, by recording and analyzing the amplitude and frequency of the tremor. Another application of tremor measures, designed to guard against excessive exposure, was adopted in a study of chlor-alkali workers. Chlor-alkali processing plants convert brine into chlorine and caustic soda by electrolysis. Huge pools of elemental mercury serve as the electrodes in this process. Even with devices to restrict the emission of mercury vapor, enough may escape to induce incipient toxicity. In this application, the investigators monitored tremor by using these advanced signal processing techniques; one indication of excessive exposure was the appearance of two peaks in the frequency distribution of the tremor rather than a single peak, and this served as a criterion for transferring the worker to another part of the plant.

Tremor is often accompanied by a group of symptoms termed 'erethism', a term derived from the Greek root for irritation or redness. The symptoms include hyperirritability, labile temperament, timidity and shyness, blushing easily, depression, insomnia, and fatigue. One description of erethism, from a report on women in a factory in which mercury was released in the production of motor components, is virtually a classic example. The worker complained of dizzy spells, weakness, fatigue, forgetfulness, grouchiness, and 'a fluttery feeling like I was scared or floating in space'.

Because the Mad Hatter in *Alice in Wonderland* exhibited some of the symptoms of erethism, he is sometimes held to be a model for the afflictions suffered by workers in the hat industry; whether Lewis Carrol intended such a parallel is still disputed, but the resemblances are uncanny. Even in the absence of identifiable symptoms, psychological testing has revealed what could be called nascent erethism in workers exposed to mercury vapor but showing no overt signs of toxicity. Tests of coordination and reaction time reveal differences between exposed and unexposed workers. Performance on elements of adult intelligence tests, such as the ability to repeat strings of digits, also shows differences.

A source of mercury other than the workplace has now assumed ascendancy in driving public unease. Recent publicity has indicted mercury amalgam dental fillings as a source of adverse health effects. Although it is true that chewing can release mercury vapor from amalgam fillings, the quantities are typically too small to produce elevations in blood or urine mercury levels that are hazardous to adults. Risks posed to the fetus and children are of greater concern but are characterized by a paucity of data.

Perhaps no other metal has aroused as much public discussion and attention as lead. It was one of the earliest metals exploited for practical uses; the word plumbing comes from the Latin word for lead. The Romans constructed cisterns and cooking utensils from lead. Lead pigments are found in glazes, even those that are used to decorate pottery; acidic foods leach the lead from the glaze. Lead permeates our current environment because of its presence in paint and in industrial products such as storage batteries and because of the lead added to gasoline.

Ancient physicians, such as Galen, were aware of lead's toxicity. Alice Hamilton, who founded the specialty of occupational medicine in the United States, termed lead the oldest of the industrial poisons except for carbon monoxide. In England, regulations designed to protect workers against the consequences of clinical lead poisoning, such as convulsions and coma, were prescribed in the nineteenth century. However, incipient lead poisoning remained a more formidable problem because the classical signs are absent. Instead, the symptoms tend to be vague; the workers may act sluggish, achy, and fatigued and be prone to errors on the job. Recent assessments of lead workers, undertaken with psychological tests, show adverse effects even in the absence of clinically overt problems. These take the form of reduced scores on tests of memory, vigilance, spatial relations, and coordination. Standardized inventories of affect and personality also show disturbances in exposed workers. Lead can also impair hearing; rises in the concentration of lead in blood, a marker of recent lead exposure, elevate hearing thresholds. The most destructive of lead's neurotoxic effects, however, is interference with brain development. This aspect will be addressed later.

Pesticides

Pesticides are another class of chemicals capable of damaging the nervous system and, even at low levels, produce deficits detectable by psychological testing. The organophosphorus insecticides, which are chemical relatives of the most potent nerve gases, are notorious poisons and, carelessly handled, as often happens in underdeveloped countries, can prove lethal. Parathion, diazinon, and malathion are representatives of this class and are widely used in the United States. Acute poisoning episodes produce signs such as eye irritation, headache, dizziness, nausea, and visual

disturbances. These gross effects fade with time, usually in days or weeks. However, when farmworkers who had undergone an episode of acute poisoning were evaluated with psychological tests 1 year later, they showed persisting sequelae. Compared to controls, who were matched on age and education, they displayed lower scores on a widely used adult intelligence scale and on a test of coordination and higher scores on a battery of psychological tests designed to measure incipient neurological impairment. These results are consistent with those from similar studies and with experiments in monkeys showing enduring effects on the electrical activity of the brain.

Other studies demonstrate that effects of even rather modest exposures can be detected with psychological tests. In one example, even though they gave no indication of overt poisoning and seemed superficially healthy, farmworkers who had been exposed to organophosphorus insecticides displayed evidence of psychological disturbances on a test constructed to measure anxiety. They selected items indicating that they experienced more tension, more restlessness, more emotional instability, more nervousness, and more fitful sleep than a sample of unexposed workers.

The organochlorine insecticides such as DDT are also potent nervous system poisons. Like the organophosphorus compounds, they interfere with the nervous systems of insects, so it is no surprise that they exert similar effects in humans. At high doses, they cause convulsions. An epidemic of convulsions in an English town, in fact, was traced to flour inadvertently contaminated with an organochlorine insecticide. At lower doses, the effects are more subtle. One organochlorine insecticide, chlordecone, was responsible for an outbreak of poisoning in a Virginia factory. After workers began to complain of health problems to local physicians, public health officials began an investigation and confirmed that poor hygiene in the plant had exposed the employees to excessive amounts of the chemical. The most intriguing facet of this episode is that the earliest index of toxicity turned out to be complaints of excessive nervousness. Would even an alert plant physician be likely to consider chemical exposure as the source of such complaints? Would not a more likely diagnosis be personal problems either at home or at the place of employment? Episodes such as these have led some observers to recommend that workers in comparable environments undergo periodic psychological assessments to detect adverse effects.

Solvents

Among the chemicals evoking the most attention from psychologists are the volatile organic solvents. Carbon disulfide, toluene, xylene, styrene, trichloroethylene, and methylene chloride are representative members of this class. They may have evoked such attention because they are demonstrably neurotoxic. Due to their volatility, they are inhaled. ACGIH exposure standards for solvents are based on this property. Because they are soluble in fatty tissues, they easily reach the brain. At high ambient levels, they produce narcosis; in fact, some have been used as surgical anesthetics. The question posed to investigators is whether low concentrations, even those meeting current workplace exposure standards, produce adverse effects.

Scandinavian investigators pioneered studies of chronically exposed workers. On the basis of their research, they posited what has been called the organic solvent syndrome, toxic encephalopathy, or painter's syndrome (because painters often work in an environment suffused with solvents). They asserted that chronic exposure in the workplace to volatile organic solvents produced permanent deficits reflected by diminished performance on psychological tests. They pointed to lower scores on tests of intelligence, memory, learning, and other cognitive functions and to elevated reaction times and personality changes. These claims were vigorously debated, with critics arguing that necessary controls, such as matching exposed and unexposed workers for education and drinking habits, were lacking. They also argued that the generous worker compensation regulations of some Scandinavian countries encouraged claims of solvent-induced impairment. Later investigations, with more rigorous controls, and in other countries, supported the original claims and have even expanded them to include an impaired sense of smell and deficits in color vision.

Solvents are ubiquitous in the workplace and are produced in the millions of kilograms annually. They also appear in many household products such as cleaners, glues, and paint thinners. Because so many workers are exposed to solvents, and because their use is so common in other settings, the US Environmental Protection Agency (EPA) proposed that solvent manufacturers undertake a comprehensive evaluation of ten solvents with high production volumes. They specified four components in the evaluation: functional observation battery, motor activity, neuropathology, and schedule-controlled operant behavior.

In the functional observation battery, rats or mice are exposed acutely and subchronically to a solvent. Technicians then make a number of systematic observations such as the response to prodding, orienting to a click, resistance to pulling, and other simple responses. A numerical score, based on the individual components, is then calculated.

In evaluating motor activity, rats or mice are exposed acutely or subchronically and tested in a device that measures amount of movement. For example, the rat may be placed in a figure-eight maze equipped with photocells at the intersections. Motor activity is scored by the number of photobeams interrupted. Although motor activity can be influenced by many factors, it is especially responsive to chemicals acting on the CNS.

Neuropathology involves sacrificing rats or mice after subchronic exposure and inspecting for lesions in the brain.

Schedule-controlled operant behavior has come to play a prominent role in behavioral toxicology because it provides a supple, flexible scheme for assessing the capacity for complex behavior. The US EPA explained its choice of schedule-controlled operant behavior by focusing on its versatility.

Solvents may have neurotoxic effects on memory, learning, and performance which can be permanent. These effects are less well understood…. The schedule-controlled operant behavior test has typically been required as a second tier test…it is proposed as a first-tier test…because of EPA's desire to obtain data on the effects of solvents on learning, memory, and performance.

The origins of this proposal from the US EPA lie in the demonstrated efficacy of performance tests as measures of psychophysiological function and in their sensitivity to the effects of chemical exposures in the workplace. These tests come from two sources. One originated in the need to provide diagnostic guidance for psychologists evaluating clients or for personnel selection. Test design and construction comprise one of psychology's major specialties; its methods have evolved over at least eight decades. Psychometric techniques provide the basis for selection tools such as the Scholastic Achievement Test. The second was the experimental psychology laboratory, the site of fundamental research on all aspects of human performance including sensory function, motor function, and cognitive functions such as memory. Contributions from these two sources always overlapped and influenced one another, but they converged especially effectively to meet the growing interest in the measurement of performance stirred by evidence that such measures could uncover toxic effects that otherwise would remain concealed.

The demonstrated sensitivity of psychological test methods to solvent exposure led the World Health Organization (WHO) to call upon experts for the design of a test battery that could be applied even in underdeveloped countries lacking such a tradition. The basic WHO battery is shown in **Table 2**. More comprehensive batteries tend to be used in the advanced industrial countries, where psychologists have tried to exploit the potential of digital computers for test design, presentation, and analysis.

Table 2 WHO neurobehavioral core test battery

Functional domain	*Core test*
Motor speed	Aiming; dot placing
Attention	Simple reaction time
Perceptual-motor	WAIS digit-symbol
Manual dexterity	Santa Ana test
Visual memory	Benton test
Auditory memory	WAIS digit span

Psychological Measures of Impaired Development

Hardly any facet of psychology claims as much attention in toxicology as brain development. Teratology describes the discipline whose dominion is the study of congenital deformities, or birth defects. By analogy, some investigators appropriated the term to label a new area they called behavioral teratology. The label became accepted practice because it graphically described what these scientists viewed as a vital but previously neglected aspect of toxicology: the functional consequences, later in life, of exposure to neurotoxic agents during gestation or infancy. Although such functional consequences might include defects as severe as mental deficiency, most of the research in this area has taken the form of questions about less blatant outcomes. Learning disabilities, conduct disorders, slower than normal language acquisition, delayed motor development, and downward shifts in the distribution of IQ scores are among the outcomes reported in the scientific literature.

In response to overwhelming public anxieties, regulatory agencies in Japan and the United Kingdom began to insist on behavioral teratology information for new drugs. The US EPA has also been active in setting guidelines for developmental toxicity that also embrace potential behavioral effects. These guidelines prescribe a range of behavioral testing protocols ranging from simple locomotor activity to tests designed to measure learning and memory. The impetus for such protocols comes from the recognized vulnerability of the developing brain to neurotoxic chemicals. The fetal alcohol syndrome is one striking example. Three agents in particular have aroused the interest of toxicologists: lead, methylmercury, and the polychlorinated biphenyls.

Lead

Severe lead poisoning in children is now a much more infrequent event in the United States than even

in the recent past. The current focus of attention is the impact of much lower exposure levels on how well children function. Twenty-five years ago, a blood lead concentration below 40 $\mu g\,dl^{-1}$ was considered acceptable. By 1991, the Centers for Disease Control (CDC), weighing all the accumulated evidence, had concluded that levels exceeding 10 $\mu g\,dl^{-1}$ gave cause for concern. The primary motive for this change stemmed from depressed scores on intelligence tests.

Attempts to construct a metric of intelligence have occupied the energies of many psychologists from the middle of the nineteenth century to the present. Definitions of intelligence continue to elicit intense debate. Intelligence testing of children, however, beyond doctrinal disagreements, has come to rest on a forthright principle: measure the child against his or her peers. Intelligence tests vary widely in the items they choose for such comparisons but typically include an assessment of vocabulary, the ability to count and calculate, the ability to discern relationships among objects, and other markers of how well the child has mastered his or her environment. The components of a leading test, the Wechsler Intelligence Scale for Children-Revised, are listed in **Table 3**. As in all psychological tests, items basically represent stimuli for the elicitation of behavior samples. They are not absolute measures of some fundamental property.

From items such as those contained in the component subtests listed in **Table 3**, a test score, equivalent to a test age, is derived. The IQ is computed as the quotient of the test age, based on the performance of a standardized population of children, divided by the child's chronological age. A child who is average for his or her age will yield an IQ of 100. An above-average child will obtain an IQ above 100. There is some dispute about the interpretation of an IQ based on a standardized population significantly different in ethnic background and socioeconomic status from the child being tested; so that exposure conditions and the child's other environmental circumstances should not be confounded.

Table 3 Components of the Wechsler intelligence scale for children (revised)

Verbal IQ	*Performance IQ*
Information	Picture completion
Vocabulary	Picture arrangement
Digit span	Block design
Arithmetic	Object assembly
Comprehension	Coding
Similarities	Mazes

IQ scores began their ascendancy in assessing the risks of childhood lead exposure as long ago as the 1950s, but poorly focused investigations, inadequate measures of exposure, and the then unrecognized scope of lead toxicity yielded little more than a stream of ambiguous studies. A pioneering report in 1979 by Herbert Needleman and colleagues marked the first of many well-designed studies showing significant IQ reductions in young children ascribable to quite modest increments of lead exposure. It adopted the then novel strategy of estimating cumulative exposure to lead by relying on baby teeth, which, like bone, store lead. The findings were so compelling that they stimulated additional investigations in many parts of the world that built further support for the lead and IQ relationship.

Subsequent investigations adopted an even more forceful strategy; they undertook prospective studies in which children with documented prenatal lead exposures were followed from birth. These studies demonstrated that even lead levels so low that they would have been considered insignificant just a few years earlier could reduce scores on IQ and analogous developmental tests.

Some critics charge that such findings possess little practical significance. They argue that a difference of a few IQ points exercises negligible influence on how well a child functions. However, such an argument neglects the implications for the population as a whole. Because of the way in which IQ scores are distributed, in a population of 100 million in which the mean IQ is 100, 2.3 million individuals will score above 130, the superior range. If the mean is shifted downward by five IQ points (5%), which the critics deem insignificant, the mean IQ becomes 95 and only 990 000 individuals will score above 130. Most observers would contend that such an impact on a society cannot be considered negligible. This perspective, gained from the results of psychological tests, made a key contribution to the CDC decision to designate a lead level of 10 $\mu g\,dl^{-1}$ in blood as a level of concern.

Methylmercury

About 26 states now disseminate fish advisories for lakes and rivers based on methylmercury contamination. Methylmercury is an organic form of mercury and a potent nervous system poison. It is especially destructive to the developing brain. Although recognized as a poison for over 100 years, its impact on the fetal brain came to attention only in the 1950s, when the population of a small Japanese fishing village, Minamata, experienced widespread methylmercury poisoning. Fish and shellfish from

Minamata bay had been contaminated by effluent from a factory that used mercury as a catalyst in the production of acetaldehyde. Many inhabitants died. Even more suffered permanent neurological damage. In addition, a much higher incidence of retarded brain development was observed in Minamata than elsewhere in Japan but the population was too small to yield a cogent answer.

The final evidence came in the form of an outbreak of methylmercury poisoning in Iraq. Because grain crops had been decimated by a severe drought in 1971, the Iraqi government ordered over 80 000 tons of seed grain from Mexico and the United States. The order specified that the grain be treated with a methylmercury fungicide, which ordinarily would dissipate into the soil after planting. Despite warnings, many farming communities in the Iraqi countryside, facing food shortages, baked the treated grain into bread. The result was a mass poisoning episode, in the winter of 1971–72, that killed as many as 5000 people. It was the largest mass chemical disaster in history.

University of Rochester investigators, led by Dr. Thomas W. Clarkson, were called upon for assistance because of their research experience with mercury and with antidotes. They established a laboratory in Bagdhad and began a project to survey the countryside. One phenomenon struck them with singular force. Offspring of mothers who had consumed large amounts of the tainted bread displayed evidence of brain damage. Some seemed afflicted with cerebral palsy. Some were prone to seizures. Others were late in speech and motor development. Because Clarkson and colleagues had discovered that growing hair took up methylmercury from the blood, hair became the ultimate measure of exposure. Because scalp hair grows ~ 1 cm (~ 0.5 in.) per month, a 12 cm length of hair had engraved on it a year's history of methylmercury blood levels, which closely reflect consumption.

With this tool, the investigators were able to establish a relationship between maternal methylmercury exposure and indices of child development. Statistical analyses of the correlation between maternal hair levels and delayed walking, for example, suggested that even slightly elevated methylmercury consumption by a pregnant woman might pose a risk for fetal brain development.

The primary repository of methylmercury in the diet is fish. Natural sources of inorganic mercury, such as volcanoes, and human contributions from fossil fuel and waste combustion contribute to a global mercury cycle that deposits the mercury in waterways. Microorganisms in the bottom sediment convert the inorganic form into methylmercury, which ascends the food chain and concentrates in the predators at the apex of the food chain. Swordfish, shark, pike, snapper, and tuna are among these predatory species.

In New Zealand, comparisons among children, whose mothers consumed different amount of fish during gestation, indicated that higher consumption levels tended to depress scores on IQ and other psychological tests. Because a single study could not be definitive, other studies have been undertaken. Their results have now begun to appear and show little evidence of adverse outcomes. Studies of this kind must occupy several years, however, because some consequences of developmental damage, such as performance on certain components of IQ tests, cannot be assayed until the child is advanced enough to be tested. In the meantime, regulatory authorities have adopted a position of caution and advised against the consumption of certain species of fish from particular sites by pregnant women, and young children.

The concerns aroused by methylmercury in fish, arising from the susceptibility of the developing brain to this neurotoxicant, led to the design and execution of two large prospective studies. One was located in the Seychelle islands, which lie in the Indian Ocean. The other was located in the Faroe Islands, which lie in the North Sea. Both communities consume large quantities of seafood. In the Seychelles, it is almost exclusively in the form of fish. In the Faroes, virtually all the methylmercury comes from the consumption of pilot whales, which are also contaminated with polychlorinated biphenyls (PCBs). Both studies assayed maternal exposures to methylmercury. In the Seychelles, maternal hair was used as the index; it reflects the history of blood levels. The Faroes study relied primarily on cord blood.

Neurobehavioral testing of the Seychelles cohort of ~ 800 children has been carried out periodically from early development to 9 years of age. The Faroes cohort was assessed at 7 years of age with a variety of neurobehavioral tests, and supplemented by electrophysiological measures at 14 years of age. The Seychelles data show little indication of adverse effects attributable to prenatal methylmercury exposure. In contrast, the Faroes data point to subtle adverse effects. Because of such effects, the EPA has concluded that limiting methylmercury intake to $0.1\ \mu g\ kg^{-1}$ daily is necessary to provide an adequate margin of safety.

Polychlorinated Biphenyls

The PCBs are as ubiquitous in the environment as lead. They also share many properties in common with other organic halogen compounds such as the

organochlorine insecticides and dioxins. Their health risks until recently have been dominated by potential carcinogenicity. Newer data sources now suggest that their most serious risks may stem from actions on the developing brain. Two poisoning episodes, one in Japan and one in Taiwan, yielded the first clues. In both instances, cooking oil had been contaminated by PCBs, which enjoyed wide use as insulating material for transformers. They are dissolved in an oil base, so contamination cannot easily be detected. Children born to mothers who had consumed the contaminated oil, besides showing skin darkening and other signs of PCB toxicity, also suffered from mental retardation. Suspicions that problems might lurk in lower levels of exposure stimulated studies equipped to measure more accurately the correlation between maternal PCB exposure and offspring development. The resulting data indicate to many scientists that current levels of tissue PCBs are disturbingly close to levels that represent a hazard to optimal brain development.

One origin of this altered point of view is a series of studies based on correlations between maternal intake of PCB-contaminated fish during pregnancy and the performance of the offspring on psychological tests. The higher the maternal PCB level (measured in blood samples or fat biopsies), the lower the IQ score. IQ scores of children whose mothers consumed Lake Michigan fish suffered a 6% (6 point) decline at 11 years of age. Additional psychological tests confirmed this relationship. Normal infants shown two pictures, one of which is familiar and one of which is novel, will tend to spend more time gazing at the novel picture. The degree of bias in the direction of novelty apparently correlates to a surprisingly degree with later IQ scores. Children later shown to be at risk for developmental retardation show little novelty bias. Maternal PCB levels are significant predictors of novelty bias; the higher the PCB level, the lower the degree of bias. These two psychological indices – IQ scores and visual recognition memory – established the PCBs, even at levels that produce no obvious indications of toxicity, as hazards to brain and behavioral development. The implications of these findings are disturbing because so many women maintain body burdens of PCBs uncomfortably close to the levels associated with lowered scores on psychological tests of developmental outcome. They are also disturbing because PCBs represent a class of chemicals, including the dioxins and DDT, that have been labeled endocrine disruptors and that have the potential to interfere with sexual differentiation of the brain, with immune system function, and with thyroid development.

Food Additives

An instructive instance of the changed perspectives that psychological measures may impose on toxicity evaluation and risk assessment emerged from claims that some foods and food additives might elicit behavioral disturbances in children. The claims were formulated by Dr. Ben Feingold, a pioneering pediatric allergist in the Kaiser-Permanente system in California. Feingold asserted that some of the children labeled as hyperactive, or suffering from what is currently called attention deficit disorder, actually were exhibiting adverse responses to certain dietary constituents. Among the additives, he singled out synthetic colors and flavors for elimination from diets because, in addition to reports in the allergy literature linking them to adverse reactions, they lacked nutritional value in any case.

The US Food and Drug Administration (FDA) does not require testing of food additives for neurobehavioral toxicity and Feingold's claims were based on clinical experience rather than on controlled clinical trials. His claims, however, generated sharp public interest, particularly on the part of agonizing parents, and provoked a series of clinical trials designed to test his hypothesis. Although the investigators adopted a wide variety of approaches, and most focused on food dyes for experimental convenience, the total published literature converges to the conclusion that, in principle, Feingold's claims were valid. The major disagreements stem from the estimated proportion of children at risk, which range from $\sim$1% to 25%, and the scope of the dietary components evoking behavioral disturbances. Another source of disagreement arises from how risks are perceived. Some critics argue that a 1% prevalence of adverse responses to food dyes, for example, is not a reason to eliminate them from the food supply. At the same time, regulatory agencies such as the US EPA strive to establish exposure levels to ensure cancer risks below one per million persons. An incidence of 1% is hardly trivial.

One of the experiments indicating the potential of food dyes to induce adverse behavioral responses was conducted by the author and coinvestigators with a sample of young California children. These children had been designated by their parents as responders. That is, their behavior had been seen to improve on a diet that eliminated food additives and some other foods. The parents enrolled in a study in which they provided daily behavioral observations of their child's response to a soft drink containing either a blend of food dyes or innocuous colorings such as caramel. The two drinks were not distinguishable. During the 11 week experimental period, the daily

drink contained the blend of dyes on eight randomly assigned occasions.

Of the 22 enrolled children, two showed consistent responses to the blend of dyes. One of the children, a 34-month-old girl, gave highly elevated scores, after drinking the challenge drink, on the following items of a ten-item list: short attention span, acts as if driven by motor, runs away, throws and breaks things, and whines. She also showed elevated scores on a standardized rating scale of attention deficit disorder. **Table 4** shows the difference between the amount of US FDA-approved food dyes evoking behavioral disturbances in sensitive children and the acceptable daily intakes based on the standard 2 years feeding study in rodents (a study required by the US FDA). The differences between conventional assays, largely based on pathology, and those based on psychological measures are about 50–60-fold.

Psychological Measures of Enhanced Chemical Sensitivity

A new array of problems closely entwined with environmental toxicology is attached to labels such as sick building syndrome, multiple chemical sensitivity, chronic fatigue syndrome, and Gulf War syndrome. They have spawned a sizable literature and gripped public interest and anxieties. For all three labels, the primary clinical manifestations consist primarily of subjective complaints; conventional medical indices are lacking. Especially for multiple chemical sensitivity and sick building syndrome, the instigators are held to be toxic chemicals, but in most instances the offending agents lack clear identification.

Patients allegedly suffering from multiple chemical sensitivities complain of depression, excessive fatigue, sleep disorders, irritability, headaches, and symptoms, such as rhinitis, similar to those associated with allergies. Although immune system disorders are hypothesized as the most frequent underlying cause, compelling evidence in support of such a mechanism is lacking. Another puzzle is the emergence of such symptoms in response to chemical agents of widely divergent classes and, typically, at rather low concentrations. The vague, malleable contours of the syndrome and the absence of an identifiable etiology have engendered a countervailing skepticism about its validity on the part of many clinicians and scientists. The absence of sound investigational protocols and experiments, except for a sparse, scattered literature, has nourished such skepticism.

Table 4 Food dyes: doses (mg day^{-1}) eliciting behavioral responses vs. FDA acceptable daily intake (ADI)

Color	*Behavior*[a]	*ADI*
Yellow 5	9.07	300
Yellow 6	10.70	300
Red 40	13.80	420
Red 3	0.57	150
Blue 1	0.80	200
Blue 2	0.15	37
Green 3	0.11	150

[a] Modified from data in Weiss B, Williams JH, Margen S, *et al.* (1980) Behavioral response to artificial food colors. *Science* 207: 1487–1489.

The sick building syndrome is another victim of sparse empirical support. The contemporary emphasis on energy efficiency has produced buildings notable for poor air quality manifested as inadequate ventilation coupled with contaminating agents ranging from infectious microorganisms to common allergens to volatile organic chemicals. Outbreaks of illness, attributed to such environmental conditions, have made their way into the biomedical literature and the popular media.

Like multiple chemical sensitivity patients, those asserted to be afflicted with the sick building syndrome exhibit a collection of largely subjective complaints; headaches, fatigue, and lightheadedness are among symptoms. They are accompanied by complaints QP persistent cough, chest tightness, wheezing, and eye and throat irritation. Although better documented than the multiple chemical sensitivity syndromes because the complaints often can be traced to a specific site, it too has often aroused suspicions of its validity. Some critics contend that most reports of widespread illness in particular buildings are more likely instances of suggestibility than of authentic illness. Others, citing unsuccessful attempts to relate variations in air quality to the quantity of complaints, also tend to belittle the syndrome as a disease entity.

US EPA scientists are among the groups that have sought to view these two syndromes from an experimental perspective. The agency's unfortunate experience with its own building renovation program, which left a residue of sick building complaints, gave this effort a substantial impetus. They exposed healthy subjects to a mixture containing 22 volatile organic chemicals commonly detected in new or newly renovated buildings and asked the subjects to rate the intensity of various responses. During the 2.75 h exposure periods, perceived odor intensity diminished and air quality ratings improved. Ratings of eye and throat irritation, headache, and dizziness either increased or remained stable. Such results indicate that odors alone, as suggested by some observers, do not trigger the symptoms of multiple chemical sensitivity or sick building syndrome.

Concurrent assessments of neurobehavioral function found that subjects reported increased fatigue and confusion. At the same time, their performance on successive administrations of a battery of 13 psychological tests remained unimpaired, a finding the authors speculate may have been influenced by the tendency to improve with practice.

Chronic fatigue syndrome, although its reality is also debated, is more firmly established as a valid entity than the other two syndromes. The label is attached to patients who suffer prolonged feelings of fatigue, weakness, and even exhaustion. They report inability to concentrate, memory loss, depression, sleep disorders, and a variety of symptoms reminiscent of influenza. The prevailing view among those who accept the syndrome is that it reflects an immune system disorder, perhaps triggered by a viral infection, but its character and etiology remain equivocal.

When psychological testing of such patients has been undertaken, some are revealed to suffer reliable cognitive deficits. For example, they exhibit slowed reaction times, reduced accuracy in searching for target letters on a page of typed letters, impaired recall of a narrative, and lowered scores on various tests of memory. Such results are intriguing and perhaps significant. They are supported by data indicating that psychological test performance is impaired by experimentally induced viral or bacterial infections and that recovery of performance, despite recovery by clinical measures, may require a prolonged period. Another clue comes from animal experiments. In response to infections, the immune system releases substances called cytokines. Interleukins are members of this class. Interleukins also exert profound effects on behavior, and some scientists speculate that the nonspecific symptoms of infection, similar to the complaints vented by chronic fatigue syndrome patients, originate from the action of these and other cytokines.

Although these three syndromes are not intrinsically linked to the traditional domain of toxicology, they illustrate the role that psychological measures are increasingly assuming when adverse effects of environmental chemicals emerge as an issue. Clinical medicine prefers to deal with specific signs pointing to specific diseases. Environmental toxicants, however, far more often now than in the past, are being indicted as the sources responsible for diffuse aberrations of function such as conduct disorders, learning disabilities, memory and concentration difficulties, feelings of listlessness, fatigue, depression, and a galaxy of other disturbances beyond the catalog of accepted medical diagnoses. Psychological test methods, developed over a period of many decades, provide the tools for making the appropriate connections.

See also: Behavioral Toxicology; Food Additives; Lead; Mercury; Metals; Methylmercury; Pesticides; Polychlorinated Biphenyls (PCBs); Sick Building Syndrome.

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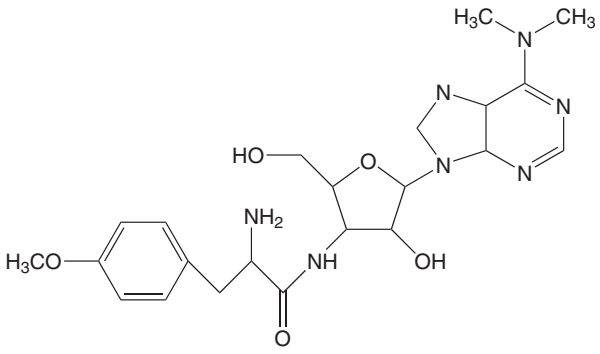
Puromycin

Midhun C Korrapati and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 53-79-2
- Synonyms: (*S*)-3′-((2-Amino-3-(4-methoxyphenyl)-1-oxopropyl)amino)-3′-deoxy-*N*,*N*-dimethyladenosine; Achromycin; Stillomycin
- Chemical/Pharmaceutical/Other Class: It is an antineoplastic and antiprotozoal (Trypanosoma) and used in research as a protein synthesis inhibitor

- Chemical Formula: $C_{22}H_{29}N_7O_5$
- Chemical Structure:



Uses

Puromycin has been widely used as a basic tool in research for studying protein synthesis. It is an antibiotic used by scientists in bioresearch to select cells modified by genetic engineering. It inhibits protein synthesis by binding to RNA. It is also an antineoplastic and antitrypanosomal agent.

Background Information

Puromycin is an aminonucleoside antibiotic produced by *Streptomyces alboniger*. It specifically inhibits peptidyl transfer on both prokaryotic and eukaryotic ribosomes. The antibiotic inhibits the growth of Gram positive bacteria and various animal and insect cells. Fungi and Gram negative bacteria are resistant due to the low permeability of the antibiotic. For more than 30 years, puromycin has been widely used as a basic tool for studying protein synthesis. Now, puromycin hydrochloride is particularly useful for the selection of cell types harboring plasmids carrying puromycin resistance genes.

Exposure Routes and Pathways

Most common exposure pathways to puromycin are via absorption through skin, inhalation, or by oral route when swallowed accidentally.

Toxicokinetics

Limited information indicates that puromycin is rapidly absorbed and distributed to the liver and is primarily excreted via the kidneys.

Mechanism of Toxicity

Puromycin is a specific metabolic inhibitor of protein synthesis and acts as an aminoacyl-tRNA analog and peptidyl acceptor. The latter causes premature chain termination of the protein and the release of nascent or growing polypeptide chains. In liver it has been shown to cause fat accumulation without causing death of the hepatocytes. Puromycin causes focal glomerular sclerosis, alters the morphology, localization of anionic sites, and metabolism of renal epithelial cells. This injury is attributable to the production of reactive oxygen species.

Acute and Short-Term Toxicity (or Exposure)

Animal

Rabbit erythrocytes exposed to low concentrations of puromycin (7×10^{-4} mol l^{-1}) caused disruption of cell membrane indicating inhibition of protein synthesis by erythrocytes. Puromycin is shown to cause nephrosis in rats. It is a glomerular nephrotoxicant and is extensively used to study pathophysiology of glomerular nephritis and nephrotic syndrome.

Human

Puromycin may cause irritation and reddening of eyes. Prolonged or repeated exposure may cause cataract and severe, permanent damage to the eyes. Puromycin causes rash, blistering, and allergic reactions if contacted with skin, and may cause nasal, gastrointestinal, and lung irritation. Exposure of erythrocytes to puromycin led to cell membrane disruption. It is a possible mutagen in humans.

Chronic Toxicity (or Exposure)

It is a possible mutagen in humans.

Clinical Management

If inhaled, the patient should be move to fresh air. If not breathing, artificial respiration should be given. If breathing is difficult, oxygen should be given. Vomiting should not be induced unless directed to do so by medical personnel. Unconscious persons should not be given anything by mouth. If large quantities of this material are swallowed, a physician should be called immediately. Tight clothing such as a collar, tie, belt or waistband should be loosened. In case of dermal exposure, the skin should be flushed with plenty of water. Contaminated clothing and shoes should be removed and clothing removed before reuse. Shoes should be cleaned thoroughly before reuse. Contact lenses should be removed. In case of contact, eyes should be flushed immediately with plenty of water for at least 15 min and medical attention sought. Repeated or prolonged exposure is not known to aggravate medical condition.

Further Reading

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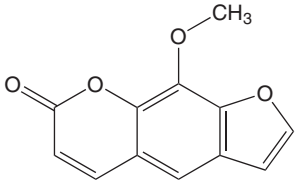
PUVA

Jean L Lim and Robert S Stern

- Chemical Abstracts Service Registry Number: CAS 298-81-7
- Synonyms: Psoralen and UVA; Photochemotherapy; Light therapy; 8-Methoxypsoralen (8-MOP); 5-Methoxypsoralen (5-MOP); 4,5′,8-Trimethylpsoralen (TMP)
- Chemical/Pharmaceutical/Other Class: Furocoumarin and UVA light
- Chemical Formula: $C_{12}H_8O_4$ (8-Methoxypsoralen)
- Chemical Structure: Psoralens are planar, tricyclic compounds, consisting of a furan ring fused to a coumarin moiety



Uses

PUVA is the acronym originally introduced to describe the combined administration of psoralen and subsequent exposure to high-intensity ultraviolet radiation from an artificial source (UVA). In the United States, orally administered 8-methoxypsoralen (8-MOP) is the psoralen most frequently used in combination with UVA light therapy and will be the focus of this discussion. However, the term PUVA also refers to therapy with other oral and topical psoralens, most commonly 4,5′,8-trimethylpsoralen (TMP), a synthetic psoralen, and 5-methoxypsoralen (5-MOP), not available in the United States.

PUVA is used most commonly in the treatment of psoriasis, vitiligo, and cutaneous T-cell lymphoma. However, up to 30 other skin diseases have been reported to be responsive to PUVA therapy. The most common of these include: palmarplantar pustulosis, polymorphous light eruption, dishydrotic eczema, atopic dermatitis, allergic contact dermatitis, actinic reticuloid, solar urticaria, pityriasis lichenoides, and graft versus host disease.

Background Information

Psoralen and psoralen derivatives are found in plants (*Psoralea corylifolia* and *Ammi majus*) and other vegetation such as limes, figs, parsnips, and certain fungi. Psoralen is a photosensitizing drug and was used with sunlight to treat skin diseases in Egypt and India as early as 1200–2000 BC. The ancient Egyptians and Indians applied plant extracts to the skin or ingested the extracts orally and then exposed themselves to sunlight to induce repigmentation in vitiligo. 8-Meythoxypsoralen, derived from *A. majus*, has been available in the United States since 1951 and was first combined with high intensity ultraviolet light from an artificial source in 1974. PUVA treatment involves the administration of psoralen (usually $0.6\,mg\,kg^{-1}$), followed by exposure to long-wave UV radiation (320–400 nm) ~1–2 h later. Clearing can usually be achieved in 8–12 weeks with two to three treatments per week. However, without maintenance therapy of up to once a week, disease will return.

Exposure Routes and Pathways

Therapeutic psoralen exposure occurs by the oral or topical route. UVA exposure occurs via epidermal contact. The patient stands upright in a vertical cylinder or similar large enclosure lined with fluorescent tubes to receive an exactly calculated UV radiation dose measured in joules per square centimeter. More than half of the energy emitted is of wavelengths in the range 340–370 nm.

Toxicokinetics

Depending on the patient, dose form, and ingestion of food, peak 8-MOP serum dose levels occur between 30 min and 4 h after ingestion, with a mean time of 90 min. Equilibrium between levels in the blood and in the skin is achieved in 1 h. Mean peak serum concentrations are $\sim 200\,\mu g\,l^{-1}$ (range: $0–500\,\mu g\,l^{-1}$) and is lower under postprandial in comparison to fasting conditions. At low doses (i.e., 40 mg or less), 8-MOP undergoes extensive first-pass elimination so that only a small amount of unchanged 8-MOP reaches the general circulation. At high doses, liver enzymes become saturated and serum concentrations

of 8-MOP increase quickly. Elimination half-life for 8-MOP ranges from 1.1 to 1.9 h and is not affected by the ingestion of food. Terminal half-life is 200 h. Psoralens are transformed to polar metabolites by hydroxylation and glucuronidation in the liver. In rats, radiolabeled 8-MOP has been recovered in high concentrations in the liver and kidney soon after oral and intravenous administration. In humans, 80% of orally administered 8-MOP is excreted in the urine within 8 h, and lesser amounts are found in the urine and feces over several days after ingestion.

Mechanism of Toxicity

The mechanisms of action and of toxicity of PUVA are not completely understood. Because of their planar structure and hydrophobicity, psoralens readily intercalate with nucleic acid basepairs when exposed to UVA light. Absorption of a single photon by psoralen results in the formation of a monofunctional adduct. A monofunctional adduct formed by the 4′5′-furan bond with the 5,6-bond of a pyrimidine can absorb a second photon leading to the formation of a bifunctional interstrand crosslink. Both monofunctional and bifunctional adducts are believed to inhibit DNA replication, which may be responsible for the antihyperproliferative effect of PUVA. In comparison to monofunctional adducts, bifunctional adducts are more strongly implicated in the toxic side effects of PUVA including irreversible damage to keratinocytes, resulting in apoptosis and cell necrosis leading to sunburn-like skin damage and blistering. Mutations arising from photoadducts in sensitive DNA sequences that encode for tumor suppressor genes may lead to the development of skin tumors. PUVA therapy has also been shown to have a suppressive effect on T cells, leading to inhibition of the delayed hypersensitivity response and decreased release of proinflammatory cytokines. This response is therapeutic for the treatment of various inflammatory and lymphoproliferative skin diseases, but the resulting immunosuppression may also explain the reported increased incidence of herpes simplex among PUVA-treated patients and may contribute to risk of squamous cell cancer.

Acute and Short-Term Toxicity (or Exposure)

Animal

In 1 day, 16 day, and 13 week studies, 8-MOP administered in high doses to rats can result in mortality, decreased body weight gain, dose-related increases in liver to body weight ratios, and fatty changes in the liver, and in male rats, atrophy of the testis, seminal vesicles, and prostate.

Human

The most common short-term side effects of PUVA are pruritus and transient nausea. Up to 25% of patients experience pruritus, which is UV dose-related and is associated with dryness of the skin. Usually, the pruritus responds well to emollients and antihistamines. Transient nausea affects $\sim$12% of patients taking 8-MOP and can be minimized by taking the medication with food or using antiemetics. PUVA pain is a rare, intermittent, severe burning pain that occurs 4–8 weeks after the onset of PUVA therapy. Because the pain worsens with ongoing therapy, PUVA must be discontinued and the pain usually resolves spontaneously in a few weeks. Other reported adverse effects include erythema and burning, maculopapular rash, exacerbation of photodermatoses, increased incidence of herpes simplex, and hepatotoxicity.

Chronic Toxicity (or Exposure)

Animal

Chronic 8-MOP administration of up to 75 mg kg^{-1} for 2 years increases the incidence of renal tubular cell hyperplasia, adenomas, and adenocarcinomas of the kidney and carcinomas of the zymbal gland in male rats. 8-MOP with the addition of UVA induces the development of squamous cell hyperplasia, squamous cell papilloma, squamous cell carcinoma, cutaneous melanoma, and cataracts in rats.

Human

Chronic PUVA exposure in humans results in premature skin aging, PUVA keratoses, PUVA lentigines, and nonmelanoma skin cancer (NMSC) typically in a dose-dependent fashion. The incidence of cataracts may also be higher among PUVA treated patients. Cardiovascular disease, noncutaneous neoplasms and teratogenic effects have not been shown to be associated with PUVA therapy.

Many studies have analyzed the effect of PUVA on the development of skin cancer. A multicenter study led by Stern and others involving 16 centers in the United States begun in 1975 has monitored the adverse effects of PUVA treatment over the past 27 years. This study has documented an increased risk of squamous cell cancer (SCC) and basal cell cancer (BCC) in a dose-dependent relationship with the number of PUVA treatments received. The overall risk of developing an NMSC among subjects in this cohort is 17 times and four times higher for the development of a first SCC and BCC, respectively, in comparison to the general population. In addition, for patients on high dose PUVA ($\geqslant$337 treatments)

compared to the general population, the risk of developing a first SCC and BCC is 104 times greater and 11 times greater, respectively. Comparable studies in Europe have shown a similar increase in the relative risk of NMSC. Japanese studies, on the other hand, report a much lower incidence of NMSC in psoriatic patients treated with PUVA. This finding might reflect the protective effect of moderately pigmented skin among Asians and greater use of topical rather than oral PUVA in Japan.

PUVA exposure also appears to be a risk factor for the development of malignant melanoma, but this finding remains somewhat controversial. A multicenter US study has shown a greater than fivefold increase in risk of melanoma among patients receiving high dose PUVA ($\geqslant$250 treatments) compared to the general population. However, a similar cohort study of Swedish patients did not show a statistically significant increase in melanoma. This difference in risk between the US and Swedish populations might be attributable to the use of different treatment protocols in the two countries, or more likely, the result of insufficient power in the Swedish study to show an increase in risk due to small numbers of patients with sufficiently large amounts of PUVA exposure.

Clinical Management

Short-term toxicity such as erythema and burning is managed by decreasing PUVA dosage, keeping sunburned areas covered by clothing while receiving PUVA, or if severe, discontinuing therapy. Long-term toxicity is prevented by the protection of genitalia in males, the protection of the face unless significant psoriasis is present, and the use of protective eyeware from the time of psoralen ingestion, in the treatment unit, and for the next 24 h if the patient is exposed to sunlight. In addition, PUVA should only be used when skin disease is a significant burden and after consideration of the risks and benefits of PUVA relative to other treatments for that patient. Efforts to limit the total number of lifetime treatments should be made, particularly in younger patients and those with a higher innate risk of skin cancer. Patients receiving PUVA or with past history of significant PUVA exposure should also have regular periodic follow-up with a dermatologist or other qualified health practitioner for skin examinations and tumor excision.

See also: Methoxypsoralen, 8-; Skin.

Further Reading

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Nijsten TEC and Stern RS (2004) The increased risk of skin cancer is persistent after discontinuation of psoralen + ultraviolet A: A cohort study. *Journal of Investigating Dermatology* 121: 252–258.

Ortel B, Maytum D, and Gange RW (1991) Long persistence of monofunctional 8-methoxypsoralen-DNA adducts in human skin *in vivo*. *Photochemistry and Photobiolology* 54: 645–650.

Parrish JA, Fitzpatrick TB, Tanenbaum L, and Pathak MA (1974) Photochemotherapy of psoriasis with oral methoxsalen and longwave ultraviolet light. *New England Journal of Medicine* 291: 1207–1211.

Sigurgeirsson BL, Tegner E, Larko O, *et al.* (1999) PUVA and cancer risk: The Swedish follow-up study. *British Journal of Dermatology* 141: 108–112.

Stern RS (2001) The risk of melanoma in association with long-term exposure to PUVA. *Journal of the American Academy of Dermatology* 44: 755–761.

Relevant Website

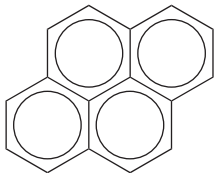
http://ntp-server.niehs.nih.gov – TR-359 Toxicology and carcinogenesis studies of 8-methoxypsoralen (CAS 298-81-7) in F344/N rats (gavage studies). National Toxicology Program. (Accessed October 9, 2003).

Pyrene

Lu Yu

- Chemical Abstracts Service Registry Number: CAS 129-00-0
- Synonyms: β-Pyrene; Coal tar pitch volatiles
- Chemical/Pharmaceutical/Other Class: Polycyclic aromatic hydrocarbon
- Chemical Formula: $C_{16}H_{10}$

- Chemical Structure:



Uses

Pyrene is used in the production of dyes and optical brighteners as a starting material. It is also produced as a result of incomplete combustion in the exhaust of motor vehicles and engines, in cigarette smoke, and in stoves and furnaces. It is found naturally in coal tar and fossil fuels.

Exposure Routes and Pathways

Inhalation and dermal contact are the major pathways for both occupational exposure and general population. Consumption of contaminated food and drinking water are also possible pathways for the general population. Persons with skin disorders are more susceptible to the toxicity of pyrene.

Toxicokinetics

Pyrene is highly lipophilic and easily penetrates cellular membranes. However, pyrene is readily metabolized to more water-soluble compounds such as 2-hydroxypyrene, 1,6-dihydroxypyrene, 1,8-dihydroxypyrene, and 4,5-dihydrodiol by liver microsomes. Two trihydroxy derivatives were also identified as metabolites of pyrene in a rat study. The high water solubility of the metabolites makes them readily excretable.

Mechanism of Toxicity

Oxidation of the rings of pyrene is an important step in its metabolism carried out by mixed function oxidases of the liver containing cytochromes P450 and P448. Epoxide intermediates are formed from oxidation. They are very reactive and can form covalent complexes with DNA and histones which serves as the ultimate carcinogenic form of pyrene.

Acute and Short-Term Toxicity (or Exposure)

Animal

It was reported that LD_{50} of rat exposed orally to pyrene was $2700\,mg\,kg^{-1}$. In a study, rats were dosed orally at concentrations near LD_{50} and succumbed in 2–5 days, and dosed through inhalation at LC_{50} in 1–2 days. Inhalation caused hepatic, pulmonary, and intragastric pathologic changes, although $10\,g\,kg^{-1}$ was considered to be less toxic through dermal contact. Cutaneous exposure for 10 days caused hyperemia, weight loss, and hepatopoietic changes. Cutaneous application for 30 days produced dermatitis and leukocytosis. In another study, four concentrations of pyrene (ranging from $5\,\mu mol\,l^{-1}$ to $5\,mmol\,l^{-1}$) were applied to the dorsal surface of six animals that received $1.0 \times 10^5\,J\,m^{-2}$ of UVA radiation. Erythematous response was evaluated after 20 h and pyrene was found to be strongly phototoxic.

Increasing dietary doses of pyrene ranging from $1000\,mg\,kg^{-1}$ food ($127\,mg\,kg^{-1}\,day^{-1}$) up to $25\,000\,mg\,kg^{-1}$ food ($917\,mg\,kg^{-1}\,day^{-1}$) for a mean dose of $426.6\,mg\,kg^{-1}\,day^{-1}$ over a 25 day period produced dilation of the renal tubules in an unspecified number of mice. The effect was not observed until the highest dose was administered, which limited the toxicological significance of this study.

Human

Pyrene is a skin, eye, and respiratory irritant. Coal tar pitch volatiles are reported to cause bronchitis.

Chronic Toxicity (or Exposure)

Animal

In a study, male and female CD-1 mice were gavaged with 0, 75, 125, or $250\,mg\,kg^{-1}\,day^{-1}$ pyrene in corn oil for 13 weeks. Minimal or mild kidney lesions (presence of multiple foci of renal tubular generation, accompanied by interstitial lymphocytic infiltrates and/or foci of renal fibrosis) were observed in all dose groups. The no-observed-adverse-effect level was obtained as $75\,ng\,kg^{-1}\,day^{-1}$, and the lowest-observed-adverse-effect level was $125\,mg\,kg^{-1}\,day^{-1}$ for nephropathy and decreased kidney weights. The animal carcinogenicity data are inadequate. Newborn male and female CD-1 mice received intraperitoneal injections of pyrene. The incidences of total liver tumors, lung tumors, or malignant lymphomas were not significantly different from control animals. Mouse skin-painting assays of pyrene as a carcinogen or as an initiator of carcinogenicity were either negative or inconclusive. Pyrene did not produce tumors in Jackson A mice 18 months after a subcutaneous injection.

Human

Pyrene is not classified as human carcinogen by the US Environmental Protection Agency (EPA) based on no human data and inadequate data from animal bioassays. Increased incidences of lung, skin, or genitourinary cancers were observed in workers exposed to a variety of polycyclic aromatic hydrocarbons. Prolonged exposure to coal tar pitch volatiles can cause dermatitis.

In Vitro Toxicity Data

Mixed results were observed for *in vitro* tests of pyrene. Negative results were obtained for pyrene in DNA damage assay in *Escherichia coli* and *Bacillus subtilis*. Both positive and negative results were observed in bacterial gene mutation test. Pyrene did not induce an increase in sex-linked recessive lethal gene in *Drosophila*. It increased the incidence of mitotic gene conversion but not other genetic endpoint in yeast.

Pyrene increased the frequency for sister chromatid exchange (SCE) in CHO cells at all treatments, but no apparent increase was observed when the concentration was increased 10-fold. Two negative results were reported for both SCE and chromosome aberrations in CHO cells at the same treatment levels. Another study also reported no increase of SCE frequency in Chinese hamster cells. Chromosome aberrations or SCE in bone marrow were not increased in several mouse strains receiving intraperitoneal injections of pyrene.

Clinical Management

The affected individual should be removed from the exposure source. A patent airway should be established. Signs of respiratory insufficiency should be monitored, and oxygen should be administered if necessary. The individual should be monitored for pulmonary edema and shock. The mouth should be rinsed and water given for dilution if patient has swallowed pyrene. Activated charcoal should be administered. Emetics should not be used. In case of eye contamination, the eyes should be flushed with water, and irrigated with normal saline during transportation.

Environmental Fate

Pyrene is immobile in soil. Volatilization from dry soil surface is extremely low with a half-life of 500 days. Biodegradation is expected to be very slow with estimated half-life of weeks to years. Photolytic degradation occurs in soil. When pyrene is disposed into the aquatic system, it is expected to adsorb to suspended soil and sediments strongly. Because of this strong adsorption, the expected volatilization from the water surface was severely attenuated. Photolytic degradation is significant at the surface of aquatic system. Biodegradation is also expected to be very slow in water. Bioaccumulation of pyrene in aquatic organisms is moderate to high. When pyrene is disposed into air, it is expected to exist in both vapor and particulate phases. The vapor-phase pyrene degrades by reaction with hydroxyl radicals and nitrate radicals in the atmosphere. Pyrene in particulate phase could be removed from air through deposition. The background concentration of pyrene in rural, agricultural, and urban soils is 1–19.7, 99–150, and 145–147 000 $\mu g\,kg^{-1}$, respectively.

Ecotoxicology

Pyrene has a moderate to high tendency to bioaccumulate in aquatic organisms from water, sediment, and food. The median threshold limit for Mosquito fish is 0.0026 $mg\,l^{-1}$ per 96 h in a static bioassay.

Other Hazards

Pyrene is a skin, eye, and respiratory irritant. It is flammable. When heated, it decomposes, and produces smoke and irritating fumes.

Exposure Standards and Guidelines

- The EPA IRIS reference dose is $0.03\,mg\,kg^{-1}\,day^{-1}$.
- The State drinking water guidelines are: Florida, 210 $\mu g\,l^{-1}$; Minnesota, 220 $\mu g\,l^{-1}$; and Wisconsin, 250 $\mu g\,l^{-1}$.
- Clean Water Act Requirements: For the maximum protection of human health from the potential carcinogenic effects due to exposure to pyrene, the ambient water criteria are 28.0, 2.8, and 0.28 $ng\,l^{-1}$, respectively, corresponding to the levels which may result in incremental increase of cancer risk over the lifetime at 1×10^{-5}, 1×10^{-6}, and 1×10^{-7}. The levels are 311, 31.1, and 3.11 $ng\,l^{-1}$, respectively, if the above estimates are made for consumption of aquatic organisms, excluding consumption of water.

See also: Polycyclic Aromatic Hydrocarbons (PAHs).

Relevant Websites

http://www.epa.gov – US EPA Integrated Risk Information System (IRIS) on pyrene. On the substance file list as of October 4, 2004.

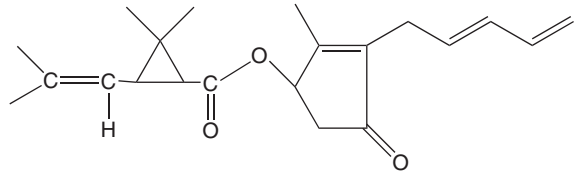
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Pyrene.

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Pyrene.

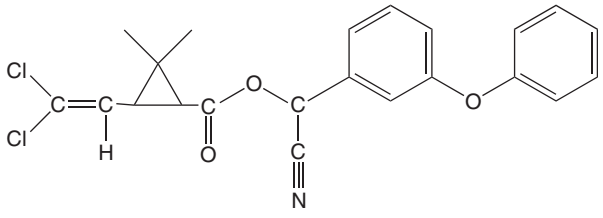
Pyrethrins/Pyrethroids

David E Ray

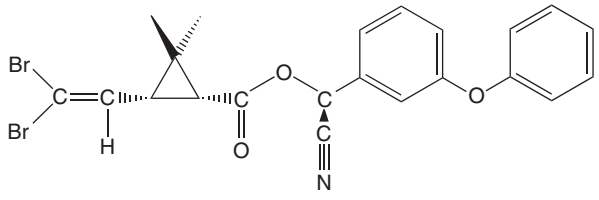
- Chemical Names: Synthetic Pyrethroids and Natural Pyrethrins
- Representative Chemicals: Pyrethrin I; Cypermethrin; Deltamethrin; Permethrin
- Chemical Abstracts Service Registry Numbers: CAS 121-21-1 (Pyrethrin I); CAS 52315-07-8 (Cypermethrin); CAS 52918-63-5 (Deltamethrin); CAS 52645-53-1 (Permethrin)
- Synonyms: The pyrethins are also known as pyrethrum
- Chemical/Pharmaceutical/Other Class: Ester insecticides
- Chemical Formulas: Pyrethrin I, $C_{21}H_{28}O_3$; Cypermethrin, $C_{22}H_{19}O_3NC1_2$; Deltamethrin, $C_{22}H_{19}Br_2NO_3$; Permethrin, $C_{21}H_{20}O_3Cl_2$
- Chemical Structures:



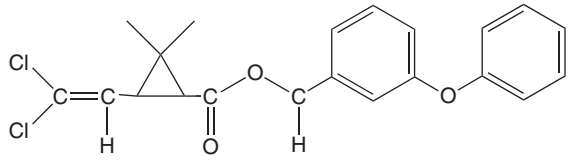
Pyrethrin I



Cypermethrin



Deltamethrin (a pure 1*R*,3,*RS* - α-isomer)



Permethrin

Uses

Both the naturally occurring pyrethrins and the synthetic pyrethroids are widely used as insecticides in agricultural, public health, and domestic applications. They are also used as ectoparisiticides in veterinary and human medicine. Attractive features are their low environmental persistence, and their rapid 'knock down' activity, whereby flying insects very quickly become uncoordinated and unable to fly before they are killed.

From the nineteenth century until the 1970s only pyrethrin mixtures obtained by solvent extraction of pyrethrum flowers (usually *Chrysanthemum cinerar-aefolum*) were available for use. However, the development by Martin Elliott of the cheaper and more light-stable synthetic pyrethroids from the 1970s led to their becoming a major pesticide class. Over a 1000 pyrethroid structures have been synthesized, some of which show considerable divergence from the original pyrethrins. The natural pyrethroids are now largely restricted to indoor domestic uses where light stability is less important. The pyrethroids are major agricultural pesticides in terms of treated land area (although not tonnage as they are more potent than most other pesticide classes). In UK arable farming, cypermethrin was the most widely used single pesticide in 2002.

Exposure Routes and Pathways

All pyrethroids and pyrethrins have low volatility (10^{-4}–10^{-8} mmHg), and so occupational and domestic use exposure is primarily via dermal contamination or inhalation/ingestion of spray droplets. Consumers are exposed to low-level residues of agricultural pyrethroids in many foods. Residues of domestic pyrethroids and pyrethrins have been found in house dust.

Toxicokinetics

Although the inherent toxic potential of pyrethroids and pyrethrins can be high (intravenous LD_{50}s range from 0.5 to $>250\,mg\,kg^{-1}$), this is limited in practice by rapid detoxification in skin, blood, and liver. The blood half-life of pyrethroids ranges from 19 min to 10 h (typically a few hours), and intoxication by

the oral route is correspondingly short lasting. The dermal toxicity of pyrethroids is further limited by low absorption through the skin and the capacity for local metabolic destruction of pyrethroids in the skin during absorption. In man the bioavailability of dermal pyrethroids is ~1%, compared to 36% for gastric absorption. Hence the dermal route of exposure presents relatively little risk of systemic poisoning, although in the few cases of very severe skin contamination that have been reported, intoxication has lasted for several weeks: possibly due to a large reservoir of pyrethroid bound to the epidermis. Once absorbed, pyrethroids rapidly distribute through the body, their high lipophylicity and lack of exclusion by the ABC multidrug transporters ensuring ready entry into the central nervous system (CNS).

The pyrethrins and allethrin are broken down mainly by oxidation of the isobutenyl side chain of the acid moiety and of the unsaturated side chain of the alcohol moiety, with ester hydrolysis playing an unimportant part, but for the other pyrethroids ester hydrolysis predominates. The acid and alcohol components of pyrethroids have very little toxic potential, so hydrolysis represents a one-step detoxification. These reactions can take place in both liver and plasma and are followed by hydroxylation and conjugation to glucuronides or sulfates, which are then excreted in urine. A number of factors modify the rate of breakdown, notably stereospecificity: with *trans* isomer hydrolysis being catalyzed by esterases but *cis* isomer hydrolysis being catalyzed at a rather lower rate by oxidases. This slower breakdown of *cis* isomers may contribute to their greater mammalian toxicity, but their higher inherent affinity for the sodium channel complex is thought to be the predominant factor. An additional influence on the rate of metabolism is the presence of an α-cyano group, which slows both hydrolysis and oxidation.

Neonatal rats are four to 17 times more vulnerable than adults to the acute lethality of both type I and II pyrethroids. This is entirely attributable to their lesser capacity for metabolic detoxification, and does not extrapolate to lower dose effects.

The relative sensitivity of insects to pyrethrins and pyrethroids is attributable (in roughly equal proportions) to their slower metabolic disposal, to their lower body temperature, and to the inherently higher sensitivity of their target sites. Although there are few, if any, toxic actions of the pyrethroids in insects that do not have their counterpart in man, these three quantitative factors combine to give insect–mammalian toxicity ratios of 2 or 3 orders of magnitude.

Mechanism of Toxicity

The pyrethrins and the pyrethroids are primarily functional toxins: causing death by hyper-excitation, and causing direct cytotoxicity in mammalian cells only at concentrations much higher than are reached in the brain of severely intoxicated animals.

The main target of the pyrethrins and the pyrethroids is the voltage-gated sodium channel family. This is responsible for the generation of the inward sodium current that produces the action potential in most cells, and is closed at normal resting potentials. Sodium channels consist of an α subunit, which forms the trans-membrane pore, resembles those of other voltage-gated ion channels, and can take several possible isoforms; and the β_1 and β_2 subunits which modify the basic function of the α subunit. There are many variant forms of the α subunit, 10 being characterized in the rat, and channels are also subject to glycosylation and phosphorylation which further modify function. These variant forms also show very different sensitivity to pyrethroids. Unfortunately there is no standard nomenclature for the many channel isoforms, and descriptions based on pharmacological properties (e.g., tetrodotoxin resistant) or tissue source (e.g., brain I, II, III) are widely used.

The interaction of pyrethroids with the sodium channel has the effect of slowing both its activation and its inactivation processes, overall causing the pyrethroid-modified channel to adopt a hyperexcitable state. This hyperexcitable condition is sustained until the pyrethroid is removed, when the channel returns to normal. Since there is a far higher density of expression of sodium channels in most cells than is needed to maintain normal excitability, only ~0.1% of sodium channels need to be modified by a pyrethroid in order for the extra current that they generate to render the whole cell hyper-excitable. This greatly increases the toxicity of pyrethroids, since they are effective well below their inherent ED_{50}s. Although activation is slowed at the single-channel level, the high density of sodium channels also means that sufficient unmodified channels are always present to ensure that the activation phase of the action potential is not appreciably delayed. However in the falling (inactivation) phase of the action potential even a low proportion of modified channels can generate enough extra current to delay inactivation. This slower rate of inactivation of pyrethroid-modified channels generates a prolonged depolarizing 'tail' current that follows the normal action potential. This 'tail' will trigger a second action potential if the current is large enough and lasts longer than the 0.5–1 ms needed for the normal

sodium channels to reactivate. In this situation, what would normally be single action potential can become multiple action potentials or a continuous uncontrolled discharge. Action potential amplitude normally remains constant, so this uncontrolled excitation produces marked functional disruption, although very high concentrations of pyrethroids or hyperactivity beyond that which the cell can sustain will eventually cause depolarization and conduction block. This depolarization is more readily produced by those pyrethroids that hold the sodium channel open longest.

An important characteristic of the pyrethroid-generated tail current is that its amplitude and duration are independent. The current amplitude is dependent only on the proportion of sodium channels modified, and hence shows a saturable relationship with pyrethroid concentration or dose. The current duration however is dependent only on the pyrethroid structure: some pyrethroids, such as permethrin holding the channel open for a few milliseconds and others, such as deltamethrin, holding it open for tens of milliseconds. Individual pyrethroids thus generate a characteristic time constant for prolongation of the sodium channel tail current that is virtually independent of dose.

Different forms of the sodium channel show differential sensitivity to pyrethroids. Pyrethroids are ~10 times more potent on the tetrodotoxin-resistant subtype of the sodium channel, which is expressed in the developing mammalian brain and in adult dorsal root ganglia. The different forms can show structure-specific sensitivity also: the rat brain IIa form being sensitive to type II, but not type I pyrethroids. Some of the selectivity of action of pyrethroids within the nervous system parallels the distribution of sensitive sodium channel subtypes, although there are at present only limited data to support this. Peripheral nerve (SNS/PN3) sodium channels are highly sensitive to pyrethroids, and action at these channels may be relevant to the production of paresthesia.

Pyrethroid action on the sodium channel shows a marked steriospecificity: the 1*R* and 1*S* *cis* isomers binding competitively to one site, and the 1*R* and 1*S* *trans* isomers binding noncompetitively to another. The 1*S* forms do not modify the channel function but do block the effect of the 1*R* isomers. In whole mammals the 1*R* isomers are thus active and the 1*S* isomers inactive and essentially nontoxic. Isomerism at the third carbon of the cyclopropane ring gives *cis* and *trans* isomers, with the *cis* isomers being about 10 times more potent than the *trans* ones. A final chiral centre is generated if a cyano substituent is added to the alcohol, giving eight possible isomers. Again this affects potency, with only the α-*S* and not the α-*R* forms being toxic to both insects and mammals. A practical consequence of this is that the toxicity of products such as permethrin, which are sold as variable isomeric mixtures, can vary from batch to batch. Thus the rat oral LD_{50} value of commercial samples of permethrin can vary from 430 to 8900 mg kg^{-1} depending on *cis* isomer content.

Many target sites other than the sodium channel have been suggested to be relevant to poisoning. Most show insufficient potency to be relevant at the concentrations of 1–30 nmol g^{-1} tissue that are seen during severe poisoning in rats, but the complex effects of pyrethroids on the CNS have led to suggestions that they also act via antagonism of γ-aminobutyric acid (GABA)-mediated inhibition, by modulation of nicotinic cholinergic transmission, or by enhancement of norepinephrine release. However, most neurotransmitter release is secondary to increased sodium entry. Actions at both voltage-dependent chloride channels and at calcium channels have also been proposed. Voltage-sensitive chloride channels are found in brain, nerve, muscle and salivary gland, and their function is to control cell excitability. The pyrethroids deltamethrin and fenvalerate decrease chloride channel currents at low enough concentrations to be relevant to mammalian poisoning, but others do not. These pyrethroids decrease chloride channel current which increases excitability, and so would indirectly synergize pyrethroid actions on the sodium channel. Voltage-dependent calcium channels are certainly pyrethroid targets in insects, and some mammalian calcium channels, such as those in the cardiac sinoatrial node, are also sensitive.

The mechanism whereby pyrethroids interact with ion channels is not yet understood, since their high lipophylicity makes investigation difficult, but type II pyrethroids have been shown to directly stimulate protein kinase C-dependent protein-phosphorylation at very low concentrations. Since ion channel activity is modulated by phosphorylation state, this is likely to be an important mechanism of action.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute effects of high oral or intravenous doses of the pyrethrins and most noncyano pyrethroids closely resemble those of DDT (which acts similarly on the sodium channel). They are characterized by fine continuous tremor, which may be severe enough to cause hyperthermia, and by marked reflex hyperexcitability. Consciousness is preserved up to the point

of death. This is variously described as T (tremor) or type I syndrome. However, with the development of the first cyano-substituted pyrethroids such as deltamethrin it was realized that these produced a very different acute poisoning syndrome in both insects and mammals known as chorea salivation (CS) or type II syndrome. This is characterized progressively by chewing, salivation, hind-limb rigidity, and finally by EEG spiking, choreoathetosis and tonic–clonic seizures with loss of consciousness. The last three are initially precipitated by sensory stimuli, but become spontaneous at near-lethal doses. In dogs, upper airway hypersecretion and gastrointestinal symptoms are more prominent than in rats. Some pyrethroids such as cyphenothrin produce mixed syndromes with both sets of symptoms superimposed, and for all pyrethroids at low doses it can be difficult to distinguish the two syndromes. Few pyrethroids have sufficient toxic potential to produce severe acute signs by the dermal route, although those with relatively long blood half-lives such as the type II pyrethroid cyhalothrin, can do so.

The nature of the poisoning syndrome can be predicted from the duration of the pyrethroid-induced sodium channel after-potential: type I pyrethroids producing shorter time constants, type II pyrethroids producing longer time constants, and mixed I/II pyrethroids having intermediate time constants. All type II pyrethroids have a cyano substituent, but not all type I pyrethroids lack one: the *trans* and *cis* isomers of flurocyphenothrin producing type I and II effects respectively. Both pyrethroid classes have a similar range of mammalian toxicity but of commercial pesticides the type II, such as deltamethrin and cypermethrin, are generally more toxic than the type I, such as permethrin. The extent to which the two classes of pyrethroids truly differ is not yet clear, but in isolated neonatal rat dorsal root ganglion cells the actions of the type I tetramethrin and the type II fenvalerate appear to be mutually exclusive.

Both type I and II pyrethroids cause marked adrenal activation in rats, probably by a direct activation of norepinephrine release. The type II pyrethroid deltamethrin causes increased corticosteroid secretion, and even moderate pyrethroid poisoning occurs against a background of profound adrenal activation – with corresponding behavioral and cardiovascular consequences. With only type II pyrethroids there is also a direct increase in cardiac contractility, although blood pressure remains well controlled.

Electrophysiological studies have shown that all of the type I motor signs of systemic pyrethroid intoxication are generated at the spinal level, although type II poisoning involves the whole CNS. Dermal application of pyrethroids can directly activate peripheral nerves however, producing paresthesia which is described in the next section.

Human

Fortunately there have been few reports of systemic poisoning, since use of adequate protective clothing will prevent intoxication even under tropical conditions. However systemic poisoning can occur under conditions of misuse or inadequate user protection.

Almost all reports of human poisoning relate to the more potent type II pyrethroids, so it is not certain how well the two syndromes seen in animals applies to man, although what has been seen in man fits quite well with the animal observations. Human type II poisoning seems to be characterized by paresthesia (if via the dermal route), dizziness, nausea, listlessness, and muscular fasciculations. More severe poisoning caused epigastric pain, nausea and vomiting (if via the oral route), hypersalivation and pulmonary edema, opisthotonos, seizures, and coma.

Given the common formulation of pyrethroids with volatile solvents such as xylene, symptoms of poisoning can be complicated by solvent toxicity, and solvents may also introduce additional skin effects. Mild poisoning symptoms may also be amplified by anxiety, which may itself be precipitated by fear or by the disconcerting paresthesia resulting from dermal contact with pyrethroids.

Although systemic toxicity is very rare, local effects are more commonly reported: skin contamination producing paresthesia, ingestion producing gastrointestinal irritation, and inhalation yielding upper respiratory tract irritation. All of these effects are reversible. The gastrointestinal irritation is rare (being limited to cases of ingestion) and has not been well studied, but presumably is a phenomenon similar to the more common dermal paresthesia. Respiratory tract irritation can be produced at comparable thresholds in rats and man, but is rarely reported. Dermal exposures far below the threshold for systemic poisoning can lead to a local paresthesia, which is evoked by all classes (the pyrethrins and type I and II pyrethroids), with a severity roughly in proportion to their systemic toxic potential. Paresthesia is by far the most commonly described toxic effect of pyrethroid exposure.

Pyrethroid-induced paresthesia is dose-dependent in severity and duration, lasting for 4–30 h after a single application. When mild the sensation is of continuous tingling or pricking, and when more severe, burning. Paresthesia is more commonly felt in the thinner skin of the face than the hands, even when the hands are the primary site of contamination. Erythema is not seen normally unless the sufferer scratches the area. Paresthesia is annoying

but not disabling and does not appear to be associated with any lasting ill effects. In animals, electrophysiological tests show peripheral nerve hyperexcitability lasting up to 24 h after exposure, and such tests have been used to monitor local pyrethroid effects in man. The mechanism of paresthesia has not been studied directly, but presumably results from abnormal pyrethroid-induced repetitive activity in skin nerve terminals.

Respiratory and dermal sensitization have both been reported after exposure to pyrethroids, but very rarely considering their widespread usage, and always in association with other potential allergens. However in the past impure pyrethrin extracts have given rise to contact dermatitis.

Chronic Toxicity (or Exposure)

Animal

Near-lethal doses of all classes of pyrethroids can give rise to an axonal degeneration in peripheral nerves closely resembling Wallerian degeneration, but this effect is inherently reversible and is only seen at dose levels which produce prolonged and severe motor signs. Central neuropathology has been described in one study of adult rats given repeated near-lethal doses of the type II pyrethroid deltamethrin, but others (including the present author) have found no such pathology. A study of repeated low doses of permethrin has also described central pathology when given in combination with other agents known to produce central neuropathology at higher doses.

A number of effects of exposure to pyrethroids during early development have been described in mice. The pyrethroids permethrin and deltamethrin (in addition to DDT, PCBs, nicotine, and paraquat) have also been reported to induce permanent changes in behavior and neurochemistry of adult mice when administered directly to the neonate. These effects were seen at dose levels, which were not acutely toxic. These results contrast with a lack of effect of longer term, dietary administration of pyrethroids in rat studies conducted for regulatory purposes using different end points, and at present the relevance of the mouse results to human health is uncertain. In other studies, delayed development of the blood–brain barrier has been reported in rat pups given pyrethroids at higher doses sufficient to reduce body weight, although it is likely that this effect was nonspecific in nature.

Human

Long-term ill-health of a variable and somewhat nonspecific nature has occasionally been ascribed to pyrethroid exposure, and this has been the subject of public concern and legal claims in Germany. Pyrethroids have been detected in domestic house dust at a low level, but no clear clinical or epidemiological studies have shown a causal relationship between pyrethrin or pyrethroid exposure and ill-health. The few descriptions of systemic acute pyrethroid poisoning and the larger number of paresthesia cases that have been reported all described complete recovery.

In Vitro Toxicity Data

Inexcitable mammalian cells are little affected by pyrethrins or pyrethroids. A number of pyrethroids produce growth inhibition at $10^{-5}\,\mathrm{mol\,l^{-1}}$ and decreased viability has been described after treatment with permethrin at $100\,\mathrm{ng}/10^6$ cells – a level which is approximately 10–100 times higher than that reached in the brain of lethally intoxicated animals. By contrast, excitable tissue (neurons, synaptosomes, and isolated nerve fibers) has proved invaluable for research into mechanisms of action: effects being seen from $10^{-10}\,\mathrm{mol\,l^{-1}}$. However the very low water solubility of pyrethroids ($\sim 10^{-9}\,\mathrm{mol\,l^{-1}}$) has meant that most such studies have been carried out using aqueous pyrethroid suspensions that then dissolve into tissue lipids, making absolute ED_{50}s difficult to determine. This solubility problem also causes most *in vitro* effects to be irreversible, since there is no route for removal of the pyrethroid – in marked contrast to the effects seen *in vivo*. Such irreversibility has been interpreted by some authors as providing potential for irreversible pyrethroid toxicity in man, but when toxicokinetic differences are taken into account, *in vitro* and *in vivo* data are generally in good agreement.

Clinical Management

The most commonly encountered sign of pyrethroid poisoning is dermal paresthesia. This can be treated by lavage of the contaminated skin with oils – but not by soap and water, as pyrethroids bind to skin and are poorly water soluble. Vitamin E cream has also been found effective for treating paresthesia in clinical trials. When applied to the skin from 29 h before to 15 min after the pyrethroid protection lasted for more than 5 h. The concentration required to give greater protection than that of the corn oil solvent alone was very high (50%) and similar relief can be obtained by use of presumably inert preparations such as corn oil or petroleum jelly. Topical treatment with local anesthetics has been described in humans and in animals, but anesthesia may be more inconvenient than the paresthesia.

If systemic toxicity does occur, the central signs of poisoning can be difficult to control and may be

confused with intoxication by other pesticides such as anticholinesterases, which also cause salivation and hyperexcitability – although pyrethroids do not inhibit acetylcholinesterase. Since pyrethroids produce no morphological damage and are rapidly removed from the body, only symptomatic treatment is needed. Two approaches are possible: to attempt to antagonize the primary ion channel effects of the pyrethroids, or to control the secondary consequences mediated by specific neurotransmitter systems. Since pyrethroids act via ion channels on multiple neurotransmitter systems, most successful attempts at therapy have been based on ion channel or membrane-stabilizing drugs. An ideal therapeutic agent would antagonize the abnormal, pyrethroid-evoked, sodium current but leave the normal one unchanged. *In vitro*, phenytoin, phenobarbitone, and valproate act equally on the pyrethroid-evoked and normal sodium current; and diazepam and mephenesin had less action on the abnormal pyrethroid-evoked current than on the normal one. Hence mephenesin and methocarbimol are effective in rats only at maximum tolerated doses, and diazepam was found to be ineffective in man. Pentobarbitone, which is both a membrane stabilizer and chloride channel agonist, was however effective against all the type II motor signs caused by deltamethrin at 25% of the anesthetic dose in rats. An equi-sedative dose of phenobarbitone (which does not act on chloride channels) was much less effective. Although phenobarbitone has been tried and found ineffective as a type II pyrethroid antidote in man, pentobarbitone does not appear to have been tested in man.

Since type II poisoning involves a combined action on the CNS, adrenals, autonomic system, and muscle, multi-drug therapy may be needed. The combination of methocarbimol and atropine prevented all deaths at LD_{80} doses of pyrethroids in rats.

Environmental Fate

Environmental residues of pyrethroids and pyrethrins are degraded by hydrolysis, and pyrethrins by photolysis, and so do not accumulate in most ecosystems. The main environmental hazard associated with pyrethroid use is contamination of freshwater by acute run-off after use as an agricultural pesticide or ectoparisitide near to water, which can lead to death of aquatic invertebrates or fish (which have very limited pyrethroid detoxification capacity).

Other Hazards

Pyrethroids and pyrethrins present no other hazards, but are usually formulated in organic solvents which may be inflammable.

Exposure Standards and Guidelines

The acceptable daily intakes set by the Joint Meeting on Pesticide Residues (JMPR) for cypermethrin, deltamethrin, and permethrin are 0–0.01 mg kg^{-1} body weight, with acute oral reference doses for deltamethrin or permethrin of 0.05 mg kg^{-1} bw. The National Institute for Occupational Safety and Health maximum allowable concentration (MAC) for pyrethrins at an 8 h time-weighted average is 5 mg m^{-3}.

Miscellaneous

The pyrethrins and some pyrethroids are commonly co-formulated with the synergist, piperonyl butoxide. This has limited toxic potential in itself but inhibits both oxidative and hydrolytic detoxification reactions and so enhances their toxicity – especially to insects.

See also: Organochlorine Insecticides.

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Soderlund DM, Clark JM, Sheets LP, *et al.* (2002) Mechanisms of pyrethroid neurotoxicity: Implications for cumulative risk assessment. *Toxicology* 171: 3–59.

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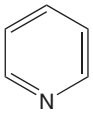
Pyridine

Kathryn J Kehoe

- Chemical Abstracts Service Registry Number: CAS 110-86-1
- Synonyms: Azabenzene; Azine
- Chemical/Pharmaceutical/Other Class: Heterocyclic nitrogen
- Chemical Formula: C_5H_5N
- Chemical Structure:



Uses

Pyridine is a solvent used in the synthesis of pharmaceuticals and other organic compounds. Approximately 50% of pyridine produced is used as an intermediate in insecticide and herbicide manufacture. Another 20% is used to produce piperidine. It can be introduced into the environment by the decomposition of many natural materials.

Exposure Routes and Pathways

Inhalation, dermal contact, and ingestion are possible routes of exposure.

Toxicokinetics

Pyridine is absorbed by the gastrointestinal tract, the skin, and the lungs. Pyridine can be excreted in the urine unchanged or it may be methylated at the N-position to form the urinary metabolite *N*-methylpyridinium hydroxide. Pyridine also may undergo oxygenation by liver microsomes (cytochrome P450) in the presence of NADPH and oxygen.

Acute and Short-Term Toxicity (or Exposure)

Animal

Dermal LD_{50}s include 1121 mg kg^{-1} for rabbits and 1 g kg^{-1} for guinea pigs. Oral LD_{50}s include 891 mg kg^{-1} for rats, 1500 mg kg^{-1} for mice, and 4 g kg^{-1} for guinea pigs. Intravenous LD_{50}s include 420 mg kg^{-1} for mice and 880 mg kg^{-1} for dogs. The LC_{Lo} (inhalation) in rats is 4000 ppm per 4 h.

Human

Despite widespread industrial use, reports of human injury as a result of pyridine are rare. The most important effect of pyridine exposure is hepatotoxicity. Acute exposures to pyridine result in irritation to the skin, nose, and throat. Central nervous system (CNS) depression results in dizziness and lightheadedness. Exposure to high concentrations may result in coma and death. Contact with the eyes causes burning and can lead to permanent damage. Ingestion of small amounts may produce narcotic effects including anorexia, nausea, fatigue, and mental depression. Larger quantities have resulted in systemic effects and death within 43 h.

Chronic Toxicity (or Exposure)

Animal

A 2 year drinking water study performed in rats and mice showed hepatocellular injury by week 13 and clear evidence of carcinogenic activity in all animals that survived 1 year or longer. Renal tubule neoplasms, mononuclear cell leukemia, hepatocellular neoplasms, and interstitial cell adenoma of the testis were noted.

Human

Chronic exposure at 6–16 ppm may result in severe liver damage and kidney injury. Permanent damage to the CNS may result and be accompanied by confusion and mental changes including headache, insomnia, and back pain. Chronic ingestion results in symptoms similar to inhalation. Chronic exposure causes liver and kidney damage.

Pyridine is an allergen and exposure may result in sensitization. It has no known human carcinogenic effects.

In Vitro Toxicity Data

In genetic toxicity screening pyridine was not mutagenic in both *Salmonella typhimurium* and mouse lymphoma cells. Negative results were also observed in sister chromatid exchange studies and no chromosomal aberrations were detected with Chinese hamster ovary cells. More recently it was noted that pyridine induced chromosomal malsegregation and increased nondisjunction in *Drosophilia melanogaster* females.

Clinical Management

The victim should be removed from the source of exposure. For inhalation exposures, fresh air should be supplied. Artificial respiration should be provided if breathing has stopped; oxygen should be administered if available. Treatment should be symptomatic, noting the narcotic effect of pyridine. Dermal exposure should be minimized by washing away all traces of the

chemical with soap or mild detergent and large amounts of water. Symptoms of dermatitis should be treated.

For ingestion, if the victim is conscious and not convulsing one or two glasses of water should be given to dilute the chemical and a hospital or poison control center called immediately. Activated charcoal may be administered. It should be noted that large doses could act as a heart poison.

Environmental Fate

Pyridine is biodegradable and not considered a threat to the environment.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration permissible exposure limit (time-weighted average) is 5 ppm.

See also: Pesticides.

Further Reading

National Toxicology Program (NTP) (2000) Toxicology and Carcinogenesis Studies of Pyridine (CAS No. 110-86-1) in F344/N Rats, Wistar Rats and B6C3F1 Mice (Drinking Water Studies). NTP Technical Report Series No. 470. NIH Publication No. 97-3960. Research Triangle Park, NC: US Department of Health and Human Services.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Pyridine.

Pyridostigmine

Teresa Dodd-Butera and Molly Broderick

- Chemical Abstracts Service Registry Number: CAS 101-26-8
- Synonyms: Mestinon; Regonol (trade names)
- Chemical/Pharmaceutical/Other Class: Anticholinesterase agent
- Chemical Formula: $C_9H_{13}BrN_2O_2$

Uses

Pyridostigmine is a carbamate that is used in the treatment of myasthenia gravis, a neuromuscular disease. It can also be used as a method of protection against nerve agent poisoning. Carbamates can occupy the catalytic site of acetylcholinesterase (AchE), which temporarily prevents phosphorylation.

Exposure Routes and Pathways

Pyridostigmine bromide is available for use by oral or parenteral routes.

Toxicokinetics

Pyridostigmine is absorbed poorly after oral administration; thus, oral doses must be higher than by the parenteral route. The drug is hydrolyzed by plasma esterases and is metabolized in the liver. Both the quarternary alcohols and parent compounds are excreted in the urine. The elimination half-life is increased with renal dysfunction.

Mechanism of Toxicity

Pyridostigmine bromide competitively binds to nerve tissue AchE. The binding is reversible and has been shown to protect AchE against irreversible inhibition by organophosphorus nerve agents. Pyridostigmine is a quarternary compound and does not readily cross the blood–brain barrier. Thus, it is not expected to affect or protect brain AchE. Cholinesterase inhibition, which is a mechanism of action, is also responsible for toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

Pyridostigmine bromide studies have been performed in dogs, guinea pigs, monkeys, rabbits, rats, and mice. Diarrhea, salivation, tremors, and respiratory failure were seen prior to death. Side effects of the drug are related to muscarinic and nicotinic effects. Toxicity is also related to cholinergic stimulation. Effectiveness of pretreatment to reduce lethality after exposure to nerve agents (in particular, soman) is dependent on the administration of atropine and pralidoxime, postexposure.

Oral administration of nonlethal doses of pyridostigmine did not alter male or female reproductive indices. Administration of pyridostigmine bromide also did not result in significant increases in congenital malformations at low and comparable doses to those used therapeutically in humans. At the high dose level, delayed ossification and missing vertebrae were noted in animal studies.

Human

Acute side effects occur from therapeutic doses in ~1% of patients. However, an excessive dose of an anticholinesterase drug results in a cholinergic crisis. The condition results from stimulation of muscarinic receptors and depolarization of the motor end plate. Symptoms of salivation, lacrimation, diaphoresis, weakness, and respiratory failure may result. Therapeutic use of pyridostigmine should be discontinued in the presence of nerve agent poisoning, as it may exacerbate symptoms in certain exposures.

Chronic Toxicity (or Exposure)

Animal

Chronic administration of therapeutic levels of pyridostigmine in mice did not demonstrate alterations in heart rate and blood pressure. Long-term carcinogenicity studies have not adequately evaluated carcinogenicity of pyridostigmine in animals.

Human

Initial and long-term follow-up found that veterans of the first Persian Gulf War reported various, unexplained symptoms termed 'Persian Gulf War Syndrome'. It is characterized by chronic fatigue, ataxia, impaired cognition, weakness, incontinence, myoneuropathy, and adenopathy. Although prophylactic use of pyridostigmine has been suspected as the causative agent (see Uses above), this syndrome has not been noted in patients with myasthenia gravis using pyridostigmine in their treatment regimen. It has been proposed, but not proven, that the combination of pyridostigmine, combustion products of pesticides, insect repellants, and post-traumatic stress disorder may be responsible for the Persian Gulf War Syndrome.

In Vitro Toxicity Data

Pyridostigmine was mutagenic in mouse lymphoma cells with metabolic activation.

Clinical Management

Symptoms of toxicity should be managed to alleviate cholinergic symptoms, with special attention to respiratory support. Atropine and pralidoxime may be needed. In a military setting, symptoms should be distinguished from nerve agent poisoning to provide proper treatment.

Potential interactions of drugs in the clinical setting for consideration include mefloquine (antimalarial), narcotics, aminoglycoside antibiotics, anesthetics, and succinylcholine. Medical conditions that warrant precaution with the use of pyridostigmine include: glaucoma, bronchial asthma and obstructive lung disease, and cardiac arrhythmias. Allergic reactions may occur in persons with bromide sensitivity.

See also: Anticholinergics; Cholinesterase Inhibition; Nerve Agents; Organophosphates; Soman.

Further Reading

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Klaassen C (ed.) (2001) *Casarett & Doull's Toxicology: The Basic Science of Poisons*, 6th edn. New York: McGraw-Hill.

Relevant Website

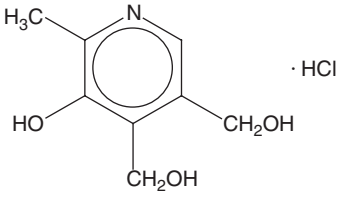
www.va.gov – Vetran's Administration (VA); search for Pyridostigmine.

Pyridoxine

Diana Ku

This article is a revision of the previous print edition article by Denise L Kurta, volume 2, pp. 612–613, © 1998, Elsevier Inc.

- Chemical Name: Pyridoxine
- Chemical Abstracts Services Registry Numbers: CAS 58-56-0; CAS 65-23-6
- Synonyms: Vitamin B_6; Pyridoxal; Pyridoxamine; Adermine hydrochloride; 3,4-Pyridinedimethanol; 5-Hydroxy-6-methyl hydrochloride
- Chemical/Pharmaceutical/Other Class: Water-soluble vitamin
- Chemical Formula: $C_8H_{12}ClNO_3$
- Chemical Structure:



Uses

Pyridoxine is a nutritional supplement used for the prophylaxis or treatment of pyridoxine deficiency resulting from conditions such as severe diarrhea, malabsorption, congenital metabolic dysfunction, hyperthyroidism, renal and hepatic disease, congestive heart failure, alcoholism, drug-induced conditions, and during pregnancy and lactation. Pyridoxine-dependent syndromes including pyridoxine-dependent seizures in infants, homocystinuria, pyridoxine-responsive anemia, and hyperoxaluria may require the clinical use of pyridoxine as well. Pyridoxine is also used as an antidote for isoniazid, hydrazine, and ethylene glycol toxicities.

Background Information

Pyridoxine deficiency was first identified in 1926; however, it was erroneously attributed to vitamin B_2. Ten years later, the active form of pyridoxine was identified and named vitamin B_6 (pyridoxal-5-phosphate, PLP).

Exposure Routes and Pathways

Routes of exposure are oral, intravenous, and intramuscular. Dietary sources of pyridoxine include bananas, potatoes, eggs, lentils, legumes, cereals, chicken, liver, and kidneys. Cooking destroys some amount of the vitamin.

Toxicokinetics

Pyridoxine is readily absorbed from the gastrointestinal tract mainly in the jejunum by passive diffusion. It is hepatically metabolized and stored mainly in the liver, muscle, and brain. Volume of distribution and protein binding are both low. The plasma half-life is 1.7 h and the biological half-life is 15–20 days. Pyridoxine is excreted renally almost entirely as metabolites. Excess amounts of pyridoxine (beyond daily needs) are excreted unchanged in the urine.

Mechanism of Toxicity

The exact mechanism of pyridoxine-induced neurotoxicity has not been established but may occur at the dorsal root and sensory ganglion.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute toxicity is not expected in animals.

Human

Acute toxic effects are not expected; however, a case report of a husband and wife who were mistakenly administered a single, large, intravenous dose ($2\,g\,kg^{-1}$) of pyridoxine shows that it resulted in permanent dorsal root and sensory ganglia deficits. Allergic reactions to the use of pyridoxine have also been reported.

Chronic Toxicity (or Exposure)

Animal

It would be unlikely for animals to be given a chronic pyridoxine overdose.

Human

Chronic doses of 200–6000 mg daily for several months may cause severe sensory neuropathy, ataxia, incoordination of hands, weakness, and paresthesias. Seizure and death have been reported with extremely large intravenous doses of pyridoxine.

In Vitro Toxicity Data

The fetus has the ability to concentrate pyridoxal phosphate 6.6 times above the maternal plasma concentration. Administration of larges doses of pyridoxine during pregnancy resulted in an infant with a requirement for supplemental vitamin B_6. In a case report, a woman administered $50\,mg\,day^{-1}$ for the

first 7 months of pregnancy was associated with an infant with phocomelia.

Clinical Management

Acute and chronic ingestions should be discontinued and any toxic effects treated symptomatically. A study on healthy volunteers reported neurotoxic symptoms to progress for 2–3 weeks upon discontinuation of pyridoxine.

See also: Ethylene Glycol; Folic Acid; Hydrazine; Isoniazid; Pyridoxine; Riboflavin; Thiamine.

Further Reading

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Pyriminil

Lynn Weber

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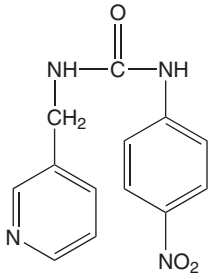
- Chemical Abstracts Service Registry Number: CAS 53558-25-1
- Synonyms: 1-(3-Pyridylmethyl-3-(4-nitrophenyl) urea; DLP-87; DLP-787; PNU; Pyrinuron; Vacor; RH-787
- Chemical/Pharmaceutical/Other Class: Substituted phenylurea
- Chemical Structure:



Uses

Pyriminil was first introduced in 1975 as a rodenticide to control rats and house mice. It is especially effective against rodents resistant to anticoagulant poisons. Pyriminil was marketed for indoor use only in the form of bait and tracking powder. Its use has been banned in the United States.

Exposure Routes and Pathways

Oral and dermal routes of exposure are possible.

Toxicokinetics

Pyriminil is absorbed from the gastrointestinal tract. Following absorption, pyriminil is distributed in the body and undergoes metabolism in the liver by cytochrome P450 1A-dependent monooxygenases. Different metabolites have been identified in the urine of poisoned rats, dogs, and humans. These metabolites include pyriminilglucuronide, aminopyriminil, acetamidopyriminil, *p*-aminophenyl urea, *p*-acetamidophenyl urea, *p*-nitroaniline, *p*-phenylenediamine, *p*-acetamidoaniline, nicotinic acid, nicotinuric acid, and nicotinamide. Dogs develop tolerance to pyriminil, which may be partially attributed to enhanced hepatic detoxification and excretion.

Mechanism of Toxicity

Pyriminil toxicity occurs primarily because it inhibits NADH:ubiquinone oxidoreductase activity of complex I in mammalian mitochondria resulting in preferential toxicity to high-energy-demanding cells such as nerves and pancreatic β-cells. However, pyriminil may also act as a nicotinamide antagonist and interfere with the synthesis of NADH/NADPH, furthering neural and β-cell toxicity. Inhibition of mitochondrial respiration in nerves causes somatic, autonomic, and central nervous system neuropathies while inhibition in β-cell causes an immediate, irreversible insulin-dependent diabetes mellitus condition. Pyriminil also acts as a noncompetitive inhibitor of rat acetylcholinesterase.

Acute and Short-Term Toxicity (or Exposure)

Animal

In rats, the LD_{50} is very low ($\sim 5\,mg\,kg^{-1}$). Mice and cats are also relatively sensitive to pyriminil toxicity with LD_{50} values of 98 and $62\,mg\,kg^{-1}$, respectively. Other species are markedly less sensitive (LD_{50} values from 0.5 to $4\,g\,kg^{-1}$). A horse was reported to show

severe muscle fasciculations, sweating, dilated pupils, and tachycardia following ingestion of 0.25–0.5 kg of pyriminil. Other signs of toxicity in horses include colic, hind limb weakness, ataxia, and persistent loss of appetite. Pyriminil intoxication in other animals causes gastrointestinal disorders (e.g., vomiting and abdominal cramp), visual problems, cardiovascular disorders, ataxia, tremor, and coma.

Human

Pyriminil appears highly toxic to humans. The lowest acute toxic dose of pyriminil in humans was estimated at 5 mg kg^{-1}. Ingestion of one-half of one 39 g packet of Vacor (2% pyriminil) reportedly led to a fatality. A seven year-old child was found dead 1 day after ingesting one packet of Vacor (2% pyriminil). In another case, two of nine people died after ingestion of 39 g of Vacor; the remaining people developed chronic hypotension and permanent diabetes mellitus. Generally, the symptoms of acute poisoning were characterized by rapid onset of ketoacidosis-prone diabetes mellitus, severe orthostatic hypotension, autonomic dysfunction, autonomic neuropathy (dysphagia, impotence, urinary retention, constipation, or diarrhea), and peripheral neuropathy. Other symptoms included nausea, vomiting, abdominal cramp, diffuse myalgias, polyuria, polydipsia, dyspnea, malaise, and general weakness. Peripheral sensory and motor neuropathies are possible signs of pyriminil exposure. Neurological effects of pyriminil can occur within hours of ingestion and may persist for months.

Clinical Management

Because pyriminil can cause early onset seizures, induction of emesis is contraindicated. Gastric lavage may be useful, if performed soon after ingestion. Activated charcoal/cathartic therapy may be adopted to retard the absorption of pyriminil from the gastrointestinal tract. According to US Food and Drug Administration guidelines, 240 ml of diluent may be mixed with 30 g of charcoal. The usual charcoal dose is 30–100 g in adults and 15–30 g in children (1 or 2 g in infants).

Conventional anticonvulsants (e.g., diazepam, phenobarbital, and phenytoin) may be administered to treat pyriminil-induced seizures. Niacinamide has been demonstrated to be an effective antidote in pyriminil poisoning in rats but little information is available regarding its antidotal efficacy in humans. Insulin therapy could be instituted as a preventive measure for possible diabetes mellitus. Orthostatic hypotension due to pyriminil exposure may be treated with conventional mineralocorticoids.

See also: Pesticides.

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Pyrrolizidine Alkaloids

Gerardo Ibanez

- Chemical Abstracts Service Registry Number: CAS 643-20-9
- Synonyms: 1-Azabicyclo(3.3.0)octane; 1*H*-Pyrrolizine; Hexahydropyrrolizine
- Chemical/Pharmaceutical/Other Class: Alkaloid
- Chemical Formula: $C_7H_{13}N$

Background Information

Pyrrolizidine alkaloids (PAs) constitute a class of plant toxin associated with disease in humans and animals. They are found in a wide variety of plant species in the world and it is estimated that ~3% of the world's flowering plants contain toxic pyrrolizidine alkaloids. The toxin is present in more than 12 higher plant families, among which three families, Compositae (Asteracea), Boraginaceae, and Leguminosae (Fabaceae), contain most toxic PAs. The wide distribution of plants containing PAs around the globe makes it difficult to prevent human and animal exposure and every year animals and people suffer from acute and chronic PA exposures.

In 1920, there was a large outbreak of food poisoning in South Africa. This incident was due to the contamination of wheat flour with toxic PAs. Also, large outbreaks have been reported in Afghanistan, India, and the former USSR. These large outbreaks were possible because PA-containing plants grow in climatic conditions in which food sources such as wheat are usually grown.

In the last decade, dietary supplement consumption has increased in Europe and the United States. This has prompted regulatory agencies to enact

regulations to protect the health of consumers. In 1992, the Federal Health Department of Germany restricted the manufacture and use of pharmaceuticals containing PAs with an unsaturated necine skeleton. Also, in 1994, the US Congress passed the Dietary Supplement Health Education Act (DSHEA), which amended the US Federal Food, Drug, and Cosmetic Act (FFDCA) and created a new regulatory category for the Food and Drug Administration (FDA) to regulate dietary supplements. Furthermore, in 1997, the US FDA published Good Manufacturing Practice (GMP) regulations that manufacturers of herbal products must follow. These regulations are meant to improve the quality of dietary supplements and minimize the risk of poisoning due to the presence of PAs in dietary supplements.

Exposure Routes and Pathways

The main route of exposure of humans and animals to PAs is the oral pathway. Human exposure occurs through consumption of food contaminated by toxic plant products or by the ingestion of herbal medicines containing the toxin. PAs have been found in wheat, milk, honey, herbal medicines, and herbal teas at different concentrations. Livestock exposure to PAs is attributed to the consumption of PA-containing plants while grazing.

Toxicokinetics

Upon ingestion, PAs are absorbed mainly through the small intestine and are carried to the liver. The most hydrophobic PAs are readily excreted unchanged in the urine within a 24 h period while less hydrophobic PAs are metabolized by cytochrome P450s and flavin monooxygenase enzymes. The metabolites are eliminated as soluble glutathione and other conjugates in the bile and urine.

Mechanism of Toxicity

The target organ for PA toxicity in experimental animals and humans is the liver. PAs cause liver toxicity and venoocclusive disease. The mechanism of hepatotoxicity has been extensively investigated and it is well established that metabolic activation of PAs to reactive metabolites is responsible for causing liver toxicity. In animals, PAs exhibit a large variety of genotoxic effects, including DNA binding, DNA cross-linking, DNA protein cross-linking, sister chromatid exchange, chromosomal aberrations, mutagenicity, and carcinogenicity. However, these effects have not been observed in humans.

Acute and Short-Term Toxicity (or Exposure)

Animal

In acute toxicity, extensive hemorrhagic necrosis of the liver is observed, which results in the death of the animal. There is conclusive evidence from studies on experimental animals that the effects of a single exposure to PAs may result in liver disease and may lead to cirrhosis of the liver. Also, animal susceptibility to PA poisoning varies among animal species depending on how fast they metabolize the parent compound. Animals more resistant to PAs, such as sheep, hamsters, and rabbits, have a higher capability toward metabolizing PAs, whereas susceptible species such as cattle, chickens, and rats have a low capability toward metabolizing the parent compound.

Human

In humans, acute poisoning causes severe liver toxicity with hemorrhagic necrosis. Acute human cases following the brief ingestion of PAs have been known to progress to cirrhosis. The most common signs of acute PA poisoning are lassitude, anorexia, nausea, vomiting, diarrhea, edema, emaciation, hepatomegaly, splenomegaly, and jaundice.

Chronic Toxicity (or Exposure)

Animal

Chronic exposure results in a number of sublethal effects on the liver, eventually leading to venoocclusive disease. The signs of chronic PA poisoning are sluggishness, loss of appetite, wasting ascites, jaundice, photosensitization, and behavioral changes. Also, chronic exposure to PAs leads to cancer in experimental animals.

Human

Chronic exposure to PAs results in hepatic damage and venoocclusive disease. Chronic poisoning takes place mainly in the liver, lungs, blood vessels, and, in some instances, kidneys, pancreas, gastrointestinal tract, bone marrow, and the brain. Some common signs of chronic exposure to PAs are cell enlargement (megalocytosis), venoocclusion in liver and lungs, fatty acid degradation, nuclei enlargement with increasing nuclear chromatin, loss of metabolic function, inhibition of mitosis, fatty acid degeneration, proliferation of biliary tract epithelium, liver cirrhosis, and hyperplasia. Liver failure due to cirrhosis and venoocclusive disease may occur months to years after the last episode of PA exposure.

Clinical Management

There is no known method to prevent PA liver damage once a hepatotoxic dose of the alkaloid has been ingested. Reversibility of chronic damage is uncertain and unpredictable. In man, it is reported that following a poisoning outbreak in which significant acute toxicity is observed, ~50% of patients will recover completely and 20% will die rapidly. Of the survivors, ~20% will appear to recover clinically but may go on to develop cirrhosis and liver failure years later. There is no clinical treatment for venoocclusive disease and the prognosis depends on the extent of damage and whether exposure to PAs recurs.

See also: Belladonna Alkaloids; Liver; Plants, Poisonous.

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QT Interval

Russell Barbare

Definition

The QT interval is a measure of the portion of time in a heartbeat starting with depolarization of the ventricles and ending with their repolarization. It is called this because standard electrocardiogram (ECG) notation uses the letters P through U to indicate various phases of the heartbeat, with the QRS complex being various stages of ventricular depolarization and T used to designate the period of repolarization (**Figure 1**). Depolarization is a lowering of the electrical potential that is normally maintained across nerve and muscle cell membranes and repolarization is the reestablishment of that potential. These changes cause various phases of the heartbeat and are caused by the exchange of calcium, potassium, sodium, and chloride ions through specific channels across the cell membranes.

Significance

The QT interval is important because notable change in it, most especially lengthening, is the most accepted indicator of potentially fatal cardiac arrhythmias. For example, excessive extension of the QT interval is strongly associated with ventricular arrhythmias including Torsade de Pointes (TdP), an acceleration in cardiac rhythm that can lead to heart attack. The root causes of heart rate and rhythm abnormalities are diverse, complex, and often difficult to evaluate. Since QT interval changes can be checked using portable, noninvasive techniques and are associated with a wide variety of cardiac problems they are a valuable indicator of cardiac function. The degree of QT interval prolongation is generally proportional to the risk.

Risk Factors

Risk factors for QT interval prolongation include genetic factors, medical history, metabolic imbalances, age, and use of pharmaceuticals. Genetically, females

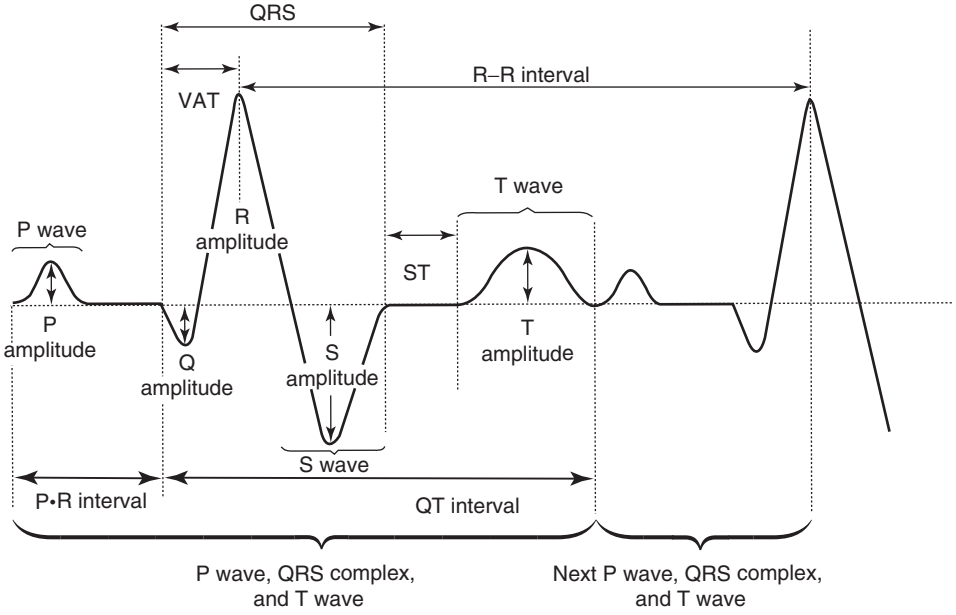

Figure 1 A typical ECG signal consists of the P wave, QRS complex, and the T wave. The time between the start of the QRS complex and the end of the T wave is called the QT interval.

are twice as likely to show prolongation as males and congenital long QT syndrome, which is actually one of six genetic defects in cellular ion channels, affects ~1 in 5000 people independent of their gender. Factors in medical history that raise the likelihood are incidences of cardiac disease, central nervous system trauma, and liver disorders. Metabolic imbalances that are risks include electrolyte imbalances, endocrine disorders, and diabetes. Using two or more QT interval-prolonging drugs together raises the chance of heart arrhythmias as well as using a QT-altering drug with another that changes the metabolism of that drug.

Measurement Standardization

Intersubject QT intervals vary noticeably, with a standard deviation of ~40 ms, or over 10% of the total. Single subject standard deviations are around 10 ms, which is still over 2.5% of the total. This is partly because the heart's rhythm is partially a hysteresis loop (i.e., previous patterns affect successive patterns) and also because of the influence by many other factors, including heart physiology, body electrolyte balance, genetics, disease, age, nutrition, and exercise.

The QT interval has an inverse relationship to the heart rate, so as the heart rate increases, QT interval decreases. The changes are not proportional to the change in heart rate, so QT measurements are often standardized by various formulas to the corrected QT interval, or QTc interval. Unfortunately, there is no standard formula that is used for the correction. Over thirty corrections have been proposed and several, including Bazette's, Fridericia's, and Van de Water's are in common use. In an ECG, both the QT interval and the QTc interval are usually examined for abnormalities.

There is also some debate as to how to interpret the exact start and end points of the various cardiac phases from an ECG. The actual changes are based on altered ionic balances across cell membranes, which move in cyclic patterns through the heart tissue and are manifested externally as minute changes in skin electrical potential. An ECG uses multiple leads to pick up the pattern of changes and so is an indirect observation that must be interpreted by a professional.

Changes in the QT interval are best interpreted using multiple measurements over time and should include the baseline or prechange observations, but data that extensive and accurate are expensive to obtain and hard to get without a controlled environment. There are portable products that digitally capture heartbeats but they have not been fully accepted by the various regulatory agencies and the data still require professional interpretation.

Various organizations, including the Food and Drug Administration (FDA), the International Conference for Harmonization, and the Committee for Proprietary Medical Products have all published guidelines for interpretation of QT interval changes. Due to the personal variability in QT interval and the uncertainties of measurement and interpretation, these are in the form of strong recommendations rather than absolutes. For example, the FDA recommends looking at both absolute QT interval length and changes from the baseline as indicators of concern in the testing of new drugs prior to any introduction into humans. In their standards, the highest levels of risk are indicated by absolute lengths greater than 500 ms, individual increases from baseline greater than 60 ms, or mean group changes from baseline greater than 20 ms.

Drugs Affecting the QT Interval

Since cardiac potential is controlled by the exchange of Ca^{2+}, K^+, Na^+, and Cl^- ions across the cellular membrane, any drugs altering the action of these ion channels directly or indirectly (for example, causing altered metabolism of another drug) can cause QT prolongation. All drugs withdrawn for TdP so far have shown inhibiting effects on the rapid potassium channel (I_{Kr}) and there are suggestions that many other drugs that cause QT interval prolongation affect K^+ exchange. This makes potassium flow inhibition the most studied basis for drug-related QT changes but not the only possible cause.

There are several classes of drugs that are specifically designed to be antiarrhythmics, to regularize or control the heart beat, and will therefore almost certainly affect the QT interval. These include over forty compounds in subclasses based on their effects on ion channels (Na, Ca, or K) or receptors (α-adrenergic, β-adrenergic, cholinergic, or adenosinergic). The mechanisms of action vary considerably and most have multiple effects so a compound designed to correct one problem might cause another.

Over 130 nonantiarrhythmic drugs now on the market have published or unpublished evidence of prolongation and ~60 of those have official warnings attached, but the same compound may have different warning levels in different countries. In one multicountry study of drugs dispensed from pharmacies, an estimated 13–20 doses per 1000 persons per day had the potential to lengthen the QT interval. Most of the drug withdrawals from marketplace since 1990 have been due either to

Table 1 Nonantiarrhythmic drugs associated with QT prolongation

Class name	*Reports*[a,b]	*Class name*	*Reports*[a,b]	*Class name*	*Reports*[a,b]
GI prokinetics		Opioids		Antiasthmatics	
Cispride	P, N, O	Levacetylmethadol	O	Fenoterol	P, O
Domperidone	*P, N*	Methadone	*N*	Procaterol	P
		Pethidine	*N*	Salbutamol	P, O
				Salmaterol	P, O
Antiemetics		Antimigraine agents			
Dolasetron	P, N, O	Naratriptan	O	Antihistamines	
Granisetron	P, N	Sumatriptan	O	Astemizole	P, N, O
Ondansetron	P, N, O	Zolmitriptan	O	Azelastine	*O*
				Cetirizine	N
Cardiovascular Drugs		Antipsychotics		Chlorpheniramine	*N*
Bepridil	P, N, O	Amisulpride	*P, O*	Clemastine	*N*
Diltiazem	N	Chlorpromazine	P, N, O	Cyproheptadine	*N*
Indapamide	P, N	Clozapine	P, N	Diphenhydramine	*P, N*
Indoramin	*P*	Droperidol	P, N, O	Ebastine	P, N
Isoprenaline	P, N	Haloperidol	P, N, O	Emedastine	*O*
Isradipine	P, N, O	Mesoridazine	*O*	Epinastine	*P, N*
Ketanserin	P, N	Olanzapine	*P, N, O*	Fexofenadine	P
Lidoflazine	N	Pimozide	P, N, O	Loratidine	N
Losartan	*N*	Prochlorperazine	*P, O*	Mizolastine	P, N, O
Methoxamine	*P, N, O*	Quetiapine	*P, O*	Oxatomide	*P*
Mibefradil	O	Risperidone	N, O	Promethazine	*P, N*
Nicardipine	*N*	Sertindole	P, N, O	Pyrilamine	*N*
Perhexiline maleate	*P, N*	Sultopride	*P, N, O*	Terfenadine	P, N, O
Prenylamine	*P, N*	Thioridazine	P, N, O		
Triamterene	P	Tiapride	*P*	Miscellanea	
Trimetaphan	*P, N, O*	Trifluoperazine	*N*	Amantadine	*P*
Verapamil	N	Ziprasidone	P, O	Antimony sodium gluconate	*P*
Vincamine	*P*	Zotepine	*O*	Arsenic trioxide	P, O
				Bupropion	*P*
Antibacterials		Antidepressants		Chloral hydrate	*P*
Clarithromycin	P, N	Amitryptyline	P, N, O	Dexfenfluramine	*N*
Clindamycin	*P*	Citalopram	*P, N*	Famotidine	*P*
Cotrimoxazole	*P, N*	Clomipramine	*P*	Felbamate	*O*
Erythromycin	P, N, O	Desipramine	P, O	Fenoxedil	*P*
Gatifloxacin	N, O	Doxepin	P, N	Foscarnet	*O*
Grepafloxacin	P, N, O	Fluoxetine	*P, O*	Fosphenytoin	*O*
Levofloxacin	P, N	Imipramine	P, N	Glibenclamide	*P, N*
Moxifloxacin	P, N, O	Maprotiline	*P*	Hydroxazine	*P, N*
Roxithromycin	P, N	Mianserin	*P, N*	Mitoxantrone	*N*
Sparfloxacin	P, N, O	Nortriptyline	P, N	Octreotide	*O*
Spiramycin	*P*	Paroxetine	*P*	Papaverine	*P*
		Protriptyline	*P*	Pentamidine	P, O
Systemic Antimycotics		Trazodone	*P*	Probucol	P, O
Fluconazole	P	Vanlafaxine	*O*	Radiographic contrast media	P, N
Ketoconazole	P, N	Zimeldine	*P, N*	Ritanserin	*P*
				Sildenafil	*N*
Agents used in general anaesthesia		Antimalarials		Tacrolimus	P, N
Enflurane	P, N	Chloroquine	P	Tamoxifen (high doses)	P, N, O
Fentanyl	*N*	Halofantrine	P, N, O	Terodiline	P, N
Halothane	P, N	Mefloquine	P, O	Tizanidine	*O*
Isoflurane	P, N	Quinine	P, O	Vasopressin	*P*
Ketamine	*N*			Vesnarinone	*N*
Pentobarbital	*N*				
Propofol	P, N				
Sevoflurane	P, N				
Sufentanil	P, N				
Thiopental	P, N				

[a] Italics indicate poorly documented evidence.

[b] P, published clinical evidence; N, published nonclinical evidence; O, official warning (from Public Assessment Reports by the European Agency for the Evaluation of Medicinal Products, the Physician Desk Reference, Dear Doctor letters from the FDA, and the British Formulary.

From information compiled by Fabrio De Ponti, Elisabetta Poluzzi, Andrea Cavalli, Maurizio Recanatini, and Nicolo Montanero.

indications of QT interval prolongation or to actual cardiac problems. Categorization shows the compounds belong to many different pharmacological classes and the mechanisms of action are diverse. The compounds themselves must be examined on an individual basis; some have QT prolongation and associated cardiac-related fatalities (terfenadine/Seldane®) while some had noticeable prolongation without any associated fatalities (moxifloxacin/Avelox®). Validated reports show that QT prolongation or cardiac arrhythmia with these other implicated compounds most often happen in combination with other compounds that also increase the risk.

The time it takes for a compound to affect the QT interval or to cease its effects upon discontinuation depends greatly on the compound. For example lidocaine and amiodarone are both drugs with antiarrhythmic properties but lidocaine's effects on the QT interval start within minutes when administered intravenously and end in a few hours as the drug clears the body, while the effects of amiodarone generally take 1–3 weeks to manifest and as long (or longer) to subside when discontinued.

QT interval testing is included in the trials of most new drugs entering the marketplace, with exceptions being mostly for compounds closely related to known compounds with no effect. The considerations for risk include the chemical and/or pharmacological class, an ion channel assay, an *in vivo* QT assay in a nonrodent species, and a repolarization assay or other follow-up studies as warranted. Despite extensive testing and consideration, it is often not clear in clinical studies whether a compound may have significant cardiotoxicity, first because the numbers used in clinical trials are often too low for the needed statistical power, and second because clinical trials do not involve the drug interactions usually associated with adverse events.

Summary

Examination of the QT interval is an important part of studies of cardiac health because of its ability to indicate potentially fatal cardiac arrhythmias before they happen even though the causes of the arrhythmias are diverse. Due to this diversity and significant normal variations in the QT interval, there is no generally accepted exacting standard of measurement or interpretation of changes in the QT interval but there are several guidelines that are in general agreement. The drug industry and its regulators regularly examine the potential of candidate drugs and released compounds to alter the QT interval.

Drugs Withdrawn for Correlation with Torsade de Pointes

Terfenadine/Seldane® (antihistamine, February 1998), Cisapride/Propulsid® (GI prokinetic, July 2000), Grepafloxacin/Raxar® (antibiotic, November 1999), Sertindole (antipsychotic, December 1998), and Astemizole (antihistamine, June 1999). See **Table 1** for a list of nonantiarrhythmic drugs associated with QT interval prolongation.

See also: Digitalis Glycosides; *hERG* (Human Ether-a-Go-Go Related Gene); Potassium; Safety Pharmacology; Sodium.

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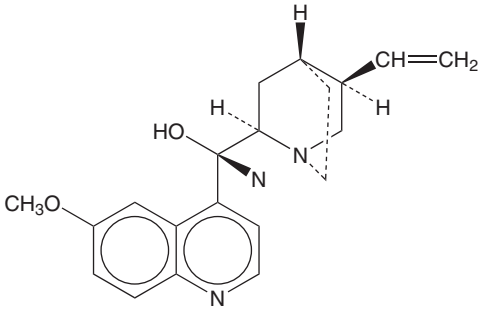
Quantitative Structure–Activity Relationship *See* Toxicity Testing, Modeling.

Quinidine

Dennis J Naas

This article is a revision of the previous print edition article by Bridget Flaherty, volume 3, pp. 2–3, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 56-54-2
- Synonyms: Cin-Quin; Quinidine sulfate; Quinidine gluconate (CAS 7054-25-3); Quinidine polygalacturanate (CAS 27555-34-6); Quinaglute; Chinidin; Cinchonan-9-ol, 6′-methoxy-; Conchinin; Conquinine; (9*s*)-6′-Methoxycinchonan-9-ol; α-(6-methoxy-4-quinolyl)-5-vinyl-2-quinuclidinemethanol; NCI-c56246; Pitayine; *β*-quinine; Quinidine hydrate (CAS 63717-04-4); Quinidex
- Chemical/Pharmaceutical/Other Class: Class IA (membrane stabilizing) antiarrhythmic agent; The dextrorotary isomer of quinine
- Chemical Structure:



Uses

Quinidine is used to treat and control atrial fibrillation and atrial flutter. Quinidine is also approved to treat premature ventricular contractions and to treat paroxysmal atrial tachycardia or paroxysmal atrioventricular junctional rhythm. It may also be used to treat malaria, although quinine is preferred.

Exposure Routes and Pathways

Ingestion is the most common route of exposure in both accidental and intentional poisonings. Quinidine is also available in intravenous and intramuscular forms.

Toxicokinetics

Quinidine undergoes extensive hepatic oxidative metabolism. The bioavailability of quinidine is up to 90%. Peak plasma effects occur in 1–3 h. Sustained-release preparations produce peak plasma levels in 5 or 6 h. Quinidine is up to 90% protein bound, but it is lower in pregnant women, and in infats and neonates it may be as low as 50–70%. The volume of distribution is 2 or $3\,l\,kg^{-1}$. Congestive heart failure can lower the volume of distribution to $0.5\,l\,kg^{-1}$ while in patients with cirrhosis of the liver this can be increased to 3 or $5\,l\,kg^{-1}$. Up to 80% of quinidine undergoes hepatic hydroxylation. The remainder ($\sim$20% of a therapeutic dose) is eliminated unchanged in the urine. Quinidine generally has a plasma half-life of 6–8 h in healthy individuals, but half-life may range from 3 to 16 h or longer. Longer half-lives are reported for malaria patients and those with chronic liver disease. Quinidine crosses the placenta and is distributed into milk.

Mechanism of Toxicity

Quinidine has direct and indirect, or antimuscarinic, effects on cardiac tissue. Quinidine decreases myocardial excitability, conduction velocity, and contractility. As quinidine concentrations increase, conduction velocity progressively decreases. This is evident in an increase in PR interval, an increase in QRS duration, and an increase in QT interval. The effective refractory period is prolonged by quinidine. The anticholinergic effect on the heart is a decrease in vagal tone. In overdose, sinus node automaticity may be depressed. It is the α-adrenergic blocking properties of quinidine that cause vasodilatation and hypotension.

Acute and Short-Term Toxicity (or Exposure)

Human

Acute overdosage can result in both cardiovascular and neurologic effects. Ventricular dysrhythmias and hypotension are the most serious toxicities. Cardiac effects occur as a result of myocardial depression and depression of atrial, atrioventricular, and ventricular conduction. EKG changes will be evident. These EKG changes include a widening of the QT, PR, and QRS complexes; ST depression; and T inversion. Myocardial depression and vasodilation can cause hypotension to develop. Syncope can result from transient Torsade de Pointes (i.e., bursts of atypical ventricular tachycardia). Ventricular tachycardia and ventricular fibrillation may develop. Possible central nervous system (CNS) effects include lethargy, seizures, and coma. Other acute effects can include apnea. Signs of toxicity are expected to occur in

adults ingesting a gram or more. Therapeutic plasma levels of quinidine range from 1 to 4 $\mu g\,ml^{-1}$. Cardiac toxicity can occur with levels of at least 14–16 $\mu g\,ml^{-1}$.

Chronic Toxicity (or Exposure)

Human

With chronic toxicity, gastrointestinal symptoms are common. Nausea, vomiting, and diarrhea are generally seen. The toxidrome known as cinchonism can occur in chronic toxicity. Effects include headache, fever, visual disturbances, mydriasis, decreased hearing or tinnitus, nausea, vomiting, hot flushed skin, rash, and CNS impairment (lethargy, memory impairment, delirium, hallucinations) and may present without cardiotoxicity, other than QT prolongation. Cinchonism can occur when quinidine plasma levels are at least 5 $\mu g\,ml^{-1}$. Loss of vision can occur when levels are at least 10 $\mu g\,ml^{-1}$.

Clinical Management

Basic and advanced life-support measures should be used as needed. Induction of emesis is not recommended due to the potential for a decreased level of consciousness, seizures, and arrhythmias. Gastric lavage followed by activated charcoal is recommended. Repeated doses of activated charcoal may enhance elimination. Serum electrolytes should be monitored in all serious exposures. Intravenous administration of sodium bicarbonate may decrease toxicity. Hypotension can be treated with fluids and vasopressors if needed. Ventricular dysrhythmias can be treated with class IB antiarrhythmics such as phenytoin or lidocaine. Persistent bradycardia and third-degree heart block are indications for insertion of a temporary pacemaker. Seizures can be treated with diazepam. If seizures are uncontrolled, phenobarbital or phenytoin can be administered.

See also: Cardiovascular System; Quinine.

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Quinine

Dennis J Naas

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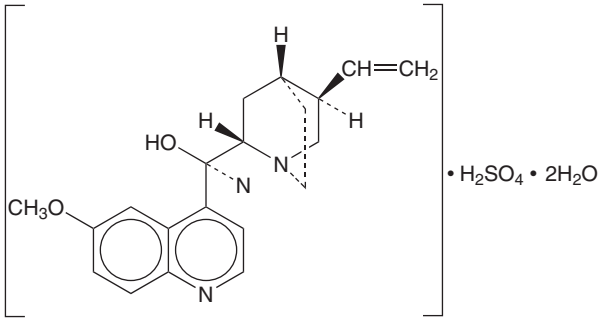
- Chemical Abstracts Service Registry Number: CAS 130-95-0
- Synonyms: Quinine sulfate (preferred name); Chininum; Quinina; Quinine bisulfate; Chinine; Cinchonan-9-ol, 6′-methoxy-; (8α,9r), chinin; 6′-Methoxycinchonan-9-ol; 6-Methoxycinchonine; α-(6-Methoxy-4-quinolyl)-5-vinyl-2-quinuclidinemethanol; α-(6-Methoxy-4-quinoyl)-5-vinyl-2-quinclidinemethanol; 6-Methoxy-α-(5-vinyl-2-quinuclidinyl)-4-quinolinemethanol; 2-Quinuclidinemethanol; α-(6-Methoxy-4-quinolyl)-5-vinyl
- Chemical/Pharmaceutical/Other Class: Antimalarial
- Chemical Structure:



Uses

Quinine is the drug of choice for the treatment of malaria; it is also used in the treatment of nocturnal leg cramps. It is often misused as an abortifacient.

Exposure Routes and Pathways

Quinine is available in oral dosage forms. Ingestion is the most common exposure pathway.

Toxicokinetics

Quinine is rapidly absorbed orally. It is metabolized in the liver by oxidation to several polar hydroxy metabolites. The volume of distribution is 1 or $2\,l\,kg^{-1}$ and protein binding is 70%, although plasma binding of 90% or more has been reported in malaria patients. Quinine is excreted by the kidneys; ~10% is excreted as unchanged drug. The therapeutic half-life of quinine is 11.1 ± 4.1 h, and may be longer in malaria patients due to hepatic impairment. The half-life can more than double at toxic doses to 26.5 ± 5.8 h.

Mechanism of Toxicity

The exact mechanism of toxicity is unknown. Quinine acts on all body muscle groups, most notably cardiac, uterine, and skeletal muscles.

Acute and Short-Term Toxicity (or Exposure)

Human

Accidental and intentional ingestions have resulted in headache, deafness, blindness, tachycardia, respiratory arrest, and death. Reversible renal failure can occur. The adult toxic dose can be as low as 2 g. Death from intentional or accidental overdose generally follows renal failure, acute hemolytic anemia, and respiratory arrest.

Chronic Toxicity (or Exposure)

Animal

Quinine has not been found to be carcinogenic in mammalian studies. Developmental effects were found when the drug was used in rabbits and guinea pigs, but not when drug was used in mice, rats, dogs, and monkeys.

Human

Cinchonism can arise from cumulative dosing. Clinical symptoms associated with the syndrome of cinchonism include headache, dizziness, tinnitus, rash, cardiovascular effects, intestinal cramping, vomiting, diarrhea, fever, confusion, and seizures. The symptoms resolve with cessation and elimination of the drug. Sensitization to quinine and quinidine have been observed (eczema, itching). Even at therapeutic dosages, an enhanced pseudohemophiliac effect can occur through the triggering of thrombocytopenia.

In Vitro Toxicity Data

There was no evidence of mutagenicity in animal studies in mice or *Salmonella typhimurium*; however, positive results were observed in studies in *S. typhimurium* when mammalian liver homogenate was added.

Clinical Management

Quinine should not be used during pregnancy. Recommended treatment includes gastric decontamination with gastric lavage and repeated doses of activated charcoal. Intensive monitoring of vital signs and the EKG are important. Quinine levels may be useful to confirm exposure. Following gastric lavage, the symptomatic therapy for acute poisonings includes atropine for bradycardia and phenytoin in the presence of tachycardic heart rhythm disorders. Forced diuresis and hemodialysis are not suitable as therapeutic measures. Monitor plasma and serum potassium levels. If refractory arrhythmia develops, assess calcium and magnesium. Other treatment may include administering a Stellate block for quinine-induced blindness and the use of vasodilators for residual visual impairment. Hemolytic–uremic syndrome following quinine ingestion is a newly described phenomenon. The reaction may be mediated by the presence of antibodies reactive against platelets in the presence of quinine. Treatment has included use of plasma exchange, prednisone, aspirin, and dipyridamole. Patients have all regained some degree of renal function. However, it is unclear whether pharmacological treatment or spontaneous resolution is responsible for the improvement.

See also: Chloramphenicol; Chloroquine.

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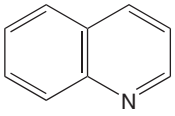
Klaassen C (ed.) (2001) *Casarett and Doull's Toxicology, The Basic Science of Poisons*, 6th edn. New York: McGraw-Hill Professional.

Quinoline

David R Wallace

- Chemical Abstracts Service Registry Number: CAS 91-22-5
- Synonyms: l-Azanaphthalene; l-Benzazine; Benzo(*b*)pyridine
- Chemical/Pharmaceutical/Other Class: Solvent and by-product of petroleum processing
- Chemical Formula: C_9H_7N
- Chemical Structure:



Uses

Quinoline is used as an intermediate in the production of quinoline-related compounds (e.g., 8-hydroxyquinoline). It is a solvent for resins and terpenes, and is used in the production of paint. Quinoline is also an antimalarial agent. Sources of quinoline include petroleum and coal processing, wood preservation and the use of shale oil.

Exposure Routes and Pathways

The major routes of exposure for quinoline are inhalation and oral ingestion. Contaminated air from petroleum distillation, coal mining, coking, and release from shale oil can lead to inhalation exposures. Quinoline is also found in cigarette smoke.

Toxicokinetics

Quinoline can undergo either detoxification (major pathway) or bioactivation (minor pathway). Less than 1% of administered quinoline is excreted unchanged in the urine.

Mechanism of Toxicity

Quinoline undergoes phase I metabolism to form an enamine oxide, a rapid transitional epoxide, which can then form DNA adducts. This epoxide is formed on the pyridine moiety of quinoline. Fluorination at position 3 completely prevents the mutagenicity of quinoline. The major metabolic enzyme is the CYP2E1 isoform with the primary end-product from this reaction being 3-hydroxyquinoline.

Acute and Short-Term Toxicity (or Exposure)

Available data have described acute or subchronic exposure. Due to higher mortality in long-term studies, little direct evidence is available to describe the actions of quinoline following chronic exposure.

Animal

Animals fed a diet that contained 0.05%, 0.1%, and 0.25% quinoline exhibit robust tumor formation and significant early mortality, which was concentration-dependent. Mean survival time decreased ~50% between the lowest and highest concentrations. Additional studies have confirmed these findings and extended them with the inclusion of proper control groups. There was an increase in the incidence of hepatic and lung tumors as well as hemangioendotheliomas. It is believed that these tumors are due to the formation of the reactive metabolic intermediate. Further investigation has shown that quinoline itself can act as a promoter of liver carcinogenicity. Treatment with quinoline in conjuction with the hepatic carcinogen diethylnitrosamine elicited significantly greater tumor formation compared to diethylnitrosamine treatment alone. Quinoline also appears to promote skin tumor formation.

The effects of quinoline on the central nervous system have also been examined. There is a structural similarity between the basic backbone of quinoline and the dopaminergic neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), but there was no evidence that quinoline was a dopaminergic neurotoxin.

Human

No acute studies of humans exposed to quinoline have been reported.

Chronic Toxicity (or Exposure)

There have been no studies of chronic quinoline effects which have exceeded 40 weeks in animals. These studies were terminated prior to this time due to the early identification of tumors and premature mortality. The studies described here relate to the carcinogenic effects of quinoline. No reports of chronic exposures in humans are available.

In Vitro Toxicity Data

In vitro mutagenicity of quinoline has been reported in *Salmonella typhimurium*, possibly through base

pair substitution. It has been determined that the cytochrome P450 monooxygenase system, particularly the CYP2E1 isoform, is responsible for the formation of the reactive epoxide intermediate. Recently, data have indicated that not only is quinoline a tumor-promoter, but is also an initiator of tumor formation. Quinoline appears to exert its toxic effects in both a genotoxic and mitogenic fashion.

Clinical Management

There are no antidotes for quinoline exposure. The patient should be removed from the source of quinoline exposure and symptomatic treatment given if necessary. Individuals exposed to quinoline may complain of severe eye irritation. Persons in occupations with possible exposure to quinoline should take measures to minimize their exposure.

Environmental Fate

If quinoline is released into water it will degrade dependent on the temperature and microbial conditions. Complete degradation can be expected to occur in less than a week. If ground soil is contaminated with quinoline, it will quickly partition to groundwater. Less than 0.5% of the quinoline will be expected to remain in the soil.

Exposure Standards and Guidelines

Quinoline has been labeled as a group B2 agent, 'probable human carcinogen, which is likely to be carcinogenic in humans based on animal data', due to significant evidence in animal models. Establishment of reference concentrations and allowable levels of exposure have been difficult to obtain due to the limited nature of animal studies (oral exposure, no inhalation studies). Computational modeling, using the Weibull model, yielded a potency of $3.07\,mg^{-1}kg^{-1}day^{-1}$ from an LED_{10} of $32.55\,\mu g\,kg^{-1}day^{-1}$. Animal experiments have suggested an oral LD_{50} of $331\,mg\,kg^{-1}$ in the rat and a dermal LD_{50} of $540\,mg\,kg^{-1}$ in rabbit.

See Also: Quinidine; Quinine.

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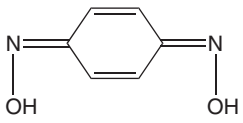
Quinone

Sachin S Devi and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 106-51-4
- Synonyms: Benzoquinone; *p*-Benzoquinone; Cyclohexadienedione; 1,4-Cyclohexadienedione; 2,5-Cyclohexadiene-1,4-dione; 1,4-Dioxybenzene; 1,4-Benzoquinone
- Chemical/Pharmaceutical/Other Class: Organic compounds
- Chemical Formula: $C_6H_4O_2$
- Chemical Structure:



Uses

Quinone is used as a chemical intermediate, a polymerization inhibitor, an oxidizing agent, a photographic chemical, a tanning agent, and a chemical reagent. It is also used in the manufacture of hydroquinone, in fungicides, as an analytical reagent, in photography, as a chemical intermediate, and as an oxidizing agent.

Background Information

Quinone is formed as yellow crystals and has a characteristic irritating odor like that of chlorine. It is slightly soluble in water, alcohol, ether, hot petroleum ether, and alkalis. Quinone is an oxidizing agent and is reduced to hydroquinone. It has been declared a federal hazardous air pollutant and was identified as a toxic air contaminant in April 1993 under AB 2728.

Exposure Routes and Pathways

Quinone may be released to the environment in effluents during its commercial production and use; and in wastewaters from the coal industry. If released to soil it is likely to leach and may volatilize and photodegrade on soil surfaces. A single degradation study found that quinone rapidly degraded in a chernozem soil to stable metabolites. If released to the aquatic environment, it may be degraded by photolysis as it absorbs ultraviolet radiation. In water, it is not expected to volatilize, adsorb to particulate matter or sediment, or bioaccumulate in aquatic organisms. Biodegradation in water may be important based upon the rapid degradation of quinone in soil. If released to the atmosphere, it will react rapidly in the vapor phase with both hydroxyl radicals and ozone with half-lives of 3.6 and 3.3 days, respectively, and may be susceptible to direct photolysis.

Toxicokinetics

There is complete reduction of a *p*- or *o*-quinone to the corresponding hydroquinone or catechol, respectively. In the human liver, carbonyl reductase may play a role in the reduction of some quinones. Catechols are primary substrates for catechol *o*-methyl transferase, but also undergo sulfation. However, for the antitumor quinones, mitomycin C, adriamycin, and daunomycin, two-electron reduction serves as an efficient bioactivation mechanism, elegantly affirming the concept of 'bioreductive alkylation' for the preferential bioactivation of antitumor prodrugs with oxygen deficient tumors.

Mechanism of Toxicity

The acute narcotic effects are due to the physical interaction of quinone itself on the cells of the central nervous system (CNS). The long-term effects are most likely due to the production of an unstable reactive intermediate during biotransformation.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute exposure of quinone leads to dermatitis, hypomelanosis, and delayed hyperpigmentation. It is a skin irritant, which may cause redness, swelling, and necrosis.

Human

Vomiting and gastrointestinal tract irritation have been seen with quinones. Nonspecific liver changes and jaundice have been reported. Ingestion of quinone results in dyspnea, anoxia, and respiratory failure. It also causes asphyxia and pulmonary damage. Cyanosis and cardiovascular collapse have also been reported.

Chronic Toxicity (or Exposure)

Animal

Quinones may lead to paralysis of the medullary centers and coma. Albuminuria and hematuria may occur. Chronic exposure of quinones to laboratory animals has resulted in skin irritation and has also caused redness of the skin.

Human

Quinone is known to cause eye irritation with chronic dust or vapor exposure. Keratitis, corneal ulceration, and discoloration of the conjunctiva may occur. Workers exposed chronically to these compounds may develop a reddish discoloration of the hair. No epidemiological data relevant to the carcinogenicity of quinone are available. There is inadequate evidence in experimental animals for the carcinogenicity of 1,4-benzoquinone. Overall evaluation: 1,4-benzoquinone is not classifiable as to its carcinogenicity to humans (group 3).

Clinical Management

1. *Emesis*: Ipecac-induced emesis is not recommended because of the potential for CNS depression and seizures.
2. *Activated charcoal*: Charcoal should be administered as a slurry (240 ml water per 30 g charcoal). Usual dose: 25–100 g in adults/adolescents, 25–50 g in children (1–12 years), and 1 g kg^{-1} in infants less than 1 year old.
3. *Gastric lavage*: Lavage should be considered after ingestion of a potentially life-threatening amount of quinones if it can be performed soon after ingestion (generally within 1 h).
4. *Eye exposure*: Decontamination: Exposed eyes should be irrigated with copious amounts of room temperature water for at least 15 min. If irritation, pain, swelling, lacrimation, or photophobia persists, the patient should be seen in a healthcare facility.
5. *Dermal exposure*: Decontamination: Contaminated clothing should be removed and the exposed area washed thoroughly with soap and water. A physician may need to examine the area if irritation or pain persists.

Environmental Fate

Quinone exists in the atmosphere in the gas phase. The dominant atmospheric loss process for quinone is expected to be by reaction with the hydroxyl (OH) radical (reaction with ozone is expected to be slow because of the $>C(O)$ substituent groups). The estimated half-life and lifetime of quinone in the atmosphere due to reaction with the OH radical are ~ 3 and 4 h, respectively.

See also: Hydroquinone.

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Quintozene *See* Pentachloronitrobenzene.

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Radiation Toxicology, Ionizing and Nonionizing

Bobby R Scott and Raymond A Guilmette

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Radiation toxicology is a specialized area of toxicology that relates to both characterizing radiation exposure of humans and evaluating the expected health consequences of the radiation exposure. It is a well-researched discipline that provides a wealth of knowledge about both the adverse and beneficial effects of radiation exposure. The indicated research covers the molecular, cellular, systemic, organism, and population levels.

Because everyone is exposed to low radiation doses from natural and human-generated ionizing radiation sources during their lifetimes, it is important to understand the potential adverse effects from such exposures as well as those that could occur as a result of high-level radiation exposures associated with infrequent events such as nuclear accidents, radiotherapy for cancer, or exposure as a result of use of a radiological (dirty bomb) or nuclear weapon.

This section provides a brief summary of the current knowledge of radiation-induced health effects together with certain basic elements of radiation toxicology needed to understand the concepts and terminology that are associated with this discipline.

Ionizing and Nonionizing Radiation

Radiation is energy in the form of waves or particles. The energy associated with any radiation can be transferred to matter. This transfer of energy can remove electrons from the orbit of atoms leading to the formation of ions. The types of radiation capable of producing ions in matter are collectively called 'ionizing radiation'. There are two general types of ionizing radiation: particulate and electromagnetic. Particulate ionizing radiations include alpha (α) particles, beta (β^-) particles, positrons (β^+; positive electrons), neutrons, protons, and heavy ions (i.e., charged heavy nuclei). Ionizing electromagnetic radiation includes X-rays and gamma rays.

Electromagnetic radiation with insufficient energy-causing ionization is called nonionizing radiation. Examples are ultraviolet (UV) radiation, radio frequency radiation (which includes microwaves), and extremely low frequency (ELF) radiation (associated with electric power lines).

The field of radiation toxicology has mainly focused on ionizing radiation. The sections that follow relate only to ionizing radiations.

Physical Characteristics of Ionizing Electromagnetic Radiation

Electromagnetic ionizing radiation energy is generally expressed in units such as kilo electron volts (keV) or million electron volts (MeV). Because gamma rays and X-rays have similar energies, they produce similar biological damage when delivered in the same manner and evaluated at the same dose. The different terminology reflects the difference in their origin, that is, gamma rays occur as a result of energy released from unstable atoms seeking a lower nuclear energy state, whereas X-rays occur as a result of energy releases by over-energized atomic electrons.

When rearrangement of orbital electrons occur as a result of ionization of an atom, the X-rays produced are called characteristic X-rays and have discrete energy values unique to the atom from which they arise. Gamma rays also have a discrete energy that is characteristic of the nuclide from which they are emitted.

When electrons that have been accelerated by a positive, high-voltage field collide with a target such as tungsten (as in an X-ray machine), the electron loses energy in the form of electromagnetic radiation (called bremsstrahlung radiation). In this type of interaction, the emitted radiation can assume a continuum of energy values up to a maximum value. Bremsstrahlung radiation is frequently encountered by astronauts in space travel as a result of the interaction of space radiation with the transport vehicle.

Gamma rays and X-rays are among the most penetrating types of radiations. Gamma rays are produced in the nuclear reactions that take place in nuclear power plants. They were also associated with the nuclear weapons that were detonated in Hiroshima and Nagasaki, Japan.

X-ray machines are used at most hospitals for diagnostic purposes. Since both X-rays and gamma

rays are not charged, they interact with matter primarily by the photoelectric effect (dislodging electrons), Compton scattering (bouncing off of an electron which is then released from its atom), and pair (negative and positive electron) production.

With pair production all of the energy of the incident photon (component of which X-rays and γ-rays are comprised) is lost in the production of the electron/positron pair. In the interaction of X-rays or γ-ray photons with atoms, electrons can therefore be ejected (e.g., via Compton scattering) from irradiated media atoms and carry with them energy transferred to them by the incidence photon. These 'secondary electrons' then interact with the surrounding media producing additional ionizations in the same manner as beta particles.

As γ- and X-ray photons pass through matter, the photon intensity decreases exponentially because of energy losses associated with interactions with electrons. The photon intensity (I) at a distance of penetration d is expressed as a fraction of the initial intensity I_0 using the following equation:

$$\frac{I}{I_0} = e^{-\mu d}$$

where μ is the media density-dependent, attenuation coefficient in the medium for the energy considered (in units of $cm^2\,g^{-1}$). The penetration distance d is expressed here in $g\,cm^{-2}$ (equal to distance in cm $\times$ density of the absorbing media).

The penetrating ability of photons depends on both the photon energy and the composition of the absorbing media, with penetration increasing with increasing energy, and decreasing density and effective atomic number of the medium. The penetrating ability of photons is sometimes described by a half thickness or half-value layer (HVL), which is the thickness of an absorbing medium that decreases the photon intensity by one-half. For example, the HVL for 1 MeV gamma rays is $\sim$9 m of air, 9.6 cm of water, 4 cm of aluminum, and 9 mm of lead.

Physical Characteristics of Ionizing Particulate Radiation

Of the various ionizing particulate radiations, the most important in terms of likelihood for human exposure are alpha particles, beta particles, protons, and neutrons. Alpha and beta particles occur as a result of the radioactive decay of unstable atoms. Neutrons generally result from nuclear reactions, such as nuclear fission (as in nuclear reactors and fission-based nuclear weapons) and charged-particle activation of target atoms (as with some accelerator-produced radioisotopes). Protons arise from atomic interactions of neutrons.

Alpha Particles

Alpha particles have a positive charge and are identical with helium nuclei and consist of two protons and two neutrons. They result from the radioactive decay of heavy elements such as radium, thorium, uranium, and plutonium. Because of their double-positive charge, alpha particles have great ionizing power, but their large mass results in very little penetration. For example, alpha particles from 4 to 10 MeV have ranges in air of 5–11 cm; the corresponding range for alpha particles in water would be from 20 to 100 μm.

Beta Particles

Beta particles are equivalent to electrons but arise from radioactive decay of unstable atoms. They are emitted with a continuous range of energies up to a maximum that is characteristic of each radionuclide. Electrons have a greater range and penetrating power but much less ionizing potential compared to alpha particles. The range of beta particles in air is $\sim$4 m per MeV of energy. In water the range in cm is approximately one-half the maximum beta energy when expressed in MeV. For example, the range of the energetic beta particles from yttrium-90 (maximum energy 2.27 MeV) is $\sim$1.15 cm in water and similarly in soft tissue.

Neutrons

Neutrons are neutrally charged particles with a mass slightly larger than that of a proton. Because they are neutrally charged, they produce ionizations indirectly. In biological material, neutrons can eject protons from the nuclei of hydrogen atoms by nuclear collisions, which in turn are charged and directly ionizing. Neutrons can also activate hydrogen and other elements by neutron capture, which results in the release of gamma rays, and sometimes radioactive by-products. Neutrons that are produced by fission or by special sources such as americium–beryllium sources have a spectrum of energies that range over many orders of magnitude. Since their ranges depend on both neutron energy and the composition of the absorbing material, it often requires complex calculations to describe the ranges of neutrons. However, as simple examples, very low-energy neutrons (called thermal neutrons) have a range of $\sim$30 cm in soft tissue, whereas high-energy or fast neutrons can penetrate tissue to about the same extent as can highly penetrating gamma rays.

Energy Loss by Electromagnetic and Particulate Ionizing Radiations

Ionization Patterns

A single initial or secondary ionizing particle passing through matter deposits its energy in a stochastic (random) and nonuniform manner on a microscopic scale. This deposited energy creates positively and negatively charged molecules and atoms (called ion pairs) along the path (or track) traveled by the ionizing particle. The density of ion pairs produced along a track varies significantly depending on the ionizing particle and the medium through which it passes and is proportional to the average energy deposited per unit path length. For example, secondary electrons produced by 200 kV X-rays create ~ 80 ions per μm path length; protons generated from interactions with 12 MeV neutrons produce ~ 300 ions μm^{-1}; and alpha particles from the decay of radium-226 produce ~ 3700 ions μm^{-1}.

Linear Energy Transfer

The ionization patterns produced by different charge particles (e.g., electrons, protons, helium ions, heavy charged nuclei) relate to their linear energy transfer (LET). LET is the average energy loss in traversing a small thickness of the target medium of interest and depends on the energy of the charged particles. Gamma rays, X-rays and neutrons have no charge. Their energy loss is mainly associated with secondary charged particles they produce. For typical X-rays (which produce electrons), LET ranges from ~ 0.2 to 15 keV μm^{-1}; for fast neutrons (which produce protons) from ~ 8 to 40 keV μm^{-1}; and for alpha particles greater than ~ 260 keV μm^{-1}. As mentioned previously, the efficiency with which a particular type of radiation produces biological effects depends strongly on LET. Further, when biological cells are hit by charged particles associated with a given radiation source, the spatial distribution of the hit cells depends strongly on LET. Because intercellular signaling can have an important influence on the outcome of radiation exposure, it is important to consider the spatial distribution of hit cells when explaining observed biological effects. The field of microdosimetry deals with the frequency and spatial distribution of cells hit by primary- and secondary-charge particles associated with ionizing radiation sources.

Dosimetric Quantities and Units

Radiotoxicology, like other disciplines of toxicology, has specialized quantities that define the relationships between exposure to radiation and the resulting dose received by specific biological entities. Some of these quantities are based on measurements and/or calculations; others, particularly those used in radiation protection, consist of theoretical quantities that include modifying factors designed to allow comparison of risks to people exposed to a variety of radiation types and with widely varying spatial patterns of dose.

Units that Apply to Radioactivity

The defining event of a radioactive nuclide is the transformation of its nucleus into the nucleus of another species, that is, radioactive decay. The number of nuclear transformations occurring per unit of time is called 'activity'. Sometimes 'radioactivity' is used instead of 'activity'. The traditional unit of activity has been the Curie (Ci), which is equal to 3.7×10^{10} nuclear transformations per second. The conversion of radiation units to the international system (Syst̨me International d'Unit or SI) has now taken place in the United States. The more fundamental unit of activity, the Becquerel (Bq), equal to 1 nuclear transformation per second, has replaced the Curie. Both units of activity are modified by prefixes such as kilo-, milli-, and micro- to achieve standard multiples of the fundamental unit. A listing of the most commonly used prefixes is given in **Table 1**.

Units that Apply to Radiation Exposure

In radiation physics, the term 'exposure' is used to describe the amount of ionization caused in air by

Table 1 Standard multiples used with radiation units

Prefix	*Multiplication factor*	*Symbol*
exa	10^{18}	E
peta	10^{15}	P
tera	10^{12}	T
giga	10^{9}	G
mega	10^{6}	M
kilo	10^{3}	k
hecto	10^{2}	h[a]
deca	10^{1}	da[a]
deci	10^{-1}	d[a]
centi	10^{-2}	c[a]
milli	10^{-3}	M
micro	10^{-6}	μ
nano	10^{-9}	n
pico	10^{-12}	p
femto	10^{-15}	f
atto	10^{-18}	a

[a] It has been suggested that all SI units be expressed in 'preferred standard form' in which the multiplier is 10^{3n} where n is a positive or negative whole number. Consequently the use of hecto, deca, deci, and centi is to be avoided wherever possible.

gamma and X-rays. The unit of exposure is the Roentgen (R), which is equal to 2.58×10^{-4} coulomb kg^{-1} of air. This quantity is most often used in diagnostic radiology and does not apply to ionizations produced by either particulate radiations or high-energy (>3 MeV) X-rays or gamma rays. For radiobiological applications, the exposure rate (e.g., in R min^{-1}) is most commonly used.

Absorbed Dose Units

The most commonly used quantity describing radiation dose is the absorbed dose (D), which is defined as the mean energy, e, imparted by ionizing radiation to matter of mass m divided by the mass, that is, $D = e/m$. This quantity is a measurement of the deposition of energy in any substance by all types of ionizing radiation. It applies to macroscopic but not to microscopic masses. The traditional unit of absorbed dose is the rad, which is equal to 100 ergs g^{-1} or 0.01 J kg^{-1}; the corresponding SI unit is the Gray (Gy), which is equal to 1 J kg^{-1} (therefore, 1 Gy is equivalent to 100 rad). The absorbed dose should be used in preference to the exposure whenever the former can be measured or reliably calculated. In this section we have used the rad unit to be consistent with our use in the 1998 publication of this section. However, in many of the current research journals, the mGy or Gy unit is preferred over the rad.

Microdosimetric Units

When viewed at the microscopic level of a cell, or smaller biological subunit, the dose D is replaced by what is called specific energy (z). While D is a macroscopic dose, z is a microscopic dose. For a single absorbed dose D to an organ or tissue, there can be many different microscopic doses z to cells in that organ or tissue. In addition, a cell may have no dose (i.e., $z = 0$) at all, while another cell in the same tissue may have a very large dose. However, when z is averaged over the microscopic targets in the macroscopic mass of interest (e.g., organ), the average value obtained should equal the absorbed dose D as defined previously.

LET Units

As previously indicated, the LET is the average rate of energy loss in traversing a small thickness of the target medium. The unit generally attributed to LET is keV μm^{-1} path length.

Quality Factor

The quality factor (Q) is a dimensionless quantity used to make adjustments for differing qualities for different radiations in producing biological damage. The factor Q takes into account the type of radiation and other factors. It also relates to LET. The greater the value for Q, the greater the biological damage produced by a given type of radiation.

Dosimetric Units Used in Radiation Protection

For radiation protection purposes, several theoretical dosimetric quantities have been created that attempt to 'normalize' the responses of different tissues and organs of the body from irradiation by different types of ionizing radiation so that uniform radiation protection guidelines can be promulgated that are insensitive to the particulars of any given irradiation scenario. The traditionally used quantity has been the dose equivalent (DE), which is defined as the absorbed dose (D) multiplied by the quality factor Q. The unit of dose equivalent has been the rem, which is dimensionally the same as the rad; the SI unit is the Sievert (Sv). Recently, the DE has been replaced by a similar concept called the equivalent dose. The equivalent dose depends on the relative biological effectiveness rather than on Q.

Current radiation protection guidelines are specified in terms of a quantity called the effective dose (E). The effective dose is presumed to have associated with it the same probability of occurrence of cancer and genetic effects whether received by the whole-body, via uniform irradiation, or by partial-body or individual-organ irradiation. To take into account the observed varying radiosensitivities of the different organ systems of the body and to adjust for nonuniformity of irradiation, a tissue weighting factor, W_t, is used. An additional radiation weighting factor, W_r, is used to adjust for the biological effectiveness of different radiations. The current weighting factors, as stated by the National Council on Radiation Protection and Measurements, are summarized in **Table 2**.

Relative Biological Effectiveness

Since equal doses of different types of ionizing radiations do not produce equivalent biological effects, a quantity called the relative biological effect (RBE) was developed to allow comparison of effects produced in identical biological systems from different types of radiations. The RBE is customarily defined as the ratio of two doses (a reference dose divided by a test dose) for producing a given level of biological effect under a given condition. The reference is often taken to be X-rays. For example, if 90% cell killing is produced by 10 Gy of X-rays (D_x), but only 0.5 Gy of neutrons (D_n) is needed for 90% killing, then the RBE in this case would be $D_x/D_n = 10\,Gy/0.5\,Gy = 20$. Thus, RBE has no units. The RBE influences both the equivalent dose and the effective dose used in radiation protection.

Table 2 Radiation and tissue weighting factors used in radiation protection guidelines[a]

Radiation type and energy range	W_r
X-rays and gamma rays, electrons and positrons	1
Neutrons, energy	5
<10 keV	10
10–100 keV	10
>100 keV to 2 MeV	20
>2–20 MeV	10
>20 MeV	5
Protons	2
Alpha particles, fission fragments	20
Organ or tissue	W_t
Gonads	0.20
Red bone marrow	0.12
Colon	0.12
Lung	0.12
Stomach	0.12
Bladder	0.05
Breast	0.05
Liver	0.05
Esophagus	0.05
Thyroid	0.05
Skin	0.01
Bone surface	0.01
Remainder	0.05

[a] W_r is the radiation weighting factor, and W_t is the tissue weighting factor.

Sources of Ionizing Radiation Exposure

Humans are routinely exposed to ionizing radiation. Some of the sources are naturally occurring, and others are due to man-made uses of radiation and radioactive materials. In general the radiation from natural sources includes cosmic radiation, external radiation from radionuclides in the earth's crust, and internal radiation from radionuclides inhaled or ingested and retained in the body. Man-made sources of radiation include X-ray equipment, particle accelerators and nuclear reactors used in the generation of nuclear energy, radionuclides used in nuclear medicine, radionuclides released to the environment as a result of nuclear weapons testing or a nuclear accident, and occupational exposure to both external and internal radiation. The magnitude of the exposure to natural sources depends mostly on geographical location, whereas exposure to man-made sources depends on human activities.

Natural Background Radiation

Exposure to natural sources of external ionizing radiation results from the levels of cosmic and terrestrial X and gamma radiation present in the environment. Cosmic radiation at the earth's surface is affected by altitude, geomagnetic latitude, and solar modulation. For example, the dose rate at 1800 m is about double that at sea level. Within the United States, the effect of latitude and solar modulation on cosmic ray dose rate is $<10\%$. Because cosmic radiation is highly penetrating, it results in relatively uniform whole-body irradiation. The average dose rate from cosmic irradiation in the United States has been estimated to be ~ 28 mrem year^{-1}.

Humans are also exposed to external gamma radiation from concentrations of naturally occurring radioactive materials in soils and rocks. These radioactive elements include uranium and thorium radionuclides plus their radioactive progeny and potassium-40, and result in widely varying dose rates that depend on the geology of the particular region. Estimates of the annual dose rate for this type of exposure in the United States averages 28 mrem year^{-1}.

Internally deposited naturally occurring radionuclides also contribute to the natural radiation dose from inhalation and ingestion of these materials when contained in air, food, and water. Included are radionuclides of lead, polonium, bismuth, radium, potassium, carbon, hydrogen, uranium, and thorium. Potassium-40 is the most prominent radionuclide in normal foods and human tissues. The dose to the total body from these internally deposited radionuclides has been estimated to be ~ 39 mrem year^{-1}.

The major exposure of the population to natural radiation arises from inhalation of the short-lived radioactive progeny of the radioactive noble gas radon-222, which in turn is a sixth-generation radioactive decay product of natural uranium. The amount of radon-222 present in the air depends on many factors (e.g., gas permeability in soil and rock, relative humidity, and barometric pressure) but is necessarily linked to the geological concentration of the uranium parent radionuclide. There is about an eightfold range of concentrations of uranium in different types of rocks and soils.

Most of the early measurements of radon levels were made outdoors; however, it has become apparent that the indoor concentrations are generally several times higher than those outdoors. Because people in Western countries spend only $\sim 15\%$ of their time outdoors, most of the exposure to radon therefore occurs indoors. Additionally, the trend toward the construction of more energy-efficient housing (more air-tight) has also enhanced the concentrations of radon-222 indoors.

The average annual radiation dose to the general population due to inhalation of radon and its progeny is estimated to be ~ 200 mrem. However, this dose can range upward by 1 or 2 orders of magnitude in cases in which the indoor radon concentrations are very high. Because of the short

half-lives of the radon progeny, and the fact that the most important radionuclides decay by α-particle emission, their radiation dose is delivered primarily to the tissues of the respiratory tract.

Man-Made Radiation Sources

Several human activities involving the production and use of radionuclides as well as the development of nuclear weapons have resulted in releases of radioactive materials into the environment. Such activities include past atmospheric testing of nuclear weapons, production of nuclear weapon materials, production of electricity by nuclear reactors, radioisotope production and use in industry and medicine, accidental releases of radionuclides at both civilian (Three Mile Island and Chernobyl) and military (Kyshtym and Windscale) nuclear installations, and intentional releases (Mayak Plutonium Production Facility). Additionally, there has been a significant increase in the types of quantities of sources of potential radiation exposure from consumer products. These include radioluminescent devices containing tritium, promethium-147, or radium-226; smoke detectors containing americium-241; static eliminators containing polonium-210; and airport X-ray baggage inspection systems. In other cases, radiation emissions are incidental or extraneous to the purpose for which the consumer product was designed, for example, television receivers, tobacco products containing polonium-210 and lead-210, combustible fuels and building materials containing uranium- and thorium-series radionuclides, and gas mantles, camera lenses, and welding rods containing thorium.

A summary of the contributions of the various natural and man-made radiation sources to our radiation background is given in **Table 3**. It can be seen that natural sources contribute ~82% of the total, with radon being the largest single source (67% of natural radiation dose). Of the 18% contributed by man-made sources, medical exposure is the most prominent (83%). Attempts to significantly reduce population radiation doses would most likely be focused on the largest contributors, that is, indoor radon and medical radiation.

Now there is growing realization of the possibility of the use of radiological weapons (dirty bombs) by terrorist organizations. With such weapons, radioactive material could be dispersed in populated areas. Such acts could lead to a variety of problematic health and environmental consequences. The National Council on Radiation Protection and Measurement has recently published a valuable reference entitled 'Management of Terrorist Events Involving Radioactive Material'. The report provides guidance on the management of radiation casualties in such circumstances.

Table 3 Radiation exposure of the US general population

Radiation source	*Per capita annual effective dose equivalent (mrem)*
Natural radiation	
Cosmic rays	28
Terrestrial, external	28
Internally deposited radionuclides (except radon)	39
Inhaled radon and progeny	200
Sources due to or enhanced by human activity	
Medical uses	53
Nuclear power[a]	0.05
Consumer products	8
Weapons fallout (averaged to year 2000)	5
Total	361

[a] Includes contributions from uranium mining and milling, fuel fabrication, power plant operation, reprocessing of spent fuel, and transportation.

Radiobiological Effects

Radiation-induced biological effects include alterations in expressed genes and proteins, oncogene activation, suppressor gene inactivation, chromosomal aberrations, mutations, cancer, genetic effects, and loss of normal tissue and organ functions. Although the biological effects of concern from exposure to ionizing radiation are described typically at the tissue or organ level, it has long been recognized that an understanding of the mechanisms by which radiation produces effects such as cancer, genetic changes, and tissue destruction are best obtained from studies performed at the cellular and molecular levels.

Direct and Indirect Radiation Effects

At the chemical level, a solute molecule (DNA, RNA, and protein) in a biological system can be affected by radiation in two different ways. When an ionization track passes either directly through a molecule or close enough so that the created ions can drift to and interact chemically with the molecule before they recombine and neutralize in solution, the phenomenon is called a direct radiation effect. On the other hand, since the largest fraction of almost any biological system consists of water (e.g., 70–80% of a typical cell), the most frequent initial radiation interactions will be with water molecules. When this occurs, ion radicals and free radicals are created.

When irradiated, water molecules become ionized in a two-step process:

$$H_2O + \text{radiation} \rightarrow H_2O^+ + e^-$$

$$e^- + H_2O \rightarrow H_2O^-$$

However, the charged water molecules are unstable, having lifetimes less than 10–15 s, and almost immediately dissociate into one smaller ion and a free radical:

$H_2O^+ \rightarrow H^+ + OH^\bullet$ (the dot symbolizes the unpaired electron of the OH free radical)

$$H_2O^- \rightarrow H^\bullet + OH^-$$

The free radicals thus produced are very reactive and diffuse through the solvent system interacting in a fairly indiscriminate manner with other free radicals, with molecules previously damaged by radiation, or, most important, with intact solute molecules previously unchanged by the radiation. Free radical reactions may also produce other more or less reactive chemical species, such as H_2, H_2O, and H_2O_2, they may react with oxygen to enhance the effect of the radiation, or they may interact with organic molecules creating organic free radicals:

$$H_2O^- + RH \rightarrow R^\bullet + H_2O_2$$

$$RH + HO_2^\bullet \rightarrow RO^\bullet + H_2O$$

This latter phenomenon is called an indirect radiation effect. If RH is an important molecule (e.g., DNA and RNA) then these interactions can affect cell functions.

Effects of Radiation on Cells

Depending on type and quantity, radiation can produce a variety of effects on cells. These effects include chromosomal aberrations, apoptosis (programmed cell death), necrotic cell death, mutations, neoplastic transformation (an early step in cancer induction), altered cell-cycle regulation, alterations of metabolic functions, and changes in intercellular signaling characteristics. As more studies are done using the tools of molecular biology, more insights into the mechanisms of radiation action are being found. This knowledge plays a key role in the development of biologically based risk models that are used to extrapolate results from studies performed at relatively high radiation doses and dose rate to low-radiation doses and dose rate. Establishing a reliable low dose and dose-rate cancer risk model is of greatest interest and importance for the protection of people and the environment from radiation damage.

Deleterious and Protective Bystander Effects

Recently it has been learned that bystander cells not directly hit by radiation can also be impacted via other mechanisms in addition to reactive oxygen species. Since the nonhit cells are bystanders, the effect has been given the special name 'bystander effect'. In the case of high-LET α-particle research, new devices called microbeams have been developed that allow a single cell to be hit by charged helium ions (same as alpha particles) and for the nearby and distant nonhit cells to be examined for possible biological effects. These studies show that some unhit cells can also develop chromosomal aberrations, mutations, and can be neoplastically transformed. Biological substances released by the hit cells appear to play an important role in occurrence of bystander effects. Simply irradiating the medium containing the cells does not lead to bystander effects.

In contrast to the observations with high-LET radiation, other studies using low dose, low-LET gamma rays (>0–10 rad) have shown that such low doses can turn on a protective process that removes spontaneous transformants (neoplastically transformed cells), presumably reducing the risk of cancer *in vivo*. This protective process has been studied in detail by German researchers at the University of Freiburg related to their cancer prevention research and involves cross-talk (signaling) between nontransformed and transformed cells. Transforming growth factor beta and reactive oxygen species are involved in cross talking. In the case of transformed cells, the net effect is that at least some neoplastically transformed cells that are present are selectively eliminated via apoptosis (i.e., the cells undergo self destruction). This process was recently given the name Protective Apoptosis-Mediated (PAM) process. This process is discussed in an in-press paper by the first author of this section and colleagues. The PAM process is a form of natural protection against problematic cells (e.g., mutant cells, neoplastically transformed cells) and seems quite important in protecting humans from cancer occurrence. However, the PAM process does not appear to be turned on by low doses of high-LET alpha radiation.

Cell Survival

Cell survival curves are used to describe the relationship between radiation dose and the proportion of cells that survive. Usually, mathematical models are used to describe cell survival data. Survival of

normal cells is an important consideration in radiation therapy. It is the cancer cells that the therapist wants to destroy, while to the extent possible, protecting normal cells.

The endpoint of survival can have two different meanings depending on whether the cell populations studied have proliferative potential. In the case of nondividing, terminally differentiated cells, survival is generally related to the ability of the cells to maintain function. In general, relatively large radiation doses are required to inactivate cell function for terminally differentiated cells. For cells that proliferate either in tissue or in culture, cell death or survival is more related to ability of that cell to continue to divide and produce clones of cells. Thus, a cell that is able to undergo no more than one or two cell divisions after irradiation would still be considered 'dead'. Doses on the order of a few rad are generally required to kill the most sensitive proliferating cells, although there is a range of radiosensitivities to this effect. For example, normal human fibroblasts irradiated with X-rays have a D_0 of 120 rad (D_0 is the dose at which survival is reduced to 37% of its original value); in comparison, cells from a patient with the disease ataxia-telangiectasia (AT) have a D_0 of $\sim$50 rad and are therefore more radiosensitive – a hallmark of AT. The heightened radiosensitivity relates to a deficiency in DNA repair for persons with AT.

Studies using experimental designs in which only certain selected portions of a cell were irradiated have shown that the sensitive sites for radiation-induced cell killing are in the nucleus as opposed to the cytoplasm. Furthermore, there is strong circumstantial evidence that the DNA in the chromosomes is the primary target for radiation-induced cell killing.

Cell and Tissue Radiosensitivity

The sensitivity of various cells and cell lines to radiation-induced lethality can differ significantly, and different organs consist of different cell populations (e.g., connective tissue and vascular, parenchymal). As early as 1906, Bergonié and Tribondeau studied cellular radiosensitivity and postulated that actively proliferating cells were most radiosensitive, that the degree of cellular differentiation was inversely related to radiosensitivity, and that radiosensitivity of cells was proportional to the duration of mitotic and developmental activity. In general, this 'law' is valid for different cell types, although there are exceptions. For example, the small lymphocyte, which is highly differentiated and has little if any proliferative potential, is one of the most radiosensitive mammalian cells. One scheme for categorizing the sensitivity of normal cells to cell killing is the following:

- *Very high*: Lymphocytes, immature hematopoietic cells, intestinal epithelium, spermatogonia, and ovarian follicular cells.
- *High*: Urinary bladder epithelium, esophageal epithelium, gastric mucosa, and mucus membranes.
- *Intermediate*: Endothelium, growing bone and cartilage, fibroblasts, glandular epithelium of breast, pulmonary, renal, pancreatic, thyroid, and hepatic epithelia.
- *Low*: Erythrocytes, muscle cells, mature connective tissue, osteocytes, chondrocytes, and ganglion cells.

The effects of radiation on more complex organ systems will depend on the effects produced on the different subpopulations of cells that comprise the organ. For example, if the parenchymal cells are most radiosensitive (as in intestinal mucosa), then loss of function of the organ may occur at the lowest doses, followed perhaps by vascular damage at higher doses. If the parenchymal cells are normally nondividing (as in the brain), then the reverse may occur, with damage to the microcirculation predominating at lower doses.

Genetic Effects

Radiation Effects on Inheritance

Radiations such as X-rays, gamma rays, and beta particles can damage genetic material in reproductive cells and cause mutations that can be transmitted from one generation to another. In the 1920s, researchers using fruit flies (*Drosophila*) found that chromosomes could be easily injured by radiation and such injury could lead to mutations that were expressed in subsequent generations. This finding was quickly confirmed in numerous species of plants and animals. Today it is known that relatively small radiation doses ($<$10 rad) can cause alterations in nucleotides and visible breaks in chromosomes of germ cells that can lead to genomic instability that can be passed on to subsequent generations.

In evaluating the effect of radiation on heredity of germ cells, two specific germ-cell stages are considered important: (1) the stem-cell spermatogonia in males and (2) the oocytes, primarily the immature ones, in the female. The spermatogonia continue to multiply throughout the reproductive lifespan of an individual. However, oocytes are not replaced during adult life.

Because of the lack of information for humans, most genetic studies have been carried out with

experimental organisms, especially mice. Radiation has been found to cause mutations in all nonhuman experimental organisms studied and therefore such effects are also expected to arise in humans.

The genetic effects that could be caused by radiation are too numerous to be considered individually. For nuclear accident risk assessment, genetic disorders have been grouped as (1) dominant and X-linked single-gene disorders, (2) chromosome disorders, and (3) multifactorial disorders.

Dominant and X-Linked Single-Gene Disorders

Most cells from humans contain two sets of chromosomes with matched pairs of genes, one gene from each parent. The matched genes can differ, with one gene being dominant over its recessive counterpart. A recessive gene can only show its effect if both matching chromosomes carry that gene. If an altered gene is present on the X chromosome it will invariably produce an effect in boys, who have only one X chromosome, but will behave as recessive in girls, who have two X chromosomes. Single-gene disorders related to damage to the X chromosome are called X-linked effects.

Dominant gene disorders that could be caused by radiation include traits such as Huntington's chorea, hypercholesterolemia, and achondroplastic dwarfism. The X-linked traits include traits such as muscular dystrophy, hemophilia, and agammaglobulinemia. However, there is no direct evidence that these diseases have been induced in humans by irradiation.

Chromosome Disorders

Two forms of genomic damage that depend on radiation quality (i.e., LET) are the induction of single-strand (SS) and double-strand breaks (DSBs). The two types of damage are considered to be important because SSBs are more easily and accurately repaired by the cell than are DSBs. Thus, DSBs result in damage that is both more lethal and more able to result in chromosome disorders. For low-LET radiation, increased production of DSBs is a function of dose rate, as single tracks are so sparsely ionizing that breaking more than one chromosome with a single track is unlikely, especially at low radiation doses; therefore, DSBs arise as a consequence of multiple tracks occurring sufficiently close in time and space. On the other hand, high-LET radiation produces a high enough ionization density within its tracks that DSBs can occur from single traversals of a cell nucleus. This in part is responsible for the greater RBE for high-LET radiation for cell killing, mutation induction, cell transformation, and cancer induction.

Damage that is produced by radiation can be chromosomal or chromatid, depending on whether the cell is in a pre- or postreplication state. In either case, sufficient energy is imparted to break a chromosome or chromatid, usually into a major and a minor fragment. Once this has occurred, (1) the broken ends may rejoin to restore the original configuration of the chromosome; (2) a fragment may fail to rejoin, resulting in a deletion, which is sometimes large enough to be scored as a micronucleus; or (3) broken ends may rejoin with other broken ends to yield abnormal forms that are subsequently scored at the following mitosis as rings, dicentrics, anaphase bridges, or symmetric and asymmetric translocations.

Chromosome anomalies and aberrations can influence heredity. Most somatic cells of humans contain 23 pairs of chromosomes, with one member of each pair contributed by the sperm and the other contributed by the egg. When the process of sperm or egg cell production goes awry as a result of radiation damage, abnormal chromosome numbers (aneuploidy) can arise. Aneuploidy is a form of genetic instability.

It has been estimated that in $\sim$90% of cases, aneuploidy will result in spontaneous loss of pregnancy. In the remaining 10% of cases, a severely affected child would be expected because of the inherited genomic instability. Conditions such as Down's syndrome and both Klinefelter and Turner anomalies are the result of genomic instability associated with aneuploidy. These defects are relatively severe – in terms of both life expectancy ($\sim$45 years) and level of disability ($\sim$50%). Persons born with aneuploidy usually are physiologically and morphologically abnormal and do not have children. Thus, their genomic instability tends not to be passed on to other generations.

Chromosomes can be easily broken by radiation which can lead to a structural rearrangement (called a translocation). Translocations are also a form of genomic instability. When translocations occur in germ cells, they can be transmitted to the offspring. Translocations normally yield chromosomes with too little or too much genetic information. If a child is born with a balanced translocation (not too little or too much information) he or she would not normally be affected but could pass on genomic instability to future generations. Those born with such genomic instability could suffer from severe physical and mental disabilities.

Multifactorial Disorders

Multifactorial diseases involve complex patterns of inheritance and represent a very large class of genetic

diseases. For such diseases to arise, a specific combination of mutant genes must be present. Environmental factors can also be important. Examples of multifactorial diseases include congenital malformations (e.g., spina bifida and cleft palate), constitutional diseases, and degenerative diseases.

Genetic Effects in Irradiated Populations

Epidemiology has not detected hereditary effects of radiation in humans with a statistically significant degree of confidence. Nevertheless, there can be no doubt of the existence of hereditary effects in man. Following radiation exposure of a large population (e.g., as occurred in the former Soviet Union (FSU) after the Chernobyl nuclear accident in 1986), an increase in the incidence of genetic disease would be expected to occur. The genetic damage would show up both early (as an increased incidence of birth defects among some children of the exposed population) and late (through latent mutations expressed in their grandchildren, great-grandchildren, and subsequent generations). It has been estimated that $\sim 50\%$ of all genetic damage introduced by radiation exposure following a major nuclear accident will be manifest within the first three to five subsequent generations, with the remaining damage dispersed over future generations.

Early and Continuing Deterministic Effects

If a person is exposed to a large amount of radiation (i.e., large radiation dose) delivered to the entire body, cells in tissues can be destroyed in large numbers. Because tissues have important functions, the destruction of significant numbers of cells can lead to impairment in one or more of these functions. The biological effects that arise when large numbers of cells are destroyed by radiation are called 'acute somatic effects' if they occur in a relatively short period of time (e.g., within a few weeks) after brief exposure. Acute somatic effects are a subset of what is now formally called 'early and continuing deterministic effects' (once called nonstochastic effects).

Deterministic effects are those that increase in severity as the radiation dose increases and for which a threshold is presumed to exist. Besides acute somatic effects, deterministic effects also include radiation effects (other than cancer and genetic effects) that continue to occur after an extended period (e.g., years) of chronic exposure. Such chronic exposures can arise from long-lived radionuclides (e.g., isotopes of plutonium and cesium) ingested via contaminated food or inhaled via contaminated air and retained in the body. Populations in Russia, Ukraine, and Belarus continue to ingest and inhale long-lived radionuclides that were released during the 1986 nuclear accident at Chernobyl. Firemen who fought the reactor fire during the Chernobyl accident and plant workers present at the time of the accident were chronically exposed to large radiation doses from inhaled radionuclides.

Examples of deterministic effects are hypothyroidism arising from large radiation doses to the thyroid gland; skin burns arising from exposure of small or large areas of the skin; permanent suppression of ovulation in females; temporary suppression of sperm production in males; growth and mental retardation caused by exposure of a fetus during pregnancy; and death from severe damage to critical organs such as the bone marrow, lung, or small intestine.

Thresholds arise for deterministic effects because large numbers of cells usually must be simultaneously destroyed to produce such effects, which is highly unlikely at low doses. The threshold dose for a specific deterministic effect depends on the type of radiation, on the rate at which the dose is delivered (dose rate), and, for some effects, on other factors.

Factors Affecting the Production of Deterministic Effects

The type of radiation is important because different types of radiation interact with body tissue differently. Gamma rays and X-rays can easily penetrate into body tissue and therefore can produce deterministic effects in all body organs if the dose and amount of tissue irradiated are both large enough. Beta radiation can cause skin burns and ulcers when beta-emitting hot particles (highly radioactive, very small particles) are deposited on the skin, but little damage is likely to be done to other tissue unless the beta-emitting particles are taken into the body in large amounts (e.g., by inhalation or ingestion). Alpha radiation does not cause skin burns or ulcers when alpha-emitting particles are deposited on the skin because alpha radiation does not have enough energy to penetrate the dead layer of tissue that covers the skin surface. However, when taken into the body in large amounts, alpha-emitting particles can cause deterministic effects.

For total-body exposure to X-rays or gamma rays, organs and tissue at risk include all organs and tissue in the body. For inhalation or ingestion exposure to beta-emitting materials, organs and tissue at risk include the lungs and gastrointestinal tract as well as other sites depending on the metabolic fate of the radionuclide of concern. For example, strontium isotopes preferentially irradiate the skeleton, while

Table 4 Threshold gamma or X radiation doses (lower, central, and upper estimates) for specific deterministic effects[a]

Effect	*Organ/tissue*	*Lower bound (rad)*	*Central (rad)*	*Upper bound (rad)*
Vomiting	Upper abdomen	Not estimated	50	Not estimated
Diarrhea	Upper abdomen	Not estimated	100	Not estimated
Erythema	Skin[b]	200	300	400
Moist desquamation	Skin[b]	800	1000	1200
Permanently suppressed ovulation	Ovum in females	20	60	100
Suppressed sperm counts[c]	Testes in males	20	30	40
Cataracts	Lens of eye	0[d]	100	150
Hypothyroidism	Thyroid	Not estimated	200	Not estimated
Radiation pneumonitis	Lung	400	500	600
Hematopoietic death[e]	Bone marrow	120	150	180

[a] Applies to gamma rays or X-rays delivered to the indicated organ or tissue in less than 1 h.
[b] For 50–100 cm^2 area of skin and the dose evaluated at a depth of 0.1 mm.
[c] Two-year suppression of sperm counts in males.
[d] Used to include the possibility that cataracts may be a stochastic effect with no threshold.
[e] Death from lethal injury to the sensitive bone marrow.

iodine isotopes preferentially irradiate the thyroid. When considering possible deterministic effects from inhaled radionuclides, organs other than the lung should also be considered because radionuclides can translocate from the lung to other organs such as the liver and skeleton.

Radiation dose and dose rate are important because the larger the dose, the larger the amount of potentially destructive radiation energy deposited in tissue, which can lead to extensive cell death and concomitant impairment in important tissue functions. A significant impairment can lead to morbidity and lethality. Likewise, radiation dose rate is important because when it is sufficiently high, radiation can overwhelm cell repair mechanisms and organs cannot recover from tissue injury. Most efficient recovery occurs when the radiation dose rate is low and when the amount of tissue that the radiation interacts with is small. In the administration of radiation therapy to cancer patients, physicians try to minimize damage to healthy tissue by delivering the radiation in a number of fractions over a number of days or weeks. This allows damaged normal tissue to recover during the periods between the fractionated exposures. The rate of recovery differs for different organs.

Other factors that can be important in determining the impact of radiation exposure include a person's age and sex, how healthy they are, and the type of medical support received from physicians after being injured by radiation.

Thresholds Doses for Specific Deterministic Effects

For nuclear accident risk assessment, organs of primary interest because of their high sensitivity or their potential for receiving large radiation doses are bone marrow, gastrointestinal tract, thyroid gland, lungs, skin, gonads, and eyes. **Table 4** shows estimates (central, lower bound, and upper bound) of threshold doses for a variety of deterministic effects of exposure to gamma rays when the dose is delivered quickly (within 1 h). Larger doses would apply when the dose is delivered over hours, days, weeks, or longer. For example, the central estimate of the γ-ray threshold for acute lethality from radiation-induced injury to the hematopoietic system is 150 rad (see **Table 4**) when the dose is delivered within an hour. However, when the dose is delivered continuously over several years, individuals have survived γ-ray doses as high as 600–1000 rad (which would be fatal if received within a few hours). Nuclear workers in the former Soviet Union (Mayak workers) who participated, during the late 1940s through mid-1950s, in the production of plutonium for nuclear weapons received large γ-ray doses (up to $\sim$1000 rad in some cases) over several years and survived.

Table 5 shows estimates (lower bound, central, and upper bound) of thresholds for specific deterministic effects of exposure of the unborn embryo or fetus to X or gamma rays delivered quickly (within 1 h).

Late Somatic Effects

Late somatic effects are those that occur long after exposure to a DNA-damaging agent in progeny of cells other than germ cells. The late somatic effect that is of most concern is cancer.

Induction of Cancer by Ionizing Radiation

One of the first observations of cancer following irradiation was the appearance of skin cancer on the

Table 5 Thresholds (lower, central and upper estimates) for deterministic effects of exposure of the unborn embryo or fetus[a]

Effect	*Time/period* [b]	*Lower bound (rad)*	*Central (rad)*	*Upper bound (rad)*
Small head size	0–17 weeks	5	10	Not estimated
Severe mental retardation	8–15 weeks	0	10	20
	16–25 weeks	0	20	50
Death of embryo or fetus	0–18 days	0	10	50
	18–150 days	20	40	50
	150–term (days)	120	150	180

[a] Applies to X or gamma rays delivered within 1 h.
[b] Refers to time after conception in days or weeks.

hands of some of the early workers who used X-rays. Since that time, animal and epidemiological studies have shown that radiation can cause an increase in the incidence of specific cancers. They have also shown that cancer does not appear immediately after exposure to radiation but only after a delay (latent period). For humans, the latent period may be quite long (many years) for some cancers.

Mechanisms that may be involved in the induction of cancer by radiation have been proposed. These mechanisms include (1) the induction of mutations, (2) the activation of oncogenes, (3) the inactivation of tumor suppressor genes, and (4) the induction of cancer-causing viruses. Although the relative importance of the various mechanisms in the induction of cancer is not clear, more than one mechanism could be involved for a given type of cancer.

For both humans and laboratory animals, one cannot currently distinguish between a radiation-induced cancer and a spontaneously occurring cancer (i.e., from an unknown cause). Therefore, statistical methods are used to determine whether radiation exposure is associated with an increase in cancer in a given study population. There have been several epidemiological studies in which definite dose–response relationships have been established for radiation-induced cancers. The best studied populations include atomic bomb survivors, *Tinea capitis* irradiation patients, ankylosing spondylitis irradiation patients, radium dial painters, radium therapy radium-224 patients, Thorotrast patients, uranium miners, Chernobyl fallout victims, and Mayak plutonium facility workers.

Atomic Bomb Survivors

Within a 3 day period in August 1945, atomic bombs were dropped on the Japanese cities of Hiroshima and Nagasaki, killing a total of 64 000 people within 1 km of the explosions as a result of blast, thermal effects, and instantaneous gamma and neutron irradiation. Since that time, a prospective epidemiological study has been conducted by a joint group of United States and Japanese scientists (the Radiation Effects Research Foundation; RERF) on $\sim$92 000 survivors who were within 10 km of the center of the respective blasts and $\sim$27 000 others who were not in either city at the time of the explosions. The study includes detailed dose reconstruction for $\sim$76 000 individuals and medical follow-up on as many of the survivors as possible. As the follow-up has continued, the RERF has periodically published updates of the cancer incidence and mortality data for these populations. Data as of 1988 showed a total of 3435 cancers, of which 357 were radiation induced. From these data, excess cancer risks are calculated which form the basis for many of the current radiation risk factors in use today. It should be noted that a large fraction of the atomic bomb survivors are still alive, particularly those who were irradiated as children, so that additional information can be anticipated as this population continues to be studied.

Tinea capitis Irradiation

From 1905 to 1960, X-ray irradiation of the scalp for treating ringworm, *T. capitis*, was regularly performed on as many as 200 000 children worldwide. For a typical series of X-ray treatments, doses of 220–540 rad were received by the scalp, 140 rad to the brain, 380 rad to the cranial marrow, and $<$100 rad to other organs and tissues of the head and neck. Cancers of the thyroid and skin (basal cell carcinoma) were the major consequences of irradiation.

Ankylosing Spondylitis Irradiation

About 14 000 patients with the disease ankylosing spondylitis received X-ray therapy between 1935 and 1954 in Great Britain and Northern Ireland. In irradiating the spine, doses of 300–700 rad were received by tissues in the thoracic region. The major radiation-related outcome has been an excess of leukemia due to irradiation of bone marrow progenitor cells within the ribs and vertebrae and, recently, an indication of excess solid tumors in the lungs,

esophagus, and breast. The importance of this study has been in the health effects from partial-body irradiation and in the temporal pattern of appearance of solid tumors.

Radium Dial Painters

Radium, as radium-226 and radium-228, was used in luminous paints during 1920–50. Large amounts of radium were ingested by painters of watch and instrument dials as they tipped their brushes by mouth to achieve a fine point. The radium, once ingested, behaves chemically like calcium and, therefore, deposits in significant quantities in bone mineral, where it is retained for a very long time. Being an alpha-emitting radionuclide, the radium irradiates bone surface-lining cells and has resulted in an excess incidence of osteogenic sarcomas. Of interest in these patients has been the observation of a 'practical threshold' of dose and dose rate from radium-226, below which bone cancers do not appear to occur. This has also been observed in some experimental animal studies.

Radium Therapy (^{224}Ra)

In Europe, the short-lived radionuclide (3.6 day half-life) radium-224 was used for more than 40 years in the early 1900s in treating tuberculosis and ankylosing spondylitis. Because of its effectiveness as an analgesic in treating debilitating bone pain from the latter, its use has continued. Radium-224, being an alpha-emitting radionuclide that deposits on bone surfaces, delivers its radiation dose effectively to bone-lining cells, inducing an excess of osteogenic sarcomas, similar to those found in the radium dial painters. Interestingly, no excess of leukemia cases has been found in this population, even though portions of the hematopoietic precursor cell populations are purportedly within range of the alpha-particle irradiation.

Thorotrast Patients From 1928 to the 1950s, a preparation of the radioactive, colloidal thorium dioxide (Thorotrast) was used extensively as an X-ray contrast medium in angiographic studies. Because of the very high density of thorium to X-rays and the tendency of the colloidal particles to be taken up by the fixed phagocytes within the liver and spleen, it was effective in diagnostic imaging of these organs. However, because Thorotrast is chemically insoluble *in vivo* and is retained tenaciously for long times, long-term alpha irradiation of liver, spleen, and bone marrow tissues occurred, with a resultant large increased incidence of various liver carcinomas and sarcomas. In this case and unlike the results from radium exposure, an excess incidence of leukemia has been observed.

Uranium Miners

As part of the radioactive decay series of uranium, radon-222, a radioactive noble gas, emanates from geological deposits. During underground mining, this gas was released to the work space, and miners inhaled both this gas and its radioactive progeny in significant amounts. Epidemiological studies have been done on mining populations from the United States, Canada, Australia, Czechoslovakia, France, China, and Sweden. Their results have shown conclusively that inhalation exposure to radon and progeny is a strong risk factor for lung cancer, both with and without concurrent exposure to cigarette smoke. This database is used to project lung cancer risk for exposure of the general population to radon in indoor environments.

Chernobyl Fallout Victims

The nuclear reactor accident that occurred in Chernobyl in April 1986 released large quantities of radionuclides to the environment. The contamination was highest near the reactor, with significant fallout also occurring in the western part of the former Soviet Union and spreading to many parts of western Europe. At this point, the medical follow-up of the populations who lived near the reactor has found only one significant disease attributable to the radiation from the accident, that is, thyroid cancer in persons who were children at the time of the accident. The radiation dose to the thyroid was due to inhalation and ingestion of radioactive iodine isotopes released when the reactor core was breached; estimates indicate that the doses to children's thyroids ranged upward to as high as 1000 rad. The relatively high incidence of thyroid cancer is significant in that it was not expected based on extrapolation from the results of the atom bomb survivor study.

Mayak Plutonium Facility Workers

In the southern Ural Mountains region of Russia are the cities of Yekaterinburg, with a population of $\sim$1.4 million and 125 miles south, Chelyabinsk with a population of about 1.1 million. Shortly after the end of World War II, in the former Soviet Union construction was begun on a nuclear weapons production complex (called the Mayak Production Association (PA)) in the region. Established nearby to house workers was a secret, closed city. Because of the secrecy, the city was originally known by its postal destination, Chelyabinsk-40 (later Chelyabinsk-65). The city is no longer a secret and is called

Ozyorsk and has a population of ~90 000 persons. The main purpose of the Mayak PA was plutonium production for nuclear weapons. The Mayak PA complex included nuclear reactors, radiochemical and plutonium plants, and associated nuclear waste facilities. In the earliest years (1948–52) of operations at the facility, massive quantities of radioactive materials were released into the nearby Techa River leading to ingestion of mainly beta- and gamma-emitting radionuclides by inhabitants of villages along the river. In addition, workers at the Mayak facility inhaled large amounts of plutonium over a period of years. These workers were also exposed chronically to large doses of gamma rays.

International studies are being conducted to investigate cancer occurrences among the thousands of plutonium-239-exposed Mayak workers. Plutonium-239 is an alpha-emitting radionuclide. For the Mayak workers exposed to alpha radiation from inhaled plutonium-239 along with external gamma rays, excess cases of lung, liver, and bone cancers have been demonstrated. Several studies are ongoing that relate to a variety of health effects among the Mayak PA workers as well as to dose reconstruction.

Excess radiation-induced cancers have also been demonstrated in well-controlled studies using laboratory animals (e.g., mice, rats, and dogs). The data from animal studies are being used to supplement the dose–response information obtained from epidemiological studies in humans and are providing model systems for the investigations of the mechanisms of radiation-induced diseases such as cancer.

Cancer Risk Estimation

Models Used to Demonstrate Excess Cancers in Populations

Specific risk-assessment models are used to demonstrate an excess in radiation-induced cancer by relating the risk of cancer induction to radiation dose and to other variables and factors such as sex, genetic makeup, the presence of cigarette smoking, and the type of radiation considered. For example, smokers exposed in uranium mines to alpha radiation from inhaling radon and its progeny have a higher risk of lung cancer than do nonsmokers. In addition, alpha radiation is ~20 times more effective than gamma rays in producing lung cancer.

Important variables used in risk-assessment models include radiation dose and dose rate, age, and follow-up time. For example, very high dose rates of gamma or X-rays are thought to be about two times more effective in causing cancer in humans than are very low dose rates. There is also some evidence that a very low dose rate of alpha radiation can be more effective in producing lung cancer than somewhat higher dose rates. However, this phenomenon may be related to changes in the susceptibility of lung cancer induction with age. It is now known that the ability to repair DNA damage declines with increasing age.

Application of Absolute and Relative Risk Models

Two types of models are often used for conducting statistical analysis of cancer risks: (1) absolute-risk models and (2) relative-risk models. With absolute-risk models, the excess risk due to exposure to radiation does not depend on the normal risk that would arise when there is no radiation exposure. With relative-risk models, the relative risk is a multiple of the normal risk. Unlike absolute risk, which is measured on a scale that starts at 0 and goes to 1, relative risk values begin at 1 and go to infinity (i.e., very large numbers). A value of 1 for the relative risk means that there is no excess risk.

As an example of application of absolute risk, if the normal risk over the lifetime is 0.001 for a specific type of cancer, and radiation adds an additional risk of 0.01, then the absolute risk of cancer over the lifetime is $0.001 + 0.01$, or 0.011.

The relative risk takes into consideration how the normal risk changes with age. For example, if the normal risk of developing a given type of cancer between the ages of 50 and 51 years is 0.001, and radiation exposure leads to a relative risk of 2; then, the relative risk is used to multiply the normal risk so one has to calculate the product 2×0.001, or 0.002. Thus, instead of having a normal risk of 0.001 for cancer in the age interval 50–51 years, the risk is increased to 0.002 because of exposure to radiation. Similar calculations are carried out for other age intervals depending on the age of the person at exposure and the latent period for the cancer type of interest. The risk for the different age intervals would then be added to obtain a lifetime risk. However, no radiation-related risk would be counted during the latent period. Currently used lifetime risk estimates for cancer induction are largely based on either relative-risk or absolute-risk models.

Current Lifetime Risk Estimates

On the basis of available evidence, the Committee on the Biological Effects of Ionizing Radiations (called BEIR V Committee) has recommended use of a lifetime excess risk (i.e., normal risk has been subtracted) of 0.08 per 100 rad for death from γ-ray- or X-ray-induced cancer. This risk applies to the average person in the United States population (all ages considered) exposed to doses up to 10 rad, when

delivered in a short time (e.g., minutes to a few hours). When the same dose is delivered over weeks or months, the risk is expected to be reduced, possibly by a factor of 2 or more. The risk for exposure during childhood is estimated to be about twice as large as that for adults. Males and females are judged to have similar risks. However, all of the cited risk estimates should be regarded as uncertain. These same risks would apply to other radiation sources (e.g., neutrons, beta particles, and alpha particles) if the absorbed dose in rads or Gy were replaced by effective dose in rem or Sv (see Dosimetric Quantities and Units for an explanation of effective dose). The cited risk estimates do not apply to the known subpopulations that are highly sensitive to radiation.

See also: Carcinogenesis; Chromosome Aberrations; Developmental Toxicology; Gastrointestinal System; Metals; Molecular Toxicology–Recombinant DNA Technology; Occupational Toxicology; Pollution, Air Indoor; Respiratory Tract; Risk Assessment, Human Health; Skeletal System; Skin.

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Radium

Shayne C Gad

- Representative Chemicals: Radium bromide and radium chloride, both soluble in water, are two of the common forms of radium with public health concerns
- Chemical Abstracts Service Registry Number: CAS 7440-14-4
- Chemical/Pharmaceutical/Other Class: Radioactive alkaline earth metals
- Chemical Formula: Ra^{2+} (radium isotopes with molecular weights of 226 and 228 are the most common isotopic forms found in the environment)

Uses

Intentional uses of radium today are primarily in the treatment of cancer using a radiation source and as a neutron source in research and instrument calibration. Earlier uses of radium in paints and as a treatment for other illnesses and health-rejuvenating tonics were halted after its toxicity was recognized (see below).

Background Information

Radium has a particularly interesting history. It was isolated from pitchblende by the Curies in 1898, who in 1903 jointly won the Nobel Prize in physics for their studies of radiation. Many people experienced significant exposures to radium before its harmful health effects were known. In the early twentieth century, luminous paint was developed that contained radium. Workers painted 'glow in the dark' watch dials, clocks, compasses, and other instruments starting in the early part of the century through the 1960s. Many developed cancer (bone sarcoma) by ingesting significant amounts of radium if they used unsafe work practices; for example, moistening and shaping the thin tips of their delicate brushes by insertion into the mouth. Also around this time the American Medical Association accepted radium therapy as a treatment for rheumatism, mental instability, and a variety of other disorders. Over-the-counter solutions containing radium (e.g., 'Radithor') were ingested by thousands of people seeking cures for these illnesses. Other patients sought relief by exposing themselves to natural water sources containing significant amounts of radium. In post-World War I Germany, injections were given to treat tuberculosis, ankylosing spondylitis, and other

diseases. Some of these worker and patient cohorts continue to be studied in order to gain a better understanding of the long-term effects of radium exposure.

Exposure Routes and Pathways

Radium is a silvery-white radioactive metal found in most soils and rocks, although usually present in small quantities. Virtually everyone is exposed to low levels of radium in inhaled air and ingested water and food. The concentrations of radium-226 and radium-228 in drinking water are generally low, but there are specific geographic regions where high concentrations of radium occur due to geologic sources. Radium is a product of uranium and thorium breakdown and present in all uranium ores. It undergoes spontaneous disintegration to form radon. People living near industries that burn coal or other fuels or in areas where uranium is abundant can expect to have higher exposures to both radium and radon. Miners and people living or working around radioactive waste disposal sites are also exposed to higher levels of radium than the general public.

Toxicokinetics

Radium is like calcium in that it deposits in bones and teeth when taken into body. Following an accidental acute inhalation of radium, the substance deposited first in lungs, some amount was detected for a short time into soft tissues, and most of the remaining amount lodged in the skeleton. The biological half-life was determined in this case to be $\sim$120 days. When ingested, a $\sim$80% can be expected to be excreted in feces and 20% retained and distributed in the body, primarily in the skeleton. Radium is not metabolized by the body; it only decays over time. The toxicokinetics of dermal exposures to radium have not be well characterized, although it is known that the predominant radioactive α- and β-decay products of radium do not penetrate appreciably into the body following skin exposures.

Mechanism of Toxicity

The radioactive properties of radium are the greatest concern and overwhelm all else. All radioactive materials may cause harm when decay particles are released that disrupt many critical cell functions, including DNA replication. Radioactive materials may also produce toxicity not related to their radioactive behavior. Like barium compounds, radium enters teeth and bones, altering growth and causing them to be weak and brittle.

Acute and Short-Term Toxicity (or Exposure)

Injection of single high doses of radium (2000–4000 μCi kg^{-1} or 74 000–148 000 Bq kg^{-1}) into mice has caused death with a few weeks. Theoretically, similarly high acute exposures could also produce the same effect in humans.

Chronic Toxicity (or Exposure)

Responses of laboratory animals to chronic radium exposure have been studied extensively and are similar to human responses. The large amount of human exposure data available is probably most relevant. Potential symptoms of overexposure to radium include anemia, cataracts, fractured teeth, excess cavities, and cancer. Bone cancer is the most common cancer site associated with high-level radium exposure, but increased risks of liver and breast cancer have also been reported.

Ecotoxicology

Radium is almost ubiquitous in soils, water, geologic materials, plants, and foods at low concentrations. The utilization of radium, uranium, and fossil fuels has resulted in the redistribution of radium in the environment by way of air, water, and land releases. The concentration of radium in natural water is usually controlled by adsorption–desorption reactions with minerals and rocks and by the solubility of radium-containing minerals. In addition, radium is constantly being produced by the radioactive decay of its precursors, uranium and thorium. Radium does not degrade other than by radioactive decay at rates which are specific to each of four naturally occurring isotopes. Radium may be bioconcentrated and bioaccumulated by plants and animals, and it is transferred through food chains from lower trophic levels to humans. The radioactive decay half-lives of the more common radium isotopes range from 3.6 days, 11.4 days, 5.7 years, and 1600 years for 224, 223, 228, and 226, respectively.

Exposure Standards and Guidelines

The US Environmental Protection Agency (EPA) drinking water limit for radium-226 and radium-228 combined is 5 pCi l^{-1}. EPA's limit for maximum soil concentration for radium-226 in uranium and

thorium mill tailings is 5 pCi g^{-1} in the top 15 cm of soil and 15 pCi g^{-1} in deeper soil.

See also: Radon; Uranium.

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Radon

Richard A Parent

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- Chemical Abstracts Service Registry Number: CAS 10043-92-2
- Synonyms: Radon-222; Nitron; Alphatron
- Chemical/Pharmaceutical/Other Class: Unstable radioisotope
- Chemical Formula: ^{222}Ra

Uses

Radon is used in cancer treatment, as a tracer in leak detection, in flow rate measurement, in radiography, and in chemical research.

Exposure Routes and Pathways

Radon and its decay products enter the body by inhalation, dermal absorption, and ingestion. The extent to which the population is exposed to radon-222 and its daughters (polonium-218 and polonium-214) in the air, especially indoors, has recently received increased attention. Indoor radon-222 and daughter concentrations arise from outside air, building materials, water supplies, and the soil and rock underlying the building. Ventilation rates may be altered to obviate unacceptable levels of radon. Persons working with radium and its compounds are also exposed to radon.

Toxicokinetics

Radon is transported in the air by absorption on dust particles that are easily deposited in the bronchiolar areas of the pulmonary system. Deposition on the sticky surface of the bronchial epithelial tissue allows for the irradiation of that tissue with α-particles and consequent transformation to cancer tissue. Radon daughters are also easily absorbed on solid surfaces, especially colloids and dust particles present in the atmosphere.

Short-lived and long-lived radon daughters, produced within the atmosphere and the body, may become selectively distributed to various organs via the bloodstream.

The major systemic threat of these materials is to the kidneys from biotransformed radon daughters. Radon transported by the blood reaches various tissues and organs. Its distribution depends chiefly on the fat content of organs and tissues since it is lipid soluble. From 50% to 90% of the radon body burden is located in the fatty tissues. Radon daughters taken in become localized largely in active deposits in the lungs, to which they represent a grave threat.

Radon is eliminated mainly in exhaled air (~90% in the first hour and the remainder within 6 or 7 h), whereas radon daughters are eliminated mainly by excretion in feces and urine. The biological half-life of radon is reported to be 3.823 days.

Mechanism of Toxicity

Radon gas has demonstrated carcinogenicity attributed chiefly to its radioactive properties. Radon gas has been implicated in the occurrence of lung cancer in individuals engaged in mining ores. Miners who smoke cigarettes are at higher risk, indicating a possible synergistic effect between ore dust, radiation, and cigarette smoking. This situation leads to a high risk of cancer in the respiratory tract. Occupancy of radon-containing homes, particularly in the lower floor levels, might also be a cause of lung cancer. Deliberate or inadvertent intake of radioactive

elements or their compounds that concentrate in certain organs or tissues may be a cancer risk.

Radon itself is chemically inert and electrically uncharged but it is radioactive, which means it undergoes a decay process and can change into other atoms. These other atoms are called radon daughters or progeny and they are electrically charged. As a result of their being charged, they can attach to tiny dust particles in indoor air. These dust particles are respirable and can deposit in the lungs and conducting airways. Because the radon daughters are also unstable, they decay producing α-radiation, which irradiates proximate tissue causing damage to the cellular DNA and in turn can lead to cancer.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute lethal effects of radon and its daughters have been studied in mice and a 30 day LD_{50} was estimated based on a single exposure at a concentration of $2.2 \times 10^8\,pCl^{-1}$ of air for 5–40 h of exposure. All of the exposed mice died within 2 weeks after 40 h of exposure but no animals died after 26 h of exposure or less.

Human

Acute radiation syndrome involves extreme cases of radiation exposure and it is difficult to envision such exposure from environmentally generated radon and its daughters. When it does occur, however, it appears to progress in four stages: prodome, latent, manifest illness, and recovery. The prodome phase occurs $\sim$48–72 h postexposure and is characterized by nausea, vomiting, diarrhea, intestinal cramps, salivation, and dehydration with accompanying neurovascular dysfunction, which includes fatigue, weakness, apathy, fever, and hypotension. During the latency period, exposure of the bone marrow results in decreased cell counts that are dose dependent. This period lasts from 1 to 2.5 weeks. Major organ damage can occur during this phase and extends into the manifest illness phase that results in either recovery or death.

Radon is not acutely irritating to the eye or mucous membranes.

Chronic Toxicity (or Exposure)

Animal

Sprague–Dawley rats were exposed to radon progeny up to 82 days. The lung cancer incidence in rats was directly proportional to the lifetime cumulative exposure to radon progeny. Mixed adenosquamous carcinomas, bronchiolar/alveolar carcinomas, and squamous cell carcinomas were observed in treated animals and were significantly elevated above control animals. Exposed hamsters also showed increased incidences of squamous cell carcinomas. Chromosomal aberrations have also been demonstrated in animals exposed to radon. Radon and its daughters are considered to be carcinogenic in animals as exemplified by the numerous radon exposure studies resulting in bronchogenic cancers.

Human

Inhalation of dust particles contaminated with radon and its daughters represents the major hazard to human health from these materials. The absorbed material is deposited in the bronchial area of the lung. Before the dust can be cleared from the lung, some of it is absorbed and all of it has irradiated the epithelial surface of the bronchial region of the lung with α-particles, creating a significant risk of cell transformation to cancer foci. An increased risk of lung cancer has been associated with radon exposure in uranium miners. This increased risk of the development of respiratory cancer has been well documented. An additive, rather than multiplicative, model has been gaining support to illustrate the connection between smoking and radon daughter-induced lung cancer. Mostly bronchogenic cancers are produced, including squamous cell carcinomas, mixed adenocarcinomas, and, in miners, mostly oat cell carcinomas.

A case–referent study of exposure to radon from the ground and bronchial cancer was carried out on 292 female lung cancer cases and 584 controls who had lived in Stockholm for 30 or more years. Lung cancer cases were diagnosed as oat cell and other types of anaplastic pulmonary carcinomas and the study concluded that radon and daughters were a significant etiologic factor in the cancers noted.

A case–control study of 27 lung cancer subjects in Ontario, Canada, resulted in a marginally significant association between radon exposures and the lung cancers but a strong association with smoking.

Other human consequences of radon exposure include cataracts, nephritis, and dermatitis. Congenital malformations and spontaneous abortions have also been reported in miners exposed to significant concentrations of radon.

Chromosomal aberrations in peripheral lymphocytes from underground miners have also been reported at significantly increased incidence levels. Peripheral lymphocyte chromosomes from 80 underground uranium miners were studied. Significantly,

more chromosomal aberrations were observed among workers with markedly atypical bronchial cell cytology, suspected carcinoma, or carcinoma *in situ* than among miners with regular or mildly atypical cells.

Radon and its daughters have been classified by the International Agency for Research on Cancer as being carcinogenic to humans and animals based on extensive data. The Environmental Protection Agency (EPA) and the National Cancer Institute have estimated that there are 15 000 deaths annually in the United States from radon-induced lung cancer. EPA's recommended exposure guideline of $4\,\mathrm{pCi\,l^{-1}}$ of air is estimated to pose a 1–5% risk from developing lung cancer if a person is a smoker or nonsmoker. The risk of lung cancer in radon exposed individuals is 10 times greater in smokers than in nonsmokers. The National Research Council estimates lung cancer risk at from 0.8% to 1.4% in persons exposed to the EPA's lifetime exposure guideline.

In Vitro Toxicity Data

Ionizing radiation is genotoxic, causing chromosomal damage, DNA fragmentation, and large-scale changes in the DNA structure and function.

Clinical Management

Although it is imperative to provide medical surveillance for those subjected to elevated exposures to radon and its decomposition products such as miners, once a cancer has developed its treatment depends a lot on the extent of the neoplasm and its location. Cytogenetics can be used as a biological monitoring tool for exposed populations.

Most treatments are symptomatic and supportive. Prevention of infection from bone marrow depletion of cellular components is imperative. Bone marrow depression must be treated and fluids and electrolytes must be replaced as needed. Seizures can be managed with benzodiazepines or phenobarbital.

Environmental Fate

Escape of radon and its daughters from soils into the atmosphere is highly dependent on meteorological conditions and the types of soils in the particular area. Release from rock, soils, and other materials is not well understood. Radon and its daughters are readily adsorbed on various surfaces and surface waters are known to contain some amount of radon. In the atmosphere, one part of radon is thought to be present in 1×10^{21} parts of air but these concentrations vary daily and seasonally.

Other Hazards

High rates of congenital malformations and spontaneous abortions have been reported in uranium mining areas.

Exposure Standards and Guidelines

EPA proposed drinking water standard: $300\,\mathrm{pCi\,l^{-1}}$ (see Federal Register: November 2, 1999 (vol. 64, no. 211, pp. 59245–59294).

Massachusetts has a drinking water guideline of $10\,000\,\mathrm{pCi\,l^{-1}}$ of water. Radionucleotides have been designated as hazardous air pollutants under section 112 of the Clean Air Act.

Miscellaneous

Radon is a naturally occurring radioactive gas and environmental contaminant resulting from the radioactive decay of radium. It is considered an inert gas and has an atomic weight of 222. It is a colorless, tasteless, odorless, and extremely dense gas that phosphoresces when condensed into liquid. It boils at $-61.7^{\circ}\mathrm{C}$ and has a density of $9.72\,\mathrm{g\,l^{-1}}$ at $0^{\circ}\mathrm{C}$. The half-life of radon-222 is ~ 3.8 days, decaying to polonium-218 and polonium-214 among other materials. Exposures to radon and its daughters are measured in working-level months. A working-level month is defined as a 170 h working month exposure to alpha radiation from radon daughters equal to $1.3 \times 10^{+5}\,\mathrm{MeV}$ emitted in 1 l of air. There is no method for detecting radon other than laboratory sampling and measurement. Radon is quite soluble in water and is the heaviest gas known to man.

Radon in houses can come from building materials, the soil under the house, the water, and the domestic gas. Some materials such as alum shale and phospho-gypsum have significantly higher radium concentrations than others and can thus cause increased internal radon concentrations to increase. Ventilation rates in basements and in houses in general can reduce exposure significantly.

See also: Pollution, Air Indoor; Pollution, Soil; Pyrrolizidine Alkaloids.

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Ranitidine

Alexander B Baer and Christopher P Holstege

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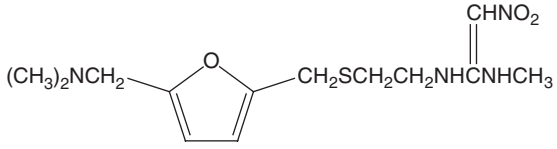
- Chemical Name: Ranitidine
- Chemical Abstracts Services Registry Number: CAS 6637-35-5
- Synonyms: Zantac; Histamine blocker; Antacid; *N*,*N*-Dimethyl-5-(2-(1-methylamino-2-nitrovinylamino)-ethylthiomethyl)furfurylamine
- Chemical/Pharmaceutical/Other Class: A competitive inhibitor of the H2 receptor located on the gastric parietal cells
- Chemical Structure:



Uses

Ranitidine is indicated for therapy of peptic ulcer disease, gastroesophageal reflux disease, pathological hypersecretory conditions (e.g., Zollinger–Ellison syndrome), erosive esophagitis, and adjunctive treatment of acute allergic reactions.

Exposure Routes and Pathways

Both injection and ingestion are the routes of both accidental and intentional exposures to ranitidine.

Toxicokinetics

Ranitidine is absorbed rapidly from the gastrointestinal tract and undergoes extensive first-pass metabolism. The absolute bioavailability of orally administered ranitidine is 39–87%. Mean peak serum concentrations occur within 2 or 3 h following oral doses of 150 mg. Ranitidine is metabolized in the liver to *N*-oxide, desmethyl ranitidine, and ranitidine *s*-oxide. Ranitidine is widely distributed throughout the body and is 10–19% protein bound. The apparent volume of distribution is $1.2–1.9\,l\,kg^{-1}$. Ranitidine is excreted principally in urine via glomerular filtration and tubular excretion. Approximately 30% of an oral dose and 70% of the parenteral dose is excreted unchanged in the urine. The elimination half-life of ranitidine is 2–2.5 h in healthy children and adults. Its half-life is prolonged in patients with renal failure (5.9–8.9 h).

Mechanism of Toxicity

Rare adverse cardiac effects have been reported secondary to ranitidine. These effects may be either due to direct ranitidine blockade of cardiac H2 receptors or due to potentiation of acetylcholine activity on the heart by ranitidine-induced inhibition of acetylcholinesterases. Ranitidine-induced hepatic injury is thought to be secondary to an idiosyncratic reaction or a hypersensitivity reaction.

Acute and Short-Term Toxicity (or Exposure)

Animal

Antihistamine toxicity in animals is usually mild, causing sedation and ataxia. Treatment consists mainly of basic supportive measures.

Human

Reports of toxicity by ranitidine are extremely rare and are primarily based on individual case reports.

Chronic Toxicity (or Exposure)

Animal

Feeding studies in mice and rats at up to $2\,g\,kg^{-1}$ have not shown evidence of carcinogenicity.

Human

Ranitidine is generally well tolerated in therapeutic doses. Ranitidine has less central nervous system (CNS) penetration, endocrine effects, and cardiovascular effects than cimetidine. Reported CNS effects associated with ranitidine include hallucinations, depression, delirium, headaches, dystonia, and choreoathetosis. Cardiac arrest during infusion, bradycardia, and progressive AV block with syncope have been reported in association with ranitidine. Abnormal liver enzymes, interstitial nephritis, parotitis, leukopenia, granulocytopenia, thrombocytopenia, pancytopenia, eosinophilia, vasculitis, dermatitis, toxic epidermal necrolysis, sexual impotence, gynecomastia, and polymyositis have also been reported in association with ranitidine therapy.

In Vitro Toxicity Data

Mutagenicity studies of ranitidine and its metabolites have not demonstrated positive effects.

Clinical Management

In addition to general supportive measures directed to the airway, breathing, and circulation, the clinician may consider measures to decrease gastrointestinal absorption in the alert patient they suspect has been exposed to a potentially toxic dose of ranitidine. Among the appropriate tests that should be obtained are an electrocardiogram, transaminases, and a complete blood count. In the vast majority of cases of ranitidine exposures, patients develop minor symptoms and no therapy is necessary.

See also: Cimetidine.

Further Reading

MacMahon B, Bakshi M, and Walash MJ (1981) Cardiac arrhythmias after intravenous cimetidine. *New England Journal of Medicine* 305: 832–833.

Price W, Coli L, and Brandstetter RD (1985) Ranitidine-associated hallucinations. *European Journal of Clinical Pharmacology* 29: 375–376.

Read Across Analysis *See* Toxicity Testing, 'Read Across Analysis'.

Recombinant DNA *See* Molecular Toxicology–Recombinant DNA Technology.

Recommended Exposure Limits (REL)

Alan J Weinrich

Published by Elsevier Inc.

Recommended exposure limit (REL) is the name used by the US National Institute for Occupational Safety and Health (NIOSH) for the occupational exposure limits (OELs) it recommends to protect workers from hazardous substances and conditions in the workplace. RELs are not regulations. While they are intended primarily as recommendations to the US Occupational Safety and Health Administration (OSHA) and the Mine Safety and Health Administration (MSHA) for use in promulgating legal standards, they also may help employers, workers, and health professionals to recognize and control occupational hazards. Most RELs have been developed for chemical air contaminants, usually represented as numerical values for airborne concentrations (expressed as ppm, $mg\,m^{-3}$, or $fibers\,cm^{-3}$). However, NIOSH has developed RELs for other hazards, including physical agents such as noise, heat, and ultraviolet radiation. Like other OELs, NIOSH expresses most RELs as time-weighted average (TWA) exposures, for up to $10\,h\,day^{-1}$ during a 40 h workweek. An REL also may be expressed as a:

- short-term exposure limit (ST) that should never be exceeded and is to be measured in a specified sampling time (usually 15 min), or
- ceiling limit (C) that should never be exceeded even instantaneously, unless specified over a given time period.

In addition to quantitative exposure recommendations, NIOSH occasionally assigns one or more

notations to selected RELs. Most prominent of these is the 'skin' designation for chemical substance RELs, indicating the potential for dermal absorption and implying a recommendation that skin exposure be prevented by using good work practices and gloves, coveralls, goggles, and other appropriate equipment.

The US Occupational Safety and Health Act of 1970, in addition to creating OSHA and NIOSH, mandated NIOSH to develop objective safety and health criteria describing RELs "at which no employee will suffer impaired health or functional capacities or diminished life expectancy as a result of his work experience." Most RELs that were independently developed by NIOSH resulted from exhaustive reviews of available data, called criteria documents, in the 1970s. However, the criteria document process almost stopped after the 1970s. NIOSH generated hundreds of RELs in the 1980s through other processes, mainly by accepting most 1989 proposed updates of the OSHA permissible exposure limits (PELs), which generally were derived indirectly from the then-current American Conference of Governmental Industrial Hygienists (ACGIH®) threshold limit values (TLVs®). NIOSH continues to review and develop RELs in much smaller numbers and to publish the RELs in various documents, most notably the frequently updated NIOSH Pocket Guide to Chemical Hazards.

NIOSH develops most RELs from qualitative and semiquantitative risk assessments, using expert judgments based on comprehensive reviews of relevant scientific literature. However, a number of RELs have been based on limits of sampling capabilities or on limits of technological feasibility. In response to an OSHA rule on carcinogens (29 CFR 1990.103), NIOSH had subscribed to a policy calling for 'no detectable exposure levels for proven carcinogenic substances'. NIOSH bases recently developed RELs for carcinogens and other chemical substances on risk evaluations using human and animal health effects data, and on feasibility assessments for engineering controls and analytical methods.

See also: American Conference of Governmental Industrial Hygienists; Occupational Exposure Limits; Occupational Safety and Health Act, US; Occupational Safety and Health Administration; Occupational Toxicology.

Further Reading

Fairchild EJ (1976) Guidelines for a NIOSH policy on occupational carcinogenesis. *Annals of the New York Academy of Sciences* 271: 200–207.

NIOSH (1992) *Recommendations for Occupational Safety and Health: Compendium of Policy Documents and Statements*. Cincinnati, OH: US Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 92-100.

NIOSH (2004) *Pocket Guide to Chemical Hazards (NPG)*. Cincinnati, OH: US Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 97-140, Fourth printing with changes and updates (2004).

US Public Law 91-596, 91st Congress, S.2193. Occupational Safety and Health Act of 1970.

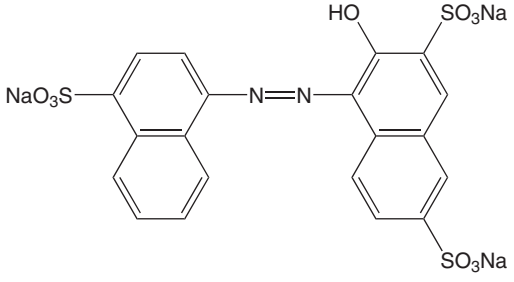
Relevant Website

http://www.cdc.gov – The current version of the NIOSH *Pocket Guide to Chemical Hazards (NPG)* is available online at an extension on this webpage.

Red Dye No. 2

Janice McKee

- Chemical Abstracts Service Registry Number: CAS 915-67-3
- Synonyms: Amaranth; FD&C No. 2; Whortleberry red; 3-Hydroxy-4-[(4-sulfo-1-naphthalenyl) azo]-2,7-naphthalenedisulfonic acid trisodium salt
- Chemical/Pharmaceutical/Other Class: Azo dye
- Chemical Formula: 20-$H_{11}N_2O10S_3Na_3$
- Chemical Structure:



Uses

Red Dye No. 2 was formerly used in food, drugs, and cosmetics but was banned by the US Food and Drug Administration (FDA) in 1976. It is currently used in the United States for dyeing wool, silk, and other textiles as well as paper, wood, and leather products. It is also used as an indicator in hydrazine titrations and is used in color photography and in the manufacture of phenol–formaldehyde resins. Red Dye No. 2 continues to be widely used in food, drugs, and cosmetics in other countries.

Background Information

In 1960, amendments to the Food, Drug, and Cosmetic Act of 1938 added the so-called Delaney anticancer clause to FDA's legal mandate. Among other things, the clause prohibits marketing any color additive the agency has found to cause cancer in animals or humans, regardless of amount. In the early 1970s, data from Russian studies raised questions about Red Dye No. 2's safety. FDA conducted its own tests, which were inconclusive. The consumer-based Health Research Group petitioned FDA to ban the color. FDA turned the matter over to its Toxicology Advisory Committee, which evaluated numerous reports and decided there was no evidence of a hazard. The committee then asked FDA to conduct follow-up analyses. Agency scientists evaluated data and concluded that "it appears that feeding FD&C Red No. 2 at a high dosage results in a statistically significant increase" in malignant tumors in female rats. FDA ultimately decided to ban the color because it had not been shown to be safe. Industry could petition FDA to list Red Dye No. 2 as a certifiable color again if animal study data adequately show safety.

Exposure Routes and Pathways

Exposure may occur through oral or dermal routes. Inhalation routes of exposure are unlikely. Occupational exposure may occur during its production and use as a dye.

Toxicokinetics

When given orally, 8% of the amaranth is absorbed from the intestinal tract. Intestinal flora have a reducing effect on amaranth, and azo reduction is also mediated by the hepatic monooxygenase system. Aromatic amine metabolites, including 1-amino-4-naphthalene sulfonic acid and 1-amino-2-hydroxy-3,6-naphthalene disulfonic acid, are excreted in the urine and bile.

Mechanism of Toxicity

Dietary amaranth in animals results in an exfoliating or solubilizing effect on the brush border membrane of the small intestine. Amaranth stimulates *in vitro* RNA synthesis by causing the dissociation of chromatin. Amaranth has also been shown to increase kidney malate dehydrogenase activity after intramuscular dosing.

Acute and Short-Term Toxicity (or Exposure)

Animal

Amaranth did not cause sensitization in guinea pigs, nor was there any significant dermal or systemic toxicity related to dermal treatment in rabbits. An allergic response was observed after intradermal stimulation in the guinea pig. The acute toxicity of amaranth is low: in rats, the intraperitoneal and intravenous LD_{50} is $1000\,mg\,kg^{-1}$, and the oral LD_{50} in mice is $10\,000\,mg\,kg^{-1}$.

Human

Some persons are sensitive to azo dyes, with reactions including recurrent urticaria. Children with sensitivity to amaranth have exhibited behavioral changes.

Chronic Toxicity (or Exposure)

Animal

Numerous chronic and transgenerational studies have shown no statistically significant increase in reproductive, developmental, or teratogenic effects due to dietary amaranth, although some behavioral changes in male mice pups have been observed. Chronic feeding studies in rats have shown increased mortality, growth inhibition, vacuolar dystrophy, granular deposits in the intestinal tract, increased kidney weight, decreased vitamin A content of the liver, and fatty degeneration of liver cells. However, no histopathological or other effects were noted in beagle dogs fed amaranth for 7 years. Carcinogenicity studies on amaranth have mixed results, with some studies showing skin carcinoma, intestinal carcinoma, lymphosarcoma, mammary tumors, hepatoma, and adenofibroma. Many other studies showed no statistically significant increase in tumors.

Human

There are no data on the carcinogenicity or chronic effects of amaranth in humans.

In Vitro Toxicity Data

Amaranth has not tested positive in a variety of *in vitro* mutagenicity studies; however, its metabolites have tested positive in some assays. DNA damage was induced in gastrointestinal organs and clastogenicity has been observed during at least three recent *in vivo* mouse mutagenicity studies.

Clinical Management

Patients exhibiting toxicity or sensitivity should be treated symptomatically. Phenobarbital has been shown to increase plasma disappearance and biliary excretion of amaranth but is not recommended for clinical treatment on a routine basis.

Environmental Fate

Red Dye No. 2 will exist in air solely in the particulate phase due to an extremely low vapor pressure. Particulate-phase Red Dye No. 2 may be physically removed from the air by wet and dry depositions. Red Dye No. 2 may have very high mobility in soil as it does not adsorb to organic carbon; however, its ionic nature may result in ion-exchange processes with clay that would retard leaching. Volatilization from dry and moist soil surfaces is not expected to be a major fate process. Red Dye No. 2 may be biodegraded anaerobically as a wide variety of anaerobic bacteria have the ability to cleave the azo linkage to produce aromatic amines. Red Dye No. 2 is not expected to volatilize from water surfaces, but it may undergo photodegradation in water. Red Dye No. 2 is not expected to bioconcentrate in aquatic organisms.

Exposure Standards and Guidelines

Red Dye No. 2 was banned by the US FDA in 1976 for use in foods, drugs, and cosmetics in the United States.

See also: Food Additives; Food and Drug Administration.

Further Reading

US Food and Drug Administration (FDA) (2001) *Color Additives Fact Sheet*. Center for Food Safety and Applied Nutrition, July 30.

World Health Organization, International Agency for Research on Cancer (IARC) (1975) *Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man*, vol. 8. Geneva: WHO.

Red Phosphorus

S Satheesh Anand and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS: 7723-14-0
- Chemical Formula: P_n

Uses

Red phosphorus (RP) is a component of matchbox strike plates and is used as an ingredient in certain commercial rat and cockroach poisons. RP is used in the manufacture of pyrotechnics, semiconductors, fertilizers, incendiary shells, smoke bombs (in combination with butyl rubber), and tracer bullets. It is also used in organic synthesis reactions and in the manufacture of phosphoric acid, phosphine, phosphoric anhydride, phosphorus pentachloride, phosphorus trichloride, and in electroluminescent coatings. RP (2–10%) is also used as a flame-retardant additive for plastics such as polyamides, polyesters, and polyurethanes. RP is combined with elemental iodine to produce hydriodic acid, which is used to reduce ephedrine or pseudoephedrine to methamphetamine.

Background Information

Phosphorus is all about fire, and means 'light bearing' in Greek. Phosphorus was first isolated in 1669 from urine and is now primarily obtained from phosphate rock ($Ca_3(PO_4)_2$). Phosphorus is the 11th most abundant element in the Earth's crust. Phosphorus is an essential nutrient in the formation of structural biomolecules, such as membrane phospholipids; functional macromolecules such as nucleic acids, and high energy storing biomolecules such as adenosine triphosphate; and metabolic intermediates, such as sugar phosphates.

Depending upon the nature of interatomic bonds established during its formation, solid elemental phosphorus could occur in three allotropic forms: black, white (yellow), or red. Other forms of phosphorus are

derived from these allotropes. Only white phosphorus (WP) and RP are of industrial importance. WP literally glows in the dark because it is always reacting with the air around it and is known to cause severe toxicity. RP is exactly the same but in a different crystalline form. RP is entirely stable and safe to keep around. Although phosphorus was isolated in 1669, it was mainly during the first part of the nineteenth century that numerous accidental or criminal poisonings by this metalloid were observed, linked to the extensive use of the WP in making matchsticks.

RP is an amorphous phosphorus polymer; it is more resistant to oxidation, less reactive, and less toxic than WP. It is denser than air and melting point ranges from 585°C to 610°C. Also, it is nonvolatile and insoluble in water. RP is manufactured in seven countries, the largest producers being Germany, India, and China.

Exposure Routes and Pathways

Exposure to RP may occur through ingestion, inhalation, and dermal contact.

Toxicokinetics

No information available.

Mechanism of Toxicity

No information available.

Reactivity

In reactivity, the red allotropic form of elemental phosphorus is intermediate to the black and white varieties. It does not ignite spontaneously. The autoignition temperature for RP is 260°C (500°F). When RP burns, the evaporated phosphorus condenses as WP, which creates fire (reignition) and is a health hazard. For this reason, RP fires should be thoroughly cleaned up.

Although it is stable at normal temperature and pressure, the substance is classified as highly flammable and may explode when exposed to heat or by chemical reaction with oxidizers. RP can also react with reducing materials and represents a moderate explosion hazard by chemical reaction or on contact with organic materials. It reacts with oxygen and water vapor to produce the toxic phosphine.

Health Hazards

Phosphorus poisoning has been a known cause of hepatic injury for more than a century. It was used as a classical hepatotoxicant along with CCl_4 to understand the mechanisms of injury and is a direct hepatotoxin. Among the three allotropic forms, only WP is shown to cause the aforementioned effects. RP, on the other hand, is poorly absorbed and does not usually represent a significant health hazard. Thus, it can be assumed as completely safe. However, RP is often contaminated with WP and poses a health hazard. Therefore, exposure to contaminated RP may result in adverse effects on health, including irritation of the skin, eyes, lungs, and gastrointestinal tract.

Individuals with preexisting skin disorders, eye problems, jaw or tooth abnormalities, or impaired liver, kidney, or respiratory function may be more susceptible to the effects of RP. There are no reports concerning the health effects of RP in children.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} of RP was reported to be greater than $10\,g\,kg^{-1}$ in Fischer 344 rats. RP caused 90% and 20% mortality in rats when exposed to $4.3\,mg\,l^{-1}$ for 1 h and $1.5\,mg\,l^{-1}$ for 4 h, respectively. The LC_{50} in Sprague–Dawley rats that were exposed to RP-BR smoke for 1 h on five consecutive days was estimated to be $2320\,mg\,m^{-3}$.

High amounts of RP may cause irritation of the skin, bronchitis, stomach pains, vomiting, and diarrhea and may affect eyes, upper respiratory tract, gastrointestinal tract, and mucous membranes if absorbed through skin, ingested, or inhaled. Effects may vary from mild irritation to severe destruction of tissue depending on the intensity and duration of exposure. Acute exposure may cause liver or kidney impairment if contaminated with WP.

No dermal and eye irritation was observed following RP exposure in rabbits. However, interdermal injection resulted in slight irritation.

One hour exposure to the combustion products of 95% RP/5% butyl rubber produced epiglottal deformation, laryngeal edema, and laryngeal and tracheal lesions in rats. A 4 h exposure produced more severe effects of a similar nature plus some hemorrhaging. Acute exposure to 97% RP/3% butadiene styrene smoke resulted in pulmonary congestion.

Human

Health effects of RP in humans in either environmental or occupational setup have not been reported. However, it is assumed that $\sim 2000\,mg\,m^{-3}$ for more than 15 min might result in death and that $700\,mg\,m^{-3}$ is the highest tolerable concentration.

Chronic Toxicity (or Exposure)

Animal

Prolonged and/or repeated skin contact may result in dermatitis and may cause eye irritation and corneal injury. Chronic exposure may cause kidney and liver damage, anemia, stomach pains, vomiting, diarrhea, blood disorders, and cardiovascular effects if RP is contaminated with WP.

Transient ocular irritation, and reddening and swelling of the eyelids were noted in rats exposed to RP-BR smoke for 5 days per week, for 12 weeks. These effects subsided by the end of the exposure.

Chronic exposure to the combustion products of 95% RP and 5% butyl rubber in male Sprague–Dawley rats for 13 weeks caused 10% mortality at high dose (1200 mg m^{-3}). Surviving animals showed dose-dependent decreases in weight gain and fibrosis of the terminal bronchioles. Guinea pigs are particularly intolerant to the effects of the smoke. Mice showed dose-dependent accumulation of alveolar macrophages.

If RP is contaminated with WP, chronic ingestion may cause necrosis of the jaw bone (Phossy jaw).

Human

According to the National Institute for Occupational Safety and Health (NIOSH), 10 occupations use RP and total number of employees exposed is 2924. There have been no studies available for the chronic effects of RP in humans. No classification data on carcinogenic properties of this material is available from the Environmental Protection Agency (EPA), International Agency for Research on Cancer (IARC), National Toxicology Program (NTP), Occupational Health and Safety Administration (OSHA), or the American Conference of Governmental Industrial Hygienists (ACGIH). There are no relevant data available for mutagenicity, genotoxicity, carcinogenicity, reproductive toxicity, and teratogenicity.

Exposure Standards and Guidelines

As a result of inadequate toxicity data, no occupational exposure limits have been set by NIOSH. Also, there are no acute or chronic reference exposure levels.

See also: Phosphorus.

Further Reading

Cal/EPA (2003) Red phosphorus. In: *Technical Support Document*: *Toxicology Clandestine Drug Labs/Methamphetamine*, vol. 1, No. 12, pp. 1–11. Office of Environmental Health Hazard Assessment.

National Research Council (1997) *Toxicity of Military Smoke and Obstructants*, vol. 1, pp. 98–126. Washington, DC: National Academy Press.

Relevant Website

http://www.cefic-efra.com – European Flame Retardants Association. Red phosphorus.

Red Squill

Alexander B Baer and Christopher P Holstege

- Chemical Name: Red squill
- Synonyms: Red squill (*Urginea maritima*); Sea onion; Oignon marin; Meerzwiebel; El-Ansal; Rat's onion; Bassel-El-Far; Wild boar's onion; Bassel-El-Khanzir; Scille; Oignon d'Egypte; Oignon de Pharaon; Scillae bulbus; Dethdiet®; Roding®

Uses

Red squill historically was used as a rodenticide, but has been replaced by newer, more effective agents. It is no longer used in medicine but may be used in folk remedies to treat cardiac insufficiency, arrhythmia, nervous heart complaints, venous complaints, edema, bronchitis, asthma, whooping cough, pain, wounds, and fractures.

Background Information

Red squill is native to the Mediterranean but has been transplanted and cultivated elsewhere. It is a member of the family Liliaceae and like most lilies produces a pear-shaped bulb that can be quite large. The bulb may be red or white in color, and this differentiates the red and white squill varieties. It produces white star-shaped flowers. It has been described as having a bitter and acrid taste. Squill was known and used by the ancients for many

purposes including treatment of coughs and arthritis, and has also been used as a diuretic, a heart tonic, and as an emetic. During the nineteenth century, medicinal use of red squill began to decline as foxglove was revealed to be both a more effective and safer alternative. Unfortunately, deaths have occurred and occur even today when humans use red squill for medicinal purposes in folk remedies. The entire plant may be toxic, but it is the bulb that is usually used and contains the greatest quantity of active compounds.

Exposure Routes and Pathways

Exposure occurs through ingestion of the plant, especially the bulb. The bulb also produces an irritating juice that can cause inflammation if rubbed onto the skin or splashed into the eye.

Mechanism of Toxicity

Red squill contains many cardiac glycosides, the most prominent being scillaren A and scilliroside. These glycosides produce digitalis-like effects when ingested and, like digitalis, inhibit Na^+/K^+ ATPase, block AV conduction, and cause sinus bradycardia.

Acute and Short-Term Toxicity (or Exposure)

Animal

Mouse LD_{50}: 0.440 mg kg^{-1}; rat LD_{50}: 0.7 mg kg^{-1}; cat LD_{50}: 100 mg kg^{-1}; dog LD_{50}: 145 mg kg^{-1}; sheep LD_{50}: 250 mg kg^{-1}. Diarrhea and vomiting are among the first signs of toxicity. Lethargy, fatigue, and anorexia are also common. Nearly any form of cardiac arrhythmia may be seen. When ingested by mice and rats, the scilliroside may also lead to seizures.

Human

Nausea, vomiting, headaches, bradycardia, almost any form of cardiac dysrhythmias, visual disturbances, and hyperkalemia may be expected to be seen in severe overdoses.

Chronic Toxicity (or Exposure)

Animal

Vomiting, diarrhea, weakness, and eventually death may be seen after exposure.

Human

Anorexia, nausea, vomiting, visual disturbances, and the cardiac effects seen in acute toxicity may be seen. In chronic exposures, patients may not demonstrate the classic finding of hyperkalemia, which is frequently seen in acute exposures. Decreased renal function may interfere with clearance of the glycosides. Patients may be more sensitive to the effects of their squill remedies if they are on medication that also slows AV conduction such as quinine, beta blockers, or calcium channel blockers.

Clinical Management

Supportive care should be provided for all cases of squill exposure. Exposures to squill can usually be confirmed by obtaining a digoxin blood level. While levels are not linear between red squill and digoxin, and cannot be used to determine toxicity, enough cross-sensitivity exists to confirm the exposure. For significant recent exposures, activated charcoal should be considered. Atropine is recommended for treatment of bradycardia. If cardiac effects are resistant to these treatments or if hyperkalemia greater than 5 mEq l^{-1} is present, treatment with digoxin-specific Fab should be considered. If inflammation occurs due to exposure of the eyes or skin to the bulb, thorough flushing should be sufficient to resolve irritant symptoms.

See also: Atropine; Charcoal; Digitalis Glycosides.

Further Reading

El Bahri L, Djegham M, and Maklouf M (2000) *Uriginea maritima* (squill): A poisonous plant of North Africa. *Veterinary Human Toxicology* 42(2): 108–110.

Tuncok Y, Kozan O, Cavdar C, *et al*. *Urginea maritima* (squill) toxicity. *Clinical Toxicology* 33(1): 83–89.

Red Tide

Robin C Guy

Background Information

Red tide is a marine event where protistas, including algae and dinoflagellates, go through a tremendous growth period, called bloom. In a 2–3 week period, it is possible for each algal cell to produce one million daughter cells. This usually takes place in the spring and summer in response to an increase in light intensity. During this time, the warm, shallow seawater tends to become discolored by the enormous concentration of algae. This discoloration is dependent on the species of algae and may be the result of the various pigments, including orange, yellow, blue, green, brown, or red. Some may not be visible at all. As red is the most common pigment, the phenomenon is called 'red tide'.

Red tides occur throughout the world, causing a negative impact to natural resources and humans. Red tides can drastically affect Scandinavian and Japanese fisheries, Caribbean and South Pacific reef fishes, and shell fishing along US coasts. The Florida red tide is caused by blooms of a type of microalgae known as a dinoflagellate. This is a single-celled alga called *Karenia brevis* and it is usually found in warm saltwater, but it can exist at lower temperatures. *K. brevis* is the new (*c.* 2000) taxonomic name for the reclassified *Gymnodinium breve* and is found almost exclusively in the Gulf of Mexico.

Most species contributing to algal blooms are harmless; however, some of the toxins produced by certain species are highly toxic. Often, the algae and the shellfish that consume them are unaffected. However, further up the food chain, these toxins can be fatal. Man, dolphins, manatees, and reptiles are potentially exposed to aerosolized toxins. Brevetoxins are potent ichthyotoxins and have been responsible for the death of billions of fish over the years. Brevetoxin is absorbed directly across the gill membranes of fish or through ingestion of *K. brevis* cells. Some of these toxicity differences will depend on the differential susceptibility of fish species to exposure to *K. brevis* strains involved, toxic components and concentration, stability of extracellular toxins, and exposure routes. Mortality typically occurs at cell concentrations of 2.5×10^5 *K. brevis* cells per liter, which is often considered to be a lethal concentration.

The symptoms of shellfish poisoning, start as soon as the victim's digestive system starts to digest the infected shellfish. Cooking does not destroy the toxins. There are different types of poisonings, with a wide variety of symptoms, depending upon the toxins(s) present, their concentrations in the shellfish and the amount of contaminated shellfish consumed. These include paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP), and neurotoxic shellfish poisoning (NSP).

In PSP, the toxin attacks the nervous system and causes effects that are primarily neurological and include tingling, burning, numbness, drowsiness, incoherent speech, and respiratory paralysis. Symptoms of the disease develop fairly rapidly, within 0.5–2 h after ingestion of the shellfish (generally mussels, clams, cockles, and scallops), depending on the amount of toxin consumed. In severe cases respiratory paralysis is common, and death may occur if respiratory support is not provided. There is no antidote. When such support is applied within 12 h of exposure, recovery usually is complete, with no lasting side effects. In unusual cases, because of the weak hypotensive action of the toxin, death may occur from cardiovascular collapse despite respiratory support.

DSP causes extreme gastrointestinal upset; DSP is less dangerous than PSP, but failure to treat the diarrhea may lead to death from dehydration or other complications. DSP is primarily observed as a generally mild gastrointestinal disorder, that is, nausea, vomiting, diarrhea, and abdominal pain accompanied by chills, headache, and fever. Onset of the disease, depending on the dose of toxin ingested, may be as little as 30 min to 2–3 h, with symptoms of the illness lasting as long as 2–3 days. Recovery is complete with no after effects; the disease is generally not life threatening. DSP is presumably caused by a group of high molecular weight polyethers, including okadaic acid, the dinophysis toxins, the pectenotoxins, and yessotoxin. DSP is generally associated with mussels, oysters, and scallops.

ASP is caused by domoic acid toxicity, which causes amnesic shellfish poisoning, binds to chemical receptors in brain cells and causes their dysfunction. The poisoning begins with gastrointestinal disorders (vomiting, diarrhea, abdominal pain) within 24 h, rapidly followed by dizziness, disorientation, and memory loss within 48 h; the symptoms may persist indefinitely and also result in seizure and coma. During a 1987 outbreak on Prince Edward Island, 1% of the reported poisonings resulted in death from brain damage. ASP is associated with the injection of

mussels. The toxicosis is particularly serious in elderly patients, and includes symptoms reminiscent of Alzheimer's disease.

NSP is the result of exposure to a group of polyethers called brevetoxins. Both gastrointestinal and neurological symptoms characterize NSP, including tingling and numbness of lips, tongue, and throat, muscular aches, dizziness, reversal of the sensations of hot and cold, diarrhea, and vomiting. Onset occurs within a few minutes to a few hours; duration is fairly short, from a few hours to several days. Recovery is complete with few after effects; no fatalities have been reported. NSP is associated with the ingestion of shellfish harvested along the Florida coast and the Gulf of Mexico.

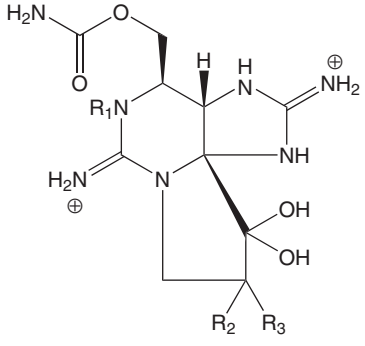
Cases are frequently misdiagnosed and, in general, infrequently reported. Of these toxicities, the most serious from a public health perspective appears to be PSP. The extreme potency of the PSP toxins has, in the past, resulted in an unusually high mortality rate.

Red tide can also pose a serious problem for public health in that the presence of airborne toxins may have an impact on the human respiratory system. Symptoms including irritation of the eyes, nose, throat, tingling lips, and tongue are common during red tides. Waves, wind, and boat propellers in high concentrations of red tides disperse toxin particles into the air causing these problems for people along the shoreline. People suffering from severe or chronic respiratory conditions such as emphysema or asthma, should try to avoid red tide areas. Symptoms usually disappear within 24 h once the exposure is discontinued. Shellfish poisoning is caused by a group of toxins elaborated by planktonic algae (dinoflagellates, in most cases) upon which the shellfish feed. The toxins are accumulated and sometimes metabolized by the shellfish. The 20 toxins responsible for PSPs are all derivatives of saxitoxin.

Since the 1950s, the Canadian–United States Conference on Shellfish Toxicology endorsed a mouse bioassay that was based on the use of purified toxins and has historically been the most universally applied technique for examining shellfish (especially for PSP); other bioassay procedures have been developed but not generally applied. The intraperitoneal minimal lethal dose of the toxin for the mouse was $\sim 9\,\mu g\,kg^{-1}$ body weight. The intravenous minimal lethal dose for the rabbit was ~ 3–$4\,\mu g\,kg^{-1}$ body weight. The minimal lethal dose of the toxin for humans is estimated to be between 1 and 4 mg.

Unfortunately, the dose-survival times for the DSP toxins in the mouse assay fluctuate considerably and fatty acids interfere with the assay, giving false-positive results; consequently, a suckling mouse assay that has been developed and used for control of DSP measures fluid accumulation after injection of the shellfish extract. Considerable effort has been applied recently to development of chemical assays to replace these bioassays. As a result a good high performance liquid chromatography (HPLC) procedure has been developed to identify individual PSP toxins (detection limit for saxitoxin = 20 fg per 100 g of meats; 0.2 ppm), an excellent HPLC procedure (detection limit for okadaic acid $= 400\,ng\,g^{-1}$; 0.4 ppm), a commercially available immunoassay (detection limit for okadaic acid = 1 fg per 100 g of meats; 0.01 ppm) for DSP, and a totally satisfactory HPLC procedure for ASP (detection limit for domoic acid $= 750\,ng\,g^{-1}$; 0.75 ppm).

Some red tides can take up several hundred square miles of water. Red tides are affected by many variables such as weather and currents; therefore, no one can predict when or where red tides will appear or how long they will last. As they tend to occur more in the spring and summer months, there may be a good reason to the folklore that warns us not to eat shellfish in months without an 'r' in their names!



STX	R_1	R_2	R_3
STX	H	H	H
GTX-II	H	H	OSO_3^-
GTX-III	H	OSO_3^-	H
NeoSTX	OH	H	H
GTX-I	OH	H	OSO_3^-
GTX-IV	OH	OSO_3^-	H

See also: Pollution, Water.

Further Reading

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Relevant Websites

http://museum.gov.ns.ca – Red Tide (from the Nova Scotia Museum).

http://vm.cfsan.fda.gov – US Food and Drug Administration, Center for Food Safety & Applied Nutrition, Foodborne Pathogenic Microorganisms and Natural Toxins Handbook.

Redbook

Robin C Guy

History

The *Redbook 2000* provides guidance for the safety of food ingredients, and is produced by the US Food and Drug Administration (FDA), Center for Food Safety and Applied Nutrition (CFSAN). The first part of this guidance was issued in July 2000. It is available electronically at the website listed at the end of the article.

The completed sections now substitute for, or supplement, guidance available in the *Redbook I* and the Draft *Redbook II*. The *Redbook I* titled *Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food*, and was prepared by the US FDA, Bureau of Foods (now CFSAN), in 1982. The 1993 Draft *Redbook II* has the same title and was published on March 29, 1993 (Notice of Availability, 58 *Federal Register* 16536).

Earlier versions of the *Redbook* focused on direct food additives and color additives used in food. The *Redbook 2000* provides guidance for the safety assessment of food ingredients, including direct food additives, color additives used in food, generally recognized as safe substances, food contact substances and constituents, or impurities of any of the above.

Current Progress

At this time, the *Redbook 2000* is an incomplete document. Sections are continuously being written and are added to the website after finalization. The current table of contents is listed in **Table 1**. Topics that have been completed are in **Table 2**.

Table 1 *Redbook 2000* table of contents

Table 1 Continued

- 8. *In Utero* Exposure Phase for Addition to Carcinogenicity Studies with Rodents
- 9. Reproduction and Developmental Toxicity Studies
  - a. Guidelines for Reproduction Studies
  - b. Guidelines for Developmental Toxicity Studies
- 10. Neurotoxicity Studies

V. Additional Studies
- A. Introduction
- B. Metabolism and Pharmacokinetic Studies
  - 1. Recommended Metabolism and Pharmacokinetic Studies
  - 2. Considerations in the Design of Pharmacokinetic Studies
    - a. Test Substance
    - b. Animals
    - c. Route of Administration
    - d. Dosage Regimen
    - e. Sampling
    - f. *In Vitro* Studies: Dose Response, Mechanism
    - g. Pregnancy/Lactation/Reproductive Studies
  - 3. Analysis and Use of Data from Pharmacokinetic Studies
    - a. Data Reporting and Parameter Estimation
    - b. Pharmacokinetic Models: Data Interpretation and Predicting Effects
  - 4. Use of Pharmacokinetic Results for Study Design and Risk Assessment
    - a. Design of Toxicity Studies
    - b. Setting Dose Levels
    - c. Determining Mechanisms of Toxicity
    - d. Improving the Risk Assessment Process/Safety Assessment
  - 5. References
- C. Immunotoxicity Studies
  - 1. Immunity: A Brief Review
  - 2. Key Concepts in Immunotoxicity Testing
  - 3. Indicators of Possible Immune Toxicity
  - 4. Expanded Type 1 Immunotoxicity Tests
  - 5. Type 2 Immunotoxicity Tests
  - 6. Relevance of Primary Indicators of Immune Toxicity to Health
  - 7. Adequacy and Reliability of Primary Indicators of Immune Toxicity
  - 8. Recommendations for Further Immunotoxicity Testing when Primary Indicators are Positive
  - 9. Animal Models for Immunotoxicity Tests
  - 10. Recommended Strategy for Assessing the Immunotoxic Potential of Food Ingredients
  - 11. Conclusion

VI. Human Studies
- A. Clinical Evaluation of Food Ingredients
  - 1. General Considerations for Clinical Studies of Food Ingredients
  - 2. Specific Considerations for Clinical Studies of Food Ingredients
  - 3. Sequence of Clinical Studies for Food Ingredients
  - 4. Submitting Reports of Clinical Studies on Food Ingredients
  - 5. Appendix A – Principles of Institutional Review and Informed Consent
- B. Epidemiology Studies

Table 1 Continued

VII. Emerging Issues
- A. Introduction
- B. Macro-Additives
- C. Safety of Food Ingredients Developed by Biotechnology
- D. Enzymes
- E. Microbially Derived Food Ingredients
- F. Advances in the Development of Alternative Toxicity Testing
- G. Heritable and Somatic Genetic Toxicity

VIII. Glossary: Acronyms and Definitions

Table 2 *Redbook 2000* completed guidelines

Preclinical Toxicity Studies
- 1. General Guidelines for Designing and Conducting Toxicity Studies
- 2. Guidelines for Reporting Results of Toxicity Studies
- 3. Pathology Considerations in Toxicity Studies
- 4. Statistical Considerations in Toxicity Studies
- 5. Short-Term Tests for Genetic Toxicity
  - a. Bacterial Reverse Mutation Test
  - b. *In Vitro* Mammalian Chromosomal Aberration Test
  - c. *In Vitro* Mouse Lymphoma $TK^{+/-}$ Gene Mutation Assay
  - d. *In Vivo* Mammalian Erythrocyte Micronucleus Test
- 6. Short-Term Toxicity Studies
  - a. Short-Term Toxicity Studies with Rodents
  - b. Short-Term Toxicity Studies with Non-Rodents
- 7. Subchronic Toxicity Studies
  - a. Subchronic Toxicity Studies with Rodents
  - b. Subchronic Toxicity Studies with Non-Rodents
- 8. One-Year Toxicity Studies with Non-Rodents
- 9. Reproduction and Developmental Toxicity Studies
  - a. Guidelines for Reproduction Studies
  - b. Guidelines for Developmental Toxicity Studies
- 10. Neurotoxicity Studies

Human Studies
- 1. Epidemiology Studies

Glossary: Acronyms and Definitions

See also: Food Additives; Food and Drug Administration; Immune System; Generally Recognized as Safe (GRAS); Good Laboratory Practices (GLP).

Relevant Website

http://www.cfsan.fda.gov – US Food and Drug Administration (FDA), Center for Food Safety and Applied Nutrition (CFSAN). *The Redbook*.

Reference Concentration (RfC)

Patricia M Nance

The reference concentration (RfC) methodology to estimate benchmark values for noncancer toxicity of inhaled chemicals was adapted for inhalation studies from the reference dose methodology used for oral exposure assessment. The same general principles were used, but the RfC methodology was expanded to account for the dynamics of the respiratory system as a portal of entry. The reference dose (RfD) methodology included dosimetric adjustments to account for species-specific relationships of exposure concentrations to deposited or delivered doses. Particles and gases are treated separately, and the type of toxicity observed influences the dosimetric adjustment applied to score the exposure concentration for animals to a human equivalent concentration.

The RfC can be defined as an estimate of continuous inhalation exposure to the human population, including some level of uncertainty (perhaps spanning an order of magnitude), and sensitive subpopulations that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a no-observed-adverse-effect level (NOAEL), lowest-observed-adverse-effect level (LOAEL), or benchmark concentration, with uncertainty factors generally applied to reflect limitations of the data used. The RfC is generally used in the US Environmental Protection Agency's (EPA) noncancer health assessments. The formula for calculating the RfC is as follows:

$$\mathrm{RfC} = \frac{\mathrm{NOAEL(or\ LOAEL)}}{\mathrm{UF} \times \mathrm{MF}}$$

The NOAEL is the highest experimental dose at which there is no statistically or biologically significant increase in frequency or severity of adverse health effects, as seen in the exposed population compared with an appropriate unexposed population. Effects may be produced at this level, but they are not considered to be adverse. If there is no suitable NOAEL available, then an LOAEL can be used instead. The LOAEL is the lowest dose or exposure level of a compound in a study that there is no statistically or biologically significant increase in frequency or severity of adverse health effect in the expose population as compared with an appropriate unexposed population.

The uncertainty factors used in the development of the RfC represent a specific area of uncertainty inherent in the extrapolation from the available data. The basis for the application of these uncertainty factors are (1) human variability; (2) animal to human extrapolation; (3) subchronic to chronic extrapolation; (4) use of an LOAEL instead of an NOAEL; and (5) database confidence. These uncertainty factors can be 1, 3, or 10, depending on the amount of uncertainty.

The modifying factor (MF) ranging from 0 to 10 is included to reflect a qualitative professional assessment of additional uncertainties in the critical study and in the entire database for the chemical not explicitly addressed by the uncertainty factors. The default value for the MF is 1.

The confidence in the RfC can be high, medium, or low. High confidence indicates the judgment that the RfC is unlikely to change in the future because there is consistency among the toxic responses observed in different sexes, species, study designs, or in dose–response relationships, or that the reasons for existing differences are well understood. High confidence is often given to RfCs that are based on human data for the exposure route of concern, since in such cases the problems of interspecies extrapolation have been avoided. Low confidence indicates the judgment that the data supporting the RfD may be of limited quality and or quantity and that additional information could result in a change in the RfC.

Occupational exposure limits (OELs) are standards based on toxicological, epidemiological, and clinical information pertaining to human exposure of airborne contaminants. OELs are generally time-weighted average concentrations of airborne substances to which a health worker can be exposed during defined work periods and under specific work conditions throughout a working lifetime, without material impairment of health. OELs also are based on a variety of assumptions and considerations, such as industrial hygienists can control workplace environments, as well as reflect the cost of controlling these environments. Because of these assumptions and considerations, the use of OELs for the derivation of RfCs is generally not done. The OELs often are not based on chronic effects and may differ in regard to the severity of the effects used for RfCs. The OELs further assume intermittent exposure, whereas RfCs are set to protect against continuous exposure. The evaluation process of toxicity data by agencies deriving OELs may differ from the US EPA's process with respect to weight-of-evidence classification, application of uncertainty factors, and other

issues. It is not recommended to use OELs in the derivation of RfCs.

See also: Occupational Exposure Limits; Reference Dose (RfD); Respiratory Tract; Risk Assessment, Human Health; Uncertainty Factors.

Relevant Website

http://www.epa.gov – US EPA (1994) *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry*, EPA/600/8-90/066F, October 1994. See also US EPA (2002) *A Review of the Reference Dose and Reference Concentration Processes. Prepared for the Risk Assessment Forum*, EPA/630/P-02/002F.

Reference Dose (RfD)

Patricia M Nance

Systemic effects have traditionally been evaluated using such terms as 'acceptable daily intake' (ADI), 'safety factor' (SF), and 'margin of safety' (MOS), concepts that are associated with certain limitations. The US Environmental Protection Agency (EPA) developed a methodology for evaluating available data pertaining to xenobiotics for purposes of developing oral reference doses (RfDs). Although similar to the intent of deriving regulatory levels of ADIs to protect exposed populations from adverse effects, RfDs were based upon a more rigorously defined methodology that adhered to the principles proposed by the US National Academy of Sciences paradigm (1983) and included guidance on the consistent application of uncertainty factors for prescribed areas of extrapolation required in the operational derivation. The RfD methodology represents a quantitative approach to assess toxicity data in order to derive a dose–response estimate.

An RfD is an estimate of the oral daily exposure to the human population, including some level of uncertainty (perhaps spanning an order of magnitude) and sensitive subpopulations that are likely to be without an appreciable risk of deleterious effects during a lifetime. It is generally expressed in units of milligrams of a compound per kilograms of body weight per day ($mg\,kg^{-1}\,day^{-1}$). The RfD is useful as a reference point from which to gauge the potential effects of the compound at other doses for long-term exposure to a compound. It is generally used in the US EPA's noncancer health assessments. The RfD is calculated by dividing the no-observed-adverse-effect level (NOAEL) (or lowest-observed-adverse-effect level, LOAEL or benchmark dose) by the product of the total amount of uncertainty factors and the modifying factor applied reflecting the limitations of the data used. The formula for calculating the RfD is as follows:

$$\mathrm{RfD} = \frac{\mathrm{NOAEL\ (or\ LOAEL)}}{\mathrm{UF} \times \mathrm{MF}}$$

The NOAEL is the highest experimental dose at which there is no statistically or biologically significant increase in frequency or severity of adverse health effects, as seen in the exposed population compared with an appropriate unexposed population. Effects may be produced at this level, but they are not considered to be adverse. If there is not a suitable NOAEL available, then a LOAEL can be used instead. The LOAEL is the lowest dose or exposure level of a compound in a study in which there is no statistically or biologically significant increase in frequency or severity of adverse health effect in the exposed population as compared with an appropriate unexposed population.

The UFs (uncertainty factors) used in the development of the RfD represent a specific area of uncertainty inherent in the extrapolation from the available data. The basis for the application of these uncertainty factors are (1) human variability; (2) animal to human extrapolation; (3) subchronic to chronic extrapolation; (4) use of a LOAEL instead of a NOAEL; and (5) database confidence. These uncertainty factors can be 1, 3, or 10, depending on the amount of uncertainty.

The MF (modifying factor) ranging from 0 to 10 is included to reflect a qualitative professional assessment of additional uncertainties in the critical study and in the entire database for the chemical not explicitly addressed by the uncertainty factors. The default value for the MF is 1.

The confidence in the RfD can be high, medium, or low. High confidence indicates the judgment that the RfD is unlikely to change in the future because there is consistency among the toxic responses observed in different sexes, species, study designs, or in dose–response relationships, or that the reasons for existing differences are well understood. High confidence is often given to RfDs that are base on human data

for the exposure route of concern, since in such cases the problems of interspecies extrapolation have been avoided. Low confidence indicates the judgment that the data supporting the RfD may be of limited quality and or quantity and that additional information could result in a change in the RfD.

See also: Reference Concentration (RfC); Risk Assessment, Human Health; Uncertainty Factors.

Further Reading

Dourson ML and Stara JF (1983) Regulatory history and experimental support of uncertainty (safety) factors. *Regulatory Toxicology and Pharmacology* 3: 224–238.

US EPA (2002) A review of the reference dose and reference concentration processes. Prepared for the Risk Assessment Forum, US Environmental Protection Agency, EPA/630/P-02/002F.

Refrigerants *See* Freons.

Regulation, Toxicology and

Michael A Kamrin

Introduction

During the last half century, due to increasing concerns about possible adverse human and environmental effects of chemicals detected in food, air, and water, a number of new laws and regulations were enacted, and old ones amended, in the United States and many other countries. In the United States, these statutes, designed to mitigate such possible adverse impacts, include the Federal Fungicide, Insecticide and Rodenticide Act, the Clean Air Act, the Clean Water Act, the Safe Drinking Water Act, the Federal Food, Drug, and Cosmetic Act, and the Toxic Substances Control Act. (Detailed descriptions of each of these can be found elsewhere in this encyclopedia.)

As can be seen from the names of the legislation, they are designed to address problems related to a particular environmental medium, such as the air or water, or to a specific type of chemical such as a drug or pesticide. In general, the regulations promulgated to carry out the intent of each of these pieces of legislation were developed independently of each other; that is, they do not consider that humans and other organisms may be simultaneously exposed to the same chemicals in a variety of media. For example, acceptable levels of a chemical in food may be calculated without consideration of concomitant exposures through air or soil – exposures that may be governed by other agencies carrying out other legislative mandates. In addition, nonregulated exposures such as those resulting from inhalation of indoor air, are also generally left out of the calculations.

There are a number of possible consequences of this medium by medium and chemical by chemical approach. One consequence might be that regulations governing a chemical in one medium are not stringent enough because humans and other organisms may also be exposed to comparable amounts of this same chemical in several other media – leading to a combined exposure that may be too high. It is also possible that this approach may have the opposite impact. For example, the regulations governing a chemical in one medium may be so stringent that they only permit exposures that are orders of magnitude lower than those allowed in other media or that result from unregulated exposures. Thus, these regulations are too stringent since they have essentially no impact on overall exposure. A good example of this is the very stringent US limits on benzene in drinking water while indoor air exposures to benzene are often orders of magnitude higher than those occurring from drinking water consumption, especially if smoking is occurring in this indoor environment.

Recognition of the problem of possible over or under regulation raises the questions of why regulations are so narrowly focused on specific chemicals or environmental media and how such large media-specific differences in allowable exposures have come about. These questions have come into greater prominence in recent decades as legislation governing environmental contaminants has been enacted in more and more countries. With the inception of multinational units, such as the European Union, it has become clear that acceptable exposures differ not only among different types of legislation within countries but also among countries – the degree of regulatory stringency can vary significantly from country to country even when dealing with the same exposures in the same environmental media.

In the case of the European Union, recognition of these problems in regulatory consistency has resulted in extensive discussions among the member states with the goal of establishing a common regulatory

metric that could apply to all. In the absence of such a metric, citizens are faced with conflicting information about what is 'safe', a conflict that is most evident when they live near borders separating countries with regulations characterized by differing stringency. In addition, with the internationalization of business and commerce, the lack of regulatory uniformity has even wider relevance and economic impact. As a result, there has been pressure for countries around the globe to harmonize environmental regulations. However, progress is slow for a variety of reasons, one of the most important being the role of policy considerations and cultural values in the application of toxicological knowledge to the generation of regulatory limits and strategies.

Role of Toxicology in Environmental Regulation

Whatever the environmental medium or chemical involved, the critical question in regulation is how to determine the maximum allowable levels of a chemical in a particular environmental medium. Answering this question is often thought of as determining the 'safe' level for that chemical. Once these 'safe' values are calculated, they are then used to promulgate legislation and regulations aimed at reducing existing 'unsafe' environmental levels and preventing the introduction of 'unsafe' levels into food, air, water, etc.

However, 'safe' and 'unsafe' are not scientific terms and so it is not obvious how to best determine them. Government agencies have responded to this problem by defining 'safe' through the issuance of guidance documents that specify what toxicological and exposure data should be considered and how these data should be interpreted. The determination of 'safe' limits is based on a process known as risk assessment. While assessments of both ecological and human health risks are performed as part of the development of environmental regulation, the discussion here will focus on human health risks. Human health risk assessment is a multistep process that combines experimental and epidemiological evidence as to the levels of a toxicant that are required to cause adverse effects with data and assumptions as to the amount, frequency and duration of exposures to that agent through each environmental medium.

While this simple description might suggest that risk assessment is a fairly straightforward process that can be performed by simply following commonly accepted guidance documents, this is not the case. One fundamental problem arises from the unavailability of toxicity data obtained directly from humans. In the absence of this most relevant scientific information, toxicological data from other species must be used in assessing risk. These data are generally collected using rodents and experiments are most often performed by administering very high doses over long periods of time to ensure an effect will be produced. To apply such animal data to humans, it is necessary to extrapolate both from high to low doses and also from other species to humans. In most cases, there is not enough scientific information and understanding to perform these extrapolations confidently. As a result, a variety of assumptions must be made in translating the risk assessment data collected from experimental animals into numbers that are applicable to humans.

To appreciate the regulatory problems this approach leads to, it is important to understand that risk assessment was developed as a tool for carrying out risk management, rather than a scientific process for understanding risk. Thus, both the selection of the data to be used and the way these data are extrapolated to the usual human exposure situation, reflect both scientific and policy considerations. As a result, risk assessment results do not represent the best scientific estimates of risk, estimates that are subject to scientific consensus, but rather 'prudent' values that incorporate margins of safety. These margins of safety are included to increase the likelihood that regulations based on these risk assessments will successfully protect the public and the environment.

However, since each governmental entity is responsible for carrying out its own unique set of legislative mandates and has a unique regulatory history, definitions of 'prudence' are often agency-specific. As a result of the differences in definition, risk assessment procedures and results often differ among agencies within countries as well as among countries. The application of such divergent procedures has contributed strongly to the diversity in risk limits established by various agencies and governments.

Another factor also contributes to the variability in limits promulgated by various agencies. This is the importance that risk is given relative to other factors, such as cost, in setting regulations. In some cases, legislation requires that risk be the only consideration; others require that risk must be balanced against benefit; still others specify that economic factors must also be taken into account. Such differences can lead to great diversity in regulatory limits even if the data utilized are the same and application of the risk assessment methodologies lead to the same result.

However, within this diversity, there are some commonalities. For example, one common element of risk assessment across agencies is the division of environmental toxicants into two categories; carcinogens and

noncarcinogens. Under most risk assessment schemes, carcinogens are evaluated under a paradigm that leads to probabilistic risk numbers; that is, the incidence of cancer expected per unit of administered dose of agent. By combining this cancer risk number with policy choices, particularly the acceptable upper bound for cancer incidence, maximum allowable limits for carcinogens in the environment can be set.

In contrast, the procedures for evaluating the risk from noncarcinogens leads to single value estimates of allowable exposures – not probabilistic risk values. These estimates have often been misinterpreted as bright lines separating 'safe' from 'unsafe'. However, a careful examination of the origins of these noncarcinogen risk values reveals that they represent prudent numbers below which adverse effects are not expected. The assessment procedure does not provide information as to what the risk will be if these values are exceeded but it is expected that the risk will be insignificant until exceedences are significantly above the established 'safe' value. Once calculated, this noncarcinogen risk value is then converted into a regulatory number by the appropriate agency, utilizing a variety of assumptions and policy judgments.

For example, in the United States, the Environmental Protection Agency's Office of Water uses both carcinogen and noncarcinogen risk numbers as inputs for establishing drinking water standards that represent the maximum acceptable levels of a variety of agents in public drinking water systems. Similarly, states in the United States may use this type of information to set maximum allowable levels of agents in soil or air, standards that apply only in that state. These national or state risk values may also be used in determining 'how clean is clean'; that is, the maximum allowed environmental levels in various media; for example, air, water, soil, at hazardous waste sites.

Summary

In summary, many environmental regulations require the generation and interpretation of toxicological data. However, the way that these data are used can be influenced greatly by a variety of factors. These include the stipulations in the legislation as to how the risk numbers are to be utilized, the degree of prudence adopted by the agency promulgating the regulation, the data available and whether the agent is labeled a carcinogen or noncarcinogen. A number of intra- and international groups are addressing the regulatory inconsistencies that have arisen as a result of these factors and it is expected that at least some degree of regulatory harmonization will result.

See also: Clean Air Act (CAA), US; Clean Water Act (CWA), US; Federal Insecticide, Fungicide, and Rodenticide Act, US; Food, Drug, and Cosmetic Act, US; Risk Assessment, Ecological; Risk Assessment, Human Health; Risk Characterization; Risk Management; Safe Drinking Water Act, US; Toxic Substances Control Act, US.

Further Reading

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Renal Toxicology *See* Kidney.

Reproductive System, Female

Bill L Lasley

Introduction

Modern technology now creates and applies new chemicals faster than they can be tested for their potential adverse health effects. At the same time, more women work outside the home in expanding job classifications, many of which have a potential for chemical exposure that is increased above the typical exposures in the home. In addition, the 'reproductive years' for women today have been widened with the development of new medical technologies that permit older women to bear children and younger women to delay their pregnancies. This, in turn, has broadened the age that must be considered at risk for reproductive impairments. In addition, health risks associated with reproductive failure in women are now recognized to extend beyond the issues of family

planning and fertility. Reduced bone accumulation in the second and third decade, bone loss, and increased risk of heart disease in later life are just three of the nonreproductive problems which may result from abnormal ovarian function.

The need for a better understanding of female reproductive toxicology, then, is driven by issues associated with women's health in the context of today's changing society and our current knowledge of women's general health. A woman's reproductive function is often considered to be more sensitive to environmental perturbations than that of a man's. This concept is supported by studies that demonstrate that reproductive failure in women can be induced by strenuous exercise, marginal nutrition, as well as by acute physical or emotional stress. Despite concerns that women may be more adversely affected than men when confronted by the same exposures, very little information is available in terms of female reproductive toxicology. Recent reference books on reproductive toxicology generally provide much more information relating to male- than female-related issues because more information is available for male compared to female toxicology. The reason for this disparity is most likely attributable to the practical considerations in experimental design and the availability of critical research materials that make the studies of males more attractive to research programs. For example, in many experimental designs, viable gametes are the ultimate object of evaluation. Spermatozoa are plentiful and more easily collected compared to ova. Although semen collection and analyses are not simple procedures, sperm production provides a constant and quantifiable end point for male fertility. Ova, in contrast, are irregularly produced and seldom available for study except in animal studies or under complex clinical management protocols. Even when ova are made available for scientific study, there is little technology available to evaluate their quality in contrast to spermatozoa.

While it is increasingly evident that gender differences probably exist in reproductive toxicity, progress in documenting male and female differences has been slow. Perceived and real difficulties in using female animal models in controlled studies have been the major impediments to progress in this area of research. The result of these difficulties is that much more information exists for the male than the female in terms of reproductive toxicology. The inequities in the database will change only when methods are developed that permit the female system to be studied as efficiently and effectively as the male is studied. Fortunately, toxicological investigations of female reproduction are becoming more common, new techniques are being developed to monitor women's reproductive health, and the information relating to female reproductive toxicology is growing. As a consequence, the gender gap in information relating to the effects of toxic exposures is closing.

Reproductive Epidemiology

Infertility in humans is usually defined as the failure to conceive following natural attempts to achieve pregnancy for a year or more. Individuals and couples not wishing to have children are seldom evaluated or even surveyed in terms of their reproductive health. In poor economic times the desire for children decreases in developed countries and reproductive health in terms of fertility becomes a less important issue. Thus infertility rates may be higher than current estimates due to insufficient and/or inaccurate information. There is a general lack of epidemiologic information regarding reproductive function in human populations and most estimates relating to fertility are biased toward the clinic population. Most of the existing information concerning human reproduction is obtained from clinical records and does not adequately represent the general population. Only recently have tools been developed to design and evaluate population-based prospective studies assessing basic reproductive physiology.

Recognized exposures of humans to reproductive toxicants have been infrequent and, when detected, the toxicants were eliminated as quickly as possible rather than studied. Controlled and/or prospective experiments with humans have moral and ethical ramifications; therefore, most of the information relating to the mechanism of action of recognized or putative toxicants on human reproduction is limited. The lack of information relating to real-world exposures combined with the recognition that adequate animal models for human reproductive processes make the study of spontaneous reproductive failures an attractive approach to predict sites and mechanisms of action of putative reproductive toxicants. This approach assumes that induced reproductive failures resulting from toxic exposures will mimic spontaneous events since there is a finite number of control mechanisms that can fail. The spontaneous failures are thought to reveal 'weak links' and are the most susceptible targets for the adverse effects of toxic exposure.

The primary problem in studying human reproduction is that many aspects of the reproductive process occur without the knowledge of either the woman or her physician. Ovulation, fertilization, and implantation all occur as concealed events. Reproductive biologists and epidemiologists, however, have recently developed new and incisive tools to associate exposures to reproductive health. While it will be years

before the 'baseline' information is available to apply these broadly, the promise for the future is that reproductive health can be monitored as well as any other aspect of health. Meanwhile, toxicologists use animal models in invasive or terminal experiments to demonstrate the effects of documented and putative toxicants to gain insight regarding their basic mechanisms of action. Because of species differences in the expression of reproductive function, the direct application of these kinds of information and the validity of certain end points to human reproductive health are not altogether clear.

What are the risks to women in regard to reproductive toxicants at home or at work? Overall increases in reported cases of infertility are difficult to evaluate. The increasing median age of most societies and delays in family planning lead to an increase in age-related infertility, which is a natural phenomenon. However, fertility rates of young couples have decreased during the past 10 years and experts speculate that as much as 37% of the reproductive failure seen in modern society could be related to environmental factors. There is no direct evidence that women have been affected more or less than men but this is often assumed. Sperm counts have been assessed and show a decline over this same time period. No such similar assessment can be made on the potential of female fertility for comparison. It is doubtful, therefore, that the entire decline in overall fertility can be attributed to men. Traditional epidemiological studies used to monitor menstrual function do not provide information to permit an assessment of fecundity or explain the trends in female fertility. This lack of information regarding female fecundity does underscore the basic theme of this review: female reproductive toxicology has been, perhaps, one of the most neglected area of toxicology.

Problems Associated with Female Reproductive Toxicology

Female reproductive toxicology is a challenging study area for several reasons. One of the purposes of this review is to delineate some of the problems that reproductive toxicologists face when attempting to investigate putative female reproductive toxicants. This will be approached by focusing on three broad aspects or qualities of female reproduction that most limit progress in this field. These areas include the sensitivity of the female reproductive system to environmental factors, the complexity of the female reproductive system compared to that of the male, and the species specificity in regard to ovarian function. Since it is essential that human and animal female reproductive physiology be understood for the effects of toxic exposures to be studied, each overview of female reproductive toxicology must provide a brief yet detailed description of normal female reproductive physiology and types of reproductive failures. Finally, examples of known reproductive toxicants and their mechanism of action are presented.

Sensitivity of Female Reproduction to Environmental Stressors

The most obvious quality of female reproductive system that influences the way it must be studied is its sensitivity to environmental influences. As mentioned previously, the female reproductive system appears to be more sensitive to environmental perturbations and is more complex in its organization than most other physiologic systems or when compared to male reproductive physiology. Reproductive failure in female animals is often considered to be the first and only detriment resulting from nonlethal adverse environmental impacts despite the fact that the capacity to reproduce (fecundity) is considered the essential quality of an animal's fitness in its current location. Chemical or physical stressors that are not adequate to perturb other physiologic functions can interrupt or delay reproductive function. In times of acute stress, or in response to chronic challenges to the survival of the organism, reproductive function may be selectively suppressed as a short-term adaptive process. Thus, as a nonessential mechanism for short-term survival, reproductive function is sacrificed as an immediate fail-safe strategy for long-term survival. This preferential selection to curtail reproduction in response to nonlethal stressors and the sensitivity of reproduction to nonspecific physical, chemical, and emotional stressors make it difficult to identify nonreproductive toxins as being distinctly separate from specific reproductive toxicants. Agents that cause reproductive failure directly and may not be specific reproductive toxicants can lead to reduced fertility. In both males and females (but probably to a higher degree in females) the general health of the individual is likely to be reflected in reproductive capacity. Toxicants which have nonreproductive organs as targets may affect sexual development or influence reproductive processes sooner and more noticeably than they affect other physiologic processes.

Complexities of Reproductive Processes

The second issue regarding the study of female reproductive toxicology is that of the complexities within the female reproductive system. The degree that the female reproductive system is considered to be more complex, compared to the male, is not necessarily the issue since this supposition is debatable.

Female reproduction may be recognized to be complex because it has been studied in greater detail and many more aspects of the female reproductive system are defined than for the male. Certainly the female reproductive system is overtly more dynamic and, perhaps because of this dynamicism, more susceptible to physical, chemical, and emotional stressors. The discrete series of events of the ovarian cycle which requires precise coordination between the central nervous system, hypothalamus, and pituitary in order for gametogenesis and ovulation to take place provides the opportunity for environmental changes to adversely influence normal processes. If these events are delayed or altered appreciably, some form of short-term infertility will most likely result. When this is compared to the male, the relatively monotonous production of hormone and gametes is not as likely to be overtly influenced by short-term events.

Female reproductive function, in general, is intermittently expressed, cannot be completely assessed in a single individual, and is often influenced by normal environmental factors. In contrast, most other organ functions are expressed continuously, can be appraised equally well at any point of time, and respond predictably to changes in the environment. Most other physiologic processes can be studied in an individual and therefore can be characterized in terms of the biological variation within one individual. In contrast, reproduction can only be evaluated completely when pairs of individuals are studied for prolonged time periods and, in some cases, when more than one generation is studied serially. Reproductive failure can vary from reduced sexual drive to complete sterility. Infertility can be the result of either functional or organic defects and subfertility may be the result of defects at one of several levels of reproductive function (e.g., menstrual dysfunction, anovulation, early fetal loss, and pregnancy loss).

Both the nervous system and the endocrine system are involved in reproductive processes and any number of metabolic processes are essential for normal reproductive function. Both the synthesis and metabolism of neural transmitters and endocrine messengers (glycoprotein and steroid hormones) are critical for normal fertility. Reproduction is a process that includes growth and development of organ systems, gametogenesis, courtship behavior, coitus, gamete transport/interaction, internal fertilization, implantation, gestation, and nurture. Perturbations and/or derangements at any stage in this process can reduce or eliminate fertility. The complete reliance on the endocrine mechanism makes the reproductive system susceptible to the downstream effects of vascular, hepatic, and renal dysfunction.

While reproductive biology is a progressive research field, many of the physiologic mechanisms involved with gamete transport, fertilization, implantation, and gestation are still poorly defined. As much as 20% of the clinically described subfertility is classified as unexplained; this may indicate that a large portion of infertility is the result of yet undefined environmental hazards. Subfertility in human populations is estimated at frequencies as high as 20% in married couples of child-bearing age and over 10% in married noncontracepting women between 20 and 35 years of age.

Experimental designs that involve the female reproductive tract must consider the influence of changing hormonal events. Portions of the female reproductive tract are sequentially modified under the influence of pituitary and ovarian hormones. Many female tissues are induced to proliferate and then differentiate in response to steroid and protein hormone patterns. There are clearly time periods of increased sensitivity and time intervals that are specific for different toxicants in one species or for the same toxicant in different species to exert its maximal effects. The concept of precise 'sensitive' periods for toxic effects is well established for developmental toxins (teratogen) and this same concept is likely to be true for toxicants that impact female reproductive functions such as follicle recruitment, folliculogenesis, gamete transport, and endometrial maturation. Very few studies have addressed these issues directly due to the complexities of the experimental design as well as concerns relating to the adequacy of the animal models that are available to study. Long-term testing with sublethal doses is currently the approach used to ensure that exposures are delivered at all possible sensitive time periods. This kind of design may have very little relevance to real-world exposures which generally occur acutely.

Historically, toxicologists have viewed the developmental aspects of reproductive toxicology (e.g., teratogenicity and growth retardation) as the fundamental or basic component of reproductive toxicology. The effects of toxicants on adult reproductive processes and organs (those aspects which limit or perturb fertility) are often considered as less important. Because of this oversight, much of female reproductive toxicology has been focused on effects of agents that target conception and/or pregnancy. In terms of the potential for life-threatening exposures and our responsibility to safeguard the fetus, the emphasis on teratology is understandable. However, it should be recognized that the opportunity for exposure and the potential for adverse effects on reproductive processes is as great if not greater for the nonpregnant woman as it is for the fetus. Currently,

the amount of scientific information available and the degree that we understand developmental reproductive toxins is greater than those for nondevelopmental reproductive toxins. For this reason, the following discussion will be limited to perturbations to reproduction success in the adult human female.

Species Specificity of Female Reproductive Physiology

The third issue is that of species specificity of reproductive physiology. The great variation in reproductive function between species creates the greatest challenge for reproductive toxicologists who study the female. Whereas the basic events of female reproductive cycles can be compared between species, the organization of the components of these events is more varied than any other of the physiologic systems. When compared to the kinds of differences observed between species for the other systems (e.g., the cardiovascular, digestive, integumental, muscular, skeletal, immune, and respiratory), the physiologic mechanism and expression of reproductive function is more diverse than any other. Even if an abundance of good and practical models existed for human reproductive toxicology, there would still be concerns regarding species specificity of toxicants because of differences in metabolic mechanisms through other organ systems, such as the liver or kidney, and in the expression of reproduction function, that is, reproductive performance.

Extrapolating data relating to ovarian function from females of one species to females of another is of limited use. Each species of mammal (there are more than 4000 species) has developed and retained a unique organization of the physiologic processes that make up the complex set of processes that are essential for reproduction to take place. Of the more than 4000 patterns of ovarian cycle organization that we might expect, less than 100 have actually been characterized. This represents the limited numbers of species that have been domesticated or adapted to captivity. It is important to remember that most of our domestic and laboratory species have been artificially selected for reproductive performance and may be more tolerant of environmental influences on their reproductive processes. Very little is understood regarding the effects of multiple stressors on any physiologic system and for most systems this can be justified because of the substantial independence of most systems from others. The reproductive system is clearly one for which this simple logic does not apply, particularly when animal modeling is involved.

When the most basic components of female reproductive physiology are compared, such as neural and pituitary control of ovarian function and ovarian morphology and endocrinology of the ovarian cycle, the diversity becomes clear. Differences between species are most easily discussed in terms of the higher nervous center control over gonadal function and organization of the ovarian cycle. Changes in photoperiod, temperature, conditions of the substrate, nutrition, and even nonspecific stressors can modulate gonadal activity through highly species-specific control mechanisms at the level of the hypothalamus. Each species has adapted to reproduce optimally in response to unique environmental conditions that artificial enclosures cannot duplicate. These factors make interspecific comparisons of reproductive performance within a controlled setting difficult.

The laboratory macaque represents the best potential model for the human female; however, the expense of maintaining the monkey model, difficulties in handling and manipulating mature monkeys, insufficient baseline data, a limitation of animal resources, the time required to perform multigeneration studies, and inadequate experimental tools make the use of monkeys as a model severely limited. Since less than the ideal model is usually used, model selection for human reproductive toxicology must be built on a solid understanding of female reproductive physiology and modern trends in reproductive medicine and pharmacology.

Female Reproductive Development and Physiology

Development

Unlike the male phenotype, the female mammal requires little additional directing force beyond the correct genotype. Thus, fetal development in the female is similar in the presence or absence of the normal fetal gonads. Since the same somatic substrates are present in male and female fetuses, the introduction of androgenic substances to a female fetus will produce the inappropriate development of male-type secondary sex characteristics. This can occur as a result of endogenous adrenal production of weak androgens (congenital adrenal hyperplasia) or exogenous androgenic agents (anabolic steroids) which can cause the development of ambiguous or male-type genitalia, and, in the most severe cases, infertility. This ability to respond inappropriately to androgens persists throughout life and females can be virilized at any time, although the sequelae of virilization generally decrease with age.

All aspects of ovarian function and adult reproductive normalcy in the female are ultimately dependent on the process of germ cell maturation in the

adolescent and adult. Ovarian steroids are responsible for the development of secondary sex characteristics as well as the function and maintenance of the reproductive tract. The ovary can be compared to an undifferentiated organ that retains its embryonic capacity to differentiate throughout the reproductive years. Ovarian stroma cells derive their function only under the direction of a competent hypothalamic-pituitary drive and the presence of developing primary oocytes. If the oocytes are depleted, by accident or age, the ovary ceases to function and all aspects of reproductive function that depend on sex steroid support will regress. The preponderance of reproductive functions is either driven or modulated by sex steroid hormones. For this reason a clear understanding of steroid hormone production and action is essential to understand either reproductive physiology or reproductive toxicology.

Germ Cells

Germ cell numbers are finite in females and are present in the early embryo. Having multiplied by mitosis and migrated to the genital ridge, they initiate, but do not complete, meiosis immediately. Unlike spermatogonia, which continue to be replenished throughout adult life, all of the germ cells are present in a resting stage from the early fetal period to the end of the reproductive life of the female. Usually the oocytes remain in a suspended stage of meiosis which is complete just prior to fertilization. If these original germ cells are lost they cannot be replaced. If the oocytes are all lost then ovarian function and all reproductive function will irreversibly cease. Unlike the testis, in which the endocrine and gametogenic activities of the gonad are physically separated, the individual ovarian follicle comprises the combined endocrine and gametic functional unit of the ovary. This is an important difference between the sexes and is reflected in the approaches that are used to assess reproductive health in each.

The 'resting' stage of germ cells (primary oocytes) and their vestments of follicle cells are tightly clustered in the cortical portion of the ovary in what is termed the germinal epithelium. The counterpart to this in the male would be the lining of the seminiferous tubules behind the 'testis–blood barrier'. While the presence of the ovary and its hormonal products are not essential for embryonic development of the female phenotype up to the neonatal stage, the presence of viable germ cells together with their surrounding differentiated gonadal tissue is essential for complete sexual maturation. Just prior to sexual maturity and under the control of higher nervous centers that control gonadotropin secretion, increased pituitary secretion of gonadotropins stimulates some of the resting oocytes and their surrounding primitive follicle cells to mature. Both the resting oocytes and the undifferentiated follicular cells must be present in the germinal epithelium for this earliest phase of normal ovarian function to occur. In the absence of viable oocytes, follicle cells will not develop and, as a consequence, there will be no response to gonadotropin stimulation.

Ovarian Function

At the onset of puberty, the undifferentiated ovarian stromal cells in the immature ovary, in response to gonadotropin secretion and their close proximity to a viable, resting primary oocyte, differentiate and develop the capacity to produce sex steroid hormones (primarily androgens and estrogens). These sex steroids are directly responsible for the development and maturation of the secondary sex organs and complete the process of sexual maturation (puberty) at the appropriate time. The follicular events associated with this follicle activation require the differentiation of the previously undifferentiated primitive follicle cells into two separate cell types, the theca and the granulosa cells. The theca cells produce androgens (androstenedione and testosterone) from acetate and circulating cholesterol, and depend on the granulosa cells to convert the androgens to estrogens. Increased production of androgens from the theca cells or decreased ability to aromatize these androgens by the granulosa calls can lead to virilization (masculinization) and infertility.

In the earliest stages of puberty, ovarian follicles develop to the stage of producing estrogen but do not ovulate. This period of 'adolescent sterility' in primate species is associated with adequate estrogen stimulation for the development of secondary sex characteristics, general sexual development, and sexual maturity. Inappropriate release of gonadotropins (luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary gland) prior to the age of normal puberty will induce follicular development and precocious sexual development since all of the components of adult ovarian function are present at birth and only lack gonadotropin stimulation for complete, normal function.

The Ovarian Cycle

The hormonal events associated with ovulation are incompletely understood. Simplistically, a feedback loop is established between the hypothalamus-pituitary and the ovary at the onset of sexual maturity. Pituitary gonadotropins function to stimulate primitive follicles to secrete estrogen, progesterone, and

peptide hormones which modulate both the pituitary and the hypothalamic control of reproduction through positive and negative feedback loops. Collectively this is referred to the hypothalamus-pituitary-ovarian axis (HPO axis). The maturing follicle secretes increasing amounts of estrogen prior to ovulation, which modulates the secretion of gonadotropins, orchestrates sexual behavior (in some species), and prepares the reproductive tract for mating, gamete transport, and potential conception. Ovulation occurs during a limited time period within the complete ovarian cycle and the extruded ova are fertilizable for only a short time period. Thus, the synchrony of ovulation and mating is very important. Precise synchrony of these events is achieved by the action of estrogen from the maturing follicle acting upon the central nervous system, the pituitary, and the reproductive tract.

Gametogenesis in the female (folliculogenesis and ovulation) is intimately associated with hormone production patterns and can be monitored by measuring changes in hormone production rates. Although the periovulatory LH surge requires increasing estrogen in order to be elicited, the final release mechanisms vary between species. For some rodents the hypothalamic release of neural peptides (catecholamines, indolamines, and specific gonadotropin-releasing factors) is closely associated with the diurnal light–dark exposure; in other species copulation is an absolute requirement; and in yet others, such as primates and most domestic and laboratory animals, it occurs as a result of follicular maturation only. For all species, the coordination between the higher nervous centers, the pituitary, and the ovary is mediated through hormone signals from the maturing follicle cells. Thus, in the female, hormone patterns that represent HPO activity are precise and appropriate indicators of reproductive status and potential fertility. This should be contrasted to the male in which the gamete itself is usually evaluated and endocrine parameters have limited clinical significance.

Ovulation is associated with a surge of gonadotropin release that is the result of estrogen-positive feedback. This massive release of gonadotropins probably functions primarily as a fail-safe mechanism to complete the ovulatory process, which is initiated through follicle maturation and the synergism of gonadotropins and estrogen acting upon the mural granulosum. The LH surge secondarily functions to convert the original vestments of the oocyte (follicle cells) into a different cell type (the corpus luteum) that will secrete another sex steroid (progesterone) during the pregestational period. If conception does not occur, progesterone secretion by the ovary is limited to the time interval following ovulation and may be produced for as short as 2 days or as long as 3 weeks depending on the species. Progesterone action serves to prepare the reproductive tract, primarily the lining of the uterus (endometrium), for the embryo implantation and also acts centrally to prevent additional follicles from developing during this time interval.

Following ovulation the ova that are extruded onto the surface of the ovary (either into the body cavity or within a bursa) are picked up by the oviducts. The oviducts serve as conduits that transfer both fertilizable ova toward the uterus and allow selected spermatozoa from the lower reproductive tract to meet and fertilize the ova. Following fertilization the resulting zygote is transported to the uterus where successful implantation can take place. The function of the oviducts, which are responsible for gamete and zygote transport, is controlled by the ovary through estrogen and progesterone production throughout the ovarian cycle. The secretion of these sex steroids is dependent on pituitary gonadotropin support. When implantation does occur, it is usually 2–6 days following ovulation and fertilization; however, delays of implantation can be as long as several months in species such as bears, seals, most mustelids, and some edentates.

The embryo survives unattached to the uterine lining for 5 days to 7 weeks in laboratory and domestic species. During this preimplantation period, nutrient requirements are absorbed from the materials within the uterine lumen. After this time some form of stable attachment is formed between the trophoblast (primitive placenta) and the endometrium. This can range from a very superficial apposition with many cell layers separating maternal and fetal circulations to true implantation with only one cell layer separating the two vascular beds.

Hormone Action

Neuropeptides and polypeptides derived from neural tissue are largely responsible for pituitary function. The neurotransmitters (catechols, indoles, endorphins, and dopamine) act directly or indirectly to cause the release of the gonadotropins or prolactin and exert their action through synaptic junctions to alter neural activity in the hypothalamus and other areas of the brain. The polypeptide hormones (gonadotropin hormone-releasing hormone, adrenocorticoid-releasing hormone, and thyroid-releasing hormone) act through membrane receptors and transduce their signal by intracellular second messengers such as cAMP, calcium, and/or phosphoinositol. The fetal pituitary is capable of responding to higher nervous centers by midgestation but does not do so until these centers 'awake' at the time of puberty long after birth. Premature 'awakening' of these centers leads to premature sexual

development and arrest of the normal somatic growth pattern through the action of sex steroids on bone growth. The absence of the awakening of the central nervous centers that control pituitary function leads to a failure to undergo sexual development.

The primary pituitary hormones that influence female reproductive function are two glycoproteins (LH and FSH) and one protein hormone (prolactin). The glycoprotein hormones (LH and FSH) are stimulated to be synthesized and released by a single polypeptide neurohormone, gonadotropin-releasing hormone (GnRH), and are modulated by both ovarian steroids (estrogen and progesterone) as well as ovarian and pituitary peptides through positive and negative feedback loops which impinge both at the level of the hypothalamus and the pituitary. Derangements which lead to spontaneous or early release of the gonadotropins are rare as the GnRH 'drive' is an essential stimulation. The opposite is true for prolactin because it is controlled primarily for the negative actions of dopamine. Any action which decreases the domaminergic drive to the pituitary will lead to hyperprolactinemia and reproductive dysfunction relating to prolactin excess. Failure of the pituitary to release gonadotropins is common particularly in women and can be the result of inadequate hypothalamic support or inability of the pituitary to manufacture and release gonadotropins. When this occurs prior to puberty delayed maturity and infantilism are the result. If this occurs following puberty, then ovarian function and menstrual periods cease.

All aspects of female reproduction are regulated directly or indirectly by ovarian sex steroids. These small lipids, which are ubiquitous to all species, act to both develop and differentiate all secondary sex characters. The principal female sex steroids are estradiol and progesterone. Estradiol is mitogenic in estrogen-sensitive tissues, and is responsible for end organ growth and proliferation, and acts through estrogen receptors that are constitutive in all estrogen-sensitive tissue. Progesterone can be mitogenic and/or differentiating, depending on the tissue. In the uterine endometrium progesterone differentiates the 'proliferated' endometrium and decreases the action of estrogen by decreasing estrogen receptors. In the breast progesterone complements the proliferative action of estrogen. All progestational cells require the antecedent action of estrogen in order to express progesterone receptors. Exogenous compounds, either natural or synthetic, can mimic endogenous hormones and cause infertility, inappropriate somatic changes, or induce hyperplastic disease. Examples include the synthetic steroid hormones and oral contraceptives, which have been produced to artificially regulate female fertility. These compounds were created to be easily ingested or absorbed and possess unusually long biological half-lives. If an exogenous ligand has androgenic activity, then the results of female exposure to this ligand are similar to those of excessive adrenal androgen production as described previously. Estrogenic or pure progestational agents have different, nonmasculinizing effects but still can result in infertility by interfering with the normal signals that are sent by the ovary to the hypothalamus, pituitary, and reproductive organs.

The direct effects of steroid hormones are limited to cells that contain steroid hormone receptors. Each steroid has at least one specific receptor but each steroid receptor has a strong structural and functional relationship to other compounds with a similar structure. While each steroid has specific effects on specific target organs, these effects can generally be divided into three categories: mitogenic (proliferative), differentiating, or regulatory. Estrogen has all three effects, is the principal female sex steroid hormone, and will be discussed in detail.

Because of the pivotal importance of estrogens it is somewhat surprising that many naturally occurring compounds, other than hormonal estrogens, have estrogenic potential due to structural similarities to the steroidal estrogens (estrone, estradiol, and estriol) and their ability to bind estrogen receptors. It is important that toxicologists understand that many xenobiotics have estrogenic properties for the same reason. Since estradiol is the primary estrogenic hormone for all species, the estrogen receptors that transduce the 'estrogenic message' have been conserved to a great degree. Conservation of both the ligand and the receptor should permit a great deal of uniformity of estrogenic activity of different compounds between species. This, however, is not the case. Estrogens of all types have different effects in different species. In terms of toxicants, for example, 'clover disease' in sheep which results in sterility, is caused by phytoestrogens found in certain legumes and tubers does not occur in other species even though the same phytoestrogen is found at the same circulating concentrations.

In humans, estrogens are primarily responsible for the development of female sex characteristics. The development of vagina, uterus, and fallopian tubes as well as the breasts, fat deposition for body contours, the pubertal growth spurt, pubic/axillary hair, and pigmentation of the genital region and areolae are all a part of estrogen action, although androgens are also probably involved. Some overlap exists between the action of estrogen and androgen in terms of anabolic effects, but, in general, estrogens oppose or have opposite effects of androgens. The loss of estrogen at menopause leads to decreased bone deposition,

decreased turgor of the skin, and sclerosis of the blood vessels. Relative immunity to coronary disease and gout is also dependent on estrogen and is lost at menopause. In animals, estrogens are psychogenic and the desire to mate or become receptive to males is a direct effect of estrogens. The effects of estrogens on emotions are not well defined in humans, although changes in emotion can be striking following menopause.

The toxic effects of estrogens are generally considered to be the adverse effects observed when estrogen is given in supraphysiologic doses or for inappropriate time intervals. Since the normal pattern of estrogen production is quite variable and since the effects of even normal patterns have a wide range of effects in different individuals, it is difficult to separate some adverse estrogenic effects from 'normal effects', that is, swelling and soreness of the breast, morning sickness in early pregnancy, and dysfunctional uterine bleeding. In very high doses, estrogens can cause water retention, thus edema related to heart failure or renal disease could be accentuated by extremely high doses of estrogen. The effect of estrogen on liver function varies tremendously between species. In birds and fish, for instance, estrogens mobilize large amounts of lipids for egg production to the point that the serum becomes milky in appearance. In humans, estrogens change the pattern of circulating lipids (they can be considered 'protective' for circulatory diseases) and this effect is of great interest to those who study heart disease and atherosclerosis.

In women, menstrual function disturbances can be an early and accurate indicator of estrogen imbalance. In other species which do not slough the endometrium, other end points need to be assessed. Assessments of estrogen deficiency can be particularly difficult to detect in particular effects of estrogen antagonists or antiestrogens. Estrogen-induced changes are not only different between species but also different within the same species at different levels of the reproductive system. In some species a carcinogenic action of estrogens has been described as for diethylstilbestrol (a nonsteroidal estrogen). In most studies the ability of estrogens to cause tumors is largely attributed to a genetic predisposition for tumor formation and has caused unnecessary fears for its use therapeutically; the exception, however, is diethylstilbestrol, which, when taken by pregnant women, leads to hyperplastic disease in female children. Estrogens are mitogenic and tend to increase their own receptor numbers and these effects are counteracted by progesterone. Thus, prolonged exposure to estrogens in the absence of progesterone can cause abnormal growth of proliferative tissues like the endometrium.

Progestins is the generic term for compounds that exert an effect similar to that of progesterone. Progesterone is the second most important steroid hormone for female reproductive function. Progesterone receptors are induced by the action of estrogen, thus progesterone has little effect in the absence of estrogen priming. The primary role of progesterone in the uterus is to create an implantation site for the embryo by causing the final differentiation of endometrial cells. Insufficient progesterone leads to an inadequate implantation site and to implantation failure. Progesterone plays a key role in pregnancy by reducing uterine contractile tone and preserving pregnancy. Within the uterus, progesterone causes a decrease in estrogen receptors, thus attenuating the mitogenic effect of estrogen on this tissue. Similarly, progestins reverse the actions of estrogen on cervical mucus, labial color and swelling, and sexual behavior. In the breast, however, progesterone acts to augment the mitogenic effect of estrogen and acts to proliferate the epithelium of the ducts in preparation for milk production.

Female Reproductive Failure

Reproductive failure can be induced by environmental hazards at any level, although most types of infertility have not been linked to environmental factors. Since it is not ethically possible to observe or impose reproductive toxic exposures on human subjects, much of what we believe about the effects of putative toxins are based on the assumption that well-defined spontaneous reproductive failures are accurate surrogates for environmentally induced defects. Spontaneous reproductive failures, together with results from animal experiments, are used as models to predict or to understand the impact of reproductive toxicants on the human system.

Ovarian senescence due to increasing age (menopause) is another form of infertility that is a normal consequence of the aging process and can be compared to the effects of an ovarian toxicant. As oocytes are depleted through the normal process of atresia, the reproductive system responds in much the same way as it would to an ovarian toxicant that acts to destroy oocytes. In both cases, the absence of gonadal hormones results in increased pituitary drive in compensation for the decreased negative feedback from the ovaries which, without germ cells, cannot produce steroid hormones. The increased gonadotropins (hypergonadotropism) cannot, however, compensate for the irretrievable gonadal deficiency (hypogonadism).

Defects that act to suppress hypothalamic or pituitary function also lead to infertility but are

expressed differently. Kallman's syndrome (anosmia with isolated gonadotropin deficiency), for example, is a form of hypothalamic deficiency in which the pituitary is not stimulated to release gonadotropins due to a defect at the level of the hypothalamus and higher nerve centers. The gonads of affected individuals remain in a preadolescent condition, and sexual maturity is never achieved. In such case the disease simulates a central nervous system toxicant that leads to reduced gonadotropin release (hypogonadotropic) and a subsequent reduced ovarian activity (hypogonadism). Since the pituitary is not compromised in this condition the appropriate administration of the hypothalamic factor which causes the release of gonadotropins (GnRH) will restore pituitary and subsequently gonadal function as well. Physical, nutrition, or even emotional stress can lead to different degrees of 'hypothalamic amenorrhea', which is a general term for hypogonadotropic hypogonadism in women. Professional dancers, athletes, and overzealous dieters can exhibit this reversible form of infertility at any stage in life and, as a consequence, this type of reproductive failure occurs relatively frequently. Such cases can be used to model theoretical toxicants which block normal hypothalamic or pituitary function.

Many kinds of organic or functional defects can lead to postovulatory reductions in fertility. For instance, anatomical impediments to gamete transport can prevent fertilization. Poorly developed or insufficient endometria due to end organ insensitivity to steroids, insufficient steroid hormone production, or impediments to steroid action at the level of the endometrium will not adequately support the implantation site of an otherwise healthy embryo. Previous reproductive tract infections are responsible for the majority of these kinds of reproductive failures; however, developmental defects and alterations in organ function caused by inappropriate stimulation or response also contribute.

Reproductive Toxicants

In recent years public concern regarding toxicant exposure has focused on the potential of reproductive hazards resulting from exposure to agricultural and industrial chemicals. This has led to the suggestion that a significant amount of the recognized reproductive failure among humans and animals can be attributed to increased toxic exposures. The increasing number of female workers in industry as well as the recent recognition of hazards to female reproduction in the workplace has heightened concerns relating to female reproductive toxicology. Such concerns, however documented, have resulted in an increase in risk assessments of both putative and real reproductive toxicants as well as in the creation of regulations concerning disposal of and exposure to xenobiotics. Progress in this area, however, has been slow for a number of reasons.

Reproductive toxicants obey the rules of other toxicants and their effects can usually be linked to some interruption of normal physiologic mechanisms such as errors in metabolism, interfering with ligand/receptor interaction or alterations of signal transduction. They can act directly by inducing a change through their inherent chemical activity. For example, the purine analogs interfere with the normal process of oogenesis and have greatly different effects at different stages of reproductive development. Some reproductive toxicants mimic or block hormone action by virtue of their structural similarity to these hormones and mimic or antagonize endogenous messengers. Other toxicants act through receptors that may or may not have well-defined physiologic functions and interact with hormone transduction signals, trans-activating factors or response elements. Some toxicants act directly while others must be metabolized to an active form before they can exert their adverse effects. Some compounds have dissimilar adverse reactions prior to and following metabolism. Other reproductive toxicants act indirectly after being metabolized from an inert compound to a form that is chemically or biologically active. Polycyclic aromatic hydrocarbons can exert their effects indirectly by inducing hepatic and ovarian enzymes, which govern steroid production and metabolism, and act by transducing adverse signals or signals that impede normal physiologic functions. Lipophilic compounds can be sequestered in adipose tissues and exert adverse effects years after a single exposure.

The identification of chemical compounds of high concern as human reproductive and developmental toxicants was provided by a report from the Government Accounting Office (GAO) in 1991. That report reviewed the evidence that identified compounds as male, female, or developmental toxicants and what safeguards were in place to protect the public. The report lists 30 compounds, 21 of which have adverse reproductive effects in women or female animals. These compounds include industrial solvents (toluene, ethylene glycol monoethyl, and monomethyl ethers); metals (cadmium and lead), pesticides, fungicides, and fumigants (clordecone and its metabolite mirex, DDT, ethylene dibromide, ethylene oxide, hexachlorobenzene, and the pesticide contaminant dioxin); halogenated hydrocarbons (vinyl chloride, PBBs, and PCBs); products of combustion (carbon disulfide, carbon monoxide, and tobacco smoke); as well as arsenic, diethylstilbestrol, and warfarin.

The GAO list of reproductive toxicants does not include some putative reproductive toxicants which are currently highly regulated or banned for industrial use such as benzene, benzamine, chloroprene, formaldehyde, styrene, and xylene. More are added to the list of putative toxicants every year. Of the toxicants listed as female reproductive hazards, approximately half have strong evidence of direct adverse effects on human (or nonhuman primates) female reproduction separate from their action as developmental toxicants and teratogens. A number of compounds such as the glycol ethers are only now being recognized as reproductive toxicants and reports demonstrating this effect are beginning to appear in the literature. Thus, the list of compounds for which there is strong evidence of adverse effects on fertility, menstrual function, or other gynecological disorders in nonpregnant women can be theoretically condensed to the 14–16 individual or groups of compounds. These are listed in **Table 1** along with the adverse effects that are associated with each. The actual number of compounds that have adverse effects on female reproduction is undoubtedly much greater than this list indicates, and will grow as new chemicals are developed. Many of the compounds which have documented effects on males will likely have adverse effects on females once they are investigated properly. However, until the adverse effects of exposures of these are observed for women and are documented, they cannot be included.

Table 1 not only illustrates the relatively small number of documented human female reproductive toxicants but also underscores the difficulty of investigating exposures to reproductive toxicants in human populations. There is a lack of specific knowledge in terms of the targets and mechanisms of action of most reproductive toxicants because the end point for recognizing the adverse effect is 'downstream' of the actual target. The literature lists more than half of the adverse effects as only 'menstrual dysfunction', which provides little help in identifying a specific site or action. This general outcome is reported because it is the only relevant end point that is usually available during the study periods that usually follow the actual exposure. Assessment of fertility, for instance, would need to include a relatively large number of women who were simultaneously exposed to the possibility of pregnancy over an interval of time that would permit adequate pregnancies to occur and be completed.

Table 1 A condensed list of human female reproductive toxicants and their adverse effects

Benzene	Menstrual dysfunction
Benzamine	Menstrual dysfunction
Chloroprene	Menstrual dysfunction
Formaldehyde	Menstrual dysfunction
Mercury	Menstrual dysfunction
Halogenated hydrocarbons	Menstrual dysfunction
Anesthetic gases	Infertility
Toluene	Menstrual dysfunction
Styrene	Menstrual dysfunction
Diethylstilbestrol	Infertility
Ethyl oxide	Abortion
Lead	Abortion
Vinyl chloride	Ovarian dysfunction
Dioxin (TCDD)	Infertility

Regardless of the number of women exposed and the time of exposure, most reproductive toxicity data are collected in retrospect, using the subjects' recall as the source of information relating to reproduction. Practical end points for assessing the target of toxicity other than the woman's menstrual calendar have not been available historically and only general symptoms such as menstrual function can be recalled and reported. As reviewed earlier, menstruation is the normal result of an ovulatory ovarian cycle and ovulatory cycles can be quite variable in length and regularity. Irregular menstrual cycles may be typical for some women and not for others and a woman's recall of her previous 'regularity' may not be accurate. In addition, vaginal bleeding for other reasons that have characteristics of true menstruation may occur in the absence of ovulation, for example, breakthrough bleeding as a result of unopposed estrogen stimulation. While the listing of adverse effects as menstrual dysfunction may be adequate to indicate that female reproduction has been perturbed, it provides very little information as to the target or mechanism of action, nor does it provide information as to the health risk except in the most severe cases.

A deeper understanding of the site of toxicity and the mechanism of action can come only from controlled animal studies in which basic hypotheses are tested using laboratory rodents or primates. As indicated previously, there are concerns of species specificity in terms of sensitivity or response to reproductive toxicants, routes of exposure, and relevant dosage that make this less than a perfect science. However, knowledge of the similarities and differences in the reproductive physiology of the model species compared to human function as presented earlier in this section, together with a knowledge of human reproductive health and disease, permits a great deal of information to be obtained from laboratory animal studies. The complete understanding of basic reproductive physiology allows the toxicologist to focus on specific targets of toxic action. It is from the understanding of basic reproductive physiology, the experiments of nature provided by spontaneous reproductive diseases, and

laboratory experiments with animal models that targets of toxicity on functional and anatomical bases are appreciated. These targets are defined in the following sections.

Central Targets

The organs, nuclei, and organelles that are required for normal pituitary secretion of gonadotropins are considered to be the central targets of toxicity. They are generally divided into the neural tissues, nerve tracts, specific nuclei in the brain, and their organelles (including the hypothalamus and higher nervous centers) and the anterior pituitary gland. In some cases, the pineal gland would also be considered a target because of its direct effect on pituitary function.

Hypothalamus and Higher Brain Centers Toxicants that disrupt the synthesis of GnRH or its normal pulsatile release will cause reproductive failure by way of pituitary dysfunction. There are two general mechanisms for this to occur. The direct effect is one in which neural transmission is altered by other neurotransmitters or their analogs. Anesthetics, anticonvulsants, and recreational drugs are examples of agents that can cause hypothalamic dysfunction that, in most cases, decrease GnRH pulse and amplitude. These kinds of toxicants can reduce neuronal firing rate and reduce either the baseline gonadotropin secretion or block the midcycle periovulatory surge in laboratory animals. In human subjects decreased nutrition, increased exercise, as well as physical or emotional stress can lead to similar derangements of the hypothalamic–pituitary axis by increasing catecholamine, indolamine, and endorphin levels with oligomenorrhea or amenorrhea as a result. Some compounds such as the ergot derivatives can mimic dopamine action and reduce prolactin secretion. The indirect effect is one in which the normal 'long-loop' feedback mechanisms are altered. Bioactive steroid hormones or their analogs can inappropriately increase or decrease hypothalamic drive leading to alterations in pituitary gonadotropin secretion. Increased adrenal glucocorticoid, for example, is thought to decrease gonadotropin secretion although the precise mechanism is not known. Diethylstilbestrol is a model for a toxicant that might decrease hypothalamic drive because it is a potent estrogen agonist. In contrast, tamoxifen or clomiphene citrate, which are estrogen antagonists, would have the opposite effect. Some of the halogenated hydrocarbons are thought to act as estrogen antagonists and may influence hypothalamic function by acting through estrogen receptors.

Anterior Pituitary The anterior pituitary can also be adversely influenced by two separate but general mechanisms. It can be directly affected by changes in the stimulatory effect of GnRH from the hypothalamus and it can be modulated by ovarian steroid and peptide hormones from the ovary (as discussed previously). Perturbations of the hypothalamus are transduced directly to the pituitary through the primary GnRH signal; thus, normal pituitary function is unlikely when the hypothalamic drive is perturbed. Because of the location of the hypothalamus and pituitary at the base of the brain and the intimate vascular and neuronal connections between them, it is difficult to separate actions that occur at this level. Therefore, hypothalamic and pituitary failure are often considered together as simple 'central effects' as opposed to actions at the level of the ovary, reproductive tract, or related reproductive tract organs.

Inappropriate circulating levels of bioactive steroid hormones or their analogs can lead to perturbations of pituitary function. Increased blood concentrations of bioactive estrogen, progesterone, androgen, or their analogs will lead to decreased secretion of gonadotropins. Steroid antagonists will open this feedback loop and cause increased amounts of gonadotropins to be secreted. The therapeutic bases of oral contraception and one aspect of fertility enhancement are based on these principles. Many halogenated hydrocarbons are thought to act as estrogen analogs and act to either transduce false signals through the estrogen receptor or block endogenous estrogen from exerting normal action. The latter case is well defined for DDT, which causes thin egg shells in birds exposed to DDT. Some PCBs have agonistic and antagonistic action in different animal species and different organs within the same species. It is not clear how many chlorinated biphenyls have estrogenic effects or if any of these compounds are serious potential hazards to women. In many cases they are estrogen agonists when acting in the absence of steroidal estrogen and estrogen antagonists in the presence of steroidal estrogen. It is also difficult to separate actions which occur at the hypothalamus and pituitary; therefore, these are often considered collectively as 'central' effects as opposed to effects that occur downstream such as at the level of the ovary or reproductive tract organs.

New evidence is now emerging that some halogenated hydrocarbons exert their effects through receptors other than the classic estrogen receptor. Beyond the identification of alpha and beta (possibly gamma?) forms of the original estrogen receptor, other nonrelated receptors are now thought to interact with the classic estrogen receptor hormone signally, besides membrane-bound forms of the estrogen receptor as well as with nonrelated orphan

receptors. One such orphan receptor is the arylhydrocarbon (Ah) receptor and has no known physiologic role but acts much like the receptors of the steroid hormone superfamily of receptors. The binding of the Ah receptor to its ligand, which can be dioxin or related coplanar, chlorinated biphenyls, elicits transcription of new proteins and/or blockage of other proteins such as estrogen receptors.

Ovarian Targets

Ovarian tissue can be compared to embryonic tissue in that most of its functional elements are still in various stages of development. All of the endocrine aspects of the ovary are differentiated at the time that a subpopulation of germ cells matures. Both the endocrine and the germ cell populations are transient populations that must be renewed with each reproductive cycle. The ovarian targets of toxicity are therefore ever-changing populations of different cell types. For this reason it has been difficult to identify cytotoxic agents that have specific ovarian cell types as their unique target. In general, ovarian targets can be divided into two categories. The most important category is the germ cells, which are primarily primary oocytes in a resting stage of meiosis. The second category is represented by the cells which produce steroid and peptide hormones.

Germ Cells Unlike the testes, in which steroid production can proceed in the absence of spermatogenesis, the ovary can function as an endocrine organ only if viable germ cells are in residence. Toxicants that eliminate the resting germ cells automatically eliminate all endocrine function. Since all aspects of female reproduction are dependent on ovarian steroids, the growth, development, and integrity of the entire reproductive system will be disrupted by loss of the germ cells. A complete loss of ovarian function would ensue and, in humans, menstrual function would cease as it would with complete hypothalamic; pituitary dysfunction. In contrast, toxicants that adversely affect only the oocytes which have ended their resting phase and begun to mature will interrupt only the current ovarian cycles as additional oocytes can be recruited from the resting germs cells. Such compounds may be 'silent' hazards having the effect of delaying conception only slightly. Exposure to such toxicants would most likely be recognized through menstrual dysfunction, long menstrual cycles, and possibly as a delay to conception.

Ovarian Steroid-Secreting Cells Steroidogenic cells within the ovary are also transient cell populations. While the development of gonadotropin receptors and steroidogenic machinery are dependent on the proximity and continued viability of a healthy oocyte, the mature cell will survive only the length of the reproductive cycle and possibly through one pregnancy. The steroidogenic cells of the ovary are recruited in each cycle from undifferentiated ovarian stroma. Agents that arrest differentiation, block the expression of gonadotropin receptors, or block the production of steroid hormones will have adverse effects on ovarian function. Steroidogenesis can be blocked by either blocking the transport and availability of cholesterol, compromising the reducing capacity of the cell, or by direct block of steroidogenic enzymes. Such disruptions would be recognized as menstrual dysfunction ranging from irregular menstrual cycles to complete amenorrhea if steroidogenesis is completely stopped.

Reproductive Tract Targets

The female reproductive tract is completely dependent on the functioning ovary to provide estrogen and progesterone for its growth, development, and function. Reduced steroid production, increased clearance of circulating steroid hormones, or antagonism of steroid action at the level of the steroid hormone receptor will lead to decreased size and function of all aspects of the female reproductive tract. Increased circulating concentrations of sex steroids or their agonist generally lead to hypertrophy, hyperplasia, and dysfunction. Sex steroids or their analogs at relatively high circulating concentrations will disrupt pituitary function through the long-loop feedback. However, exposure to low levels of steroid analogs for prolonged time periods may have adverse effects on the reproductive tract without disrupting the HPO axis. Such theoretical toxicants could cause infertility with no other overt signs. An example of this kind of toxicant is low-dose progestin therapy which, in some women, is an effective contraceptive although relatively normal menstrual cycles are observed, suggesting adverse effects at the level of the endometrium while exerting no demonstrable effect at the level of the HPO axis. Similarly, weak sex steroids could act locally to alter cervical secretion, reducing sperm survival and transport through the reproductive tract as observed in sheep exposed to plant estrogens.

Over 300 different plants contain either compounds with estrogenic activity or precursors for the formation of nonsteroidal estrogens. Coumestrol, equol, and zearalenone are examples of phytoestrogens that are found in legumes, tubers, and fungi that infest grains. These substances clearly act as reproductive toxins in sheep (equol in clover disease) and

pigs (zearalenone in moldy corn syndrome). Evidence is not as convincing for carnivores fed commercial diets with plant 'fillers'. In humans there is some evidence that Asian diets act as a protectant for some forms of hyperplastic disease. Some claims for precocious puberty being the result of contamination with environmental estrogens have been made. Although there are structural similarities between the parent phytoestrogen molecules and DES, it is speculated that only phenolic metabolites of these compounds are active compounds since pretreatment with carbon tetrachloride (to inhibit the mixed function oxidase in the liver) reduces the estrogenic *in vivo* potency of *o*,*p*′-DDT in rats. However, *in vitro* studies indicate that *in vitro* competition of compounds, such as *o*,*p*′-DDT and methoxychlor with estradiol for binding the rat uterine estrogen receptor, is positively correlated with *in vivo* estrogenicity. The fact that the estrogenicity of either the parent compound or its metabolite competitively competes with estradiol for receptor binding may limit the action of circulating steroidal estrogens. By limiting the action of the more potent steroidal estrogens, the weaker nonsteroidal estrogens may act as antiestrogens. In addition, some DDT analogs such as *p*,*p*′-DDT are thought to act by inducing liver enzymes that metabolize endogenous steroidal estrogen, thus reducing normal estrogen delivery to the target tissue.

Substances that increase or decrease smooth muscle activity can cause adverse reproductive effects. Nicotine, for example, acting through epinephrine and oxytocin can influence tubal and uterine contractions. Theoretically, such agents could cause mistiming of gamete and/or embryo transport and failure of fertilization or implantation, respectively. Hemotoxic agents can alter menstrual flow and result in menstrual irregularities (as indicated previously) at the level of the endometrium without having any effect on the reproductive system directly.

Nonreproductive Organ Targets

Key nonreproductive organs are essential for normal reproduction. An example of this kind of interaction is the production of binding proteins that are essential for steroid hormone transport by the liver. Hepatotoxins such as ethanol can limit binding protein production and adversely alter the ability of sex steroids to be transported to their binding sites. The liver also plays the primary role in deactivating and eliminating steroid hormones. Hepatotoxins, such as the halogenated hydrocarbons, barbiturates, and anticonvulsants which alter enzymes that either conjugate or metabolize steroid hormones, can also adversely affect reproductive function. Normal thyroid function is important for normal reproduction. Thyroid hormone is essential for normal cell function in general, and thyroid disease is often associated with reproductive failure.

Summary and Conclusion

In summary, in the broadest view reproductive toxicants can impinge on the female system through changing normal sexual development, obliterating gametes, causing dysfunction of reproductive organs, interfering with the differentiation of cell types, or interrupting the hormone messages through which the processes of hormone synthesis, transduction, or metabolism occur. Reproductive toxicants can influence reproductive performance by affecting sexual or social behavior, embryo survival and development, as well as affecting reproduction indirectly by influencing general health. The primary difficulties faced in identifying reproductive toxicants are the sensitivity of female reproductive processes to normal environmental change, the lack of baseline data, the complexities of the ovarian cycle, and the species-specific nature of female reproductive physiology.

A great deal of progress is currently being made in this discipline in response to pressures exerted by the public. Real concerns are now being expressed that environmental factors are causing an increase in female infertility while more women are exposed to chemicals through the workplace. Perhaps the greatest impediment to progress in this area is adequate animal models or an *in vitro* screening test for human sensitivity to the large number of chemicals being produced.

See also: Androgens; Carcinogen–DNA Adduct Formation and DNA Repair; Chromosome Aberrations; Developmental Toxicology; Dose–Response Relationship; Endocrine System; Epidemiology; Reproductive System, Male; Risk Assessment, Human Health; Sister Chromatid Exchanges; Toxicity Testing, Developmental; Toxicity Testing, Reproductive.

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Reproductive System, Male

Marion G Miller and Shelley Brown DuTeaux

Introduction

Both the public and the scientific community have become increasingly aware of the potential for chemicals to adversely affect the male reproductive system. It has been estimated that as many as 15% of couples in the United States are infertile. For ~40% of those couples, infertility is associated with the male partner. Recent reports suggest that the rates of prostate and testicular cancer, prostatic hyperplasia, and cryptorchidism (undescended testes) are also increasing. Although a continuing subject of debate, an analysis of human sperm counts from 1938 to 1990 provided data indicating that the average sperm density in males has declined in the last 50 years. It has been proposed that exposure to estrogens and other endocrine disruptors in the environment could be associated with adverse effects on the male reproductive system.

Evidence for male reproductive toxicity in humans generally surfaces only when the outcomes are severe. For example, pesticide formulators exposed to high levels of the nematocide dibromochloropropane developed testicular atrophy and infertility. The loss of sperm production in the testes of several of the men studied became permanent. The opportunity to study such overt and devastating effects in humans is rare. It is more likely that a chemical will affect the reproductive tract in more subtle and complicated ways. Animal models are generally used to test the ability of chemicals to cause reproductive toxicity. Data generated from such experiments can supplement human epidemiological studies to help determine the safety of chemicals in the workplace and the environment. However, male reproductive toxicity testing has only been done on a fraction of the over 500 000 chemicals and mixtures currently used in commerce and industry. These data gaps have created an awareness of the need to improve the adequacy of our testing procedures and to better understand the events underlying male reproductive toxicity.

Reproduction is biologically complex. Perturbations in any number of biological processes could result in changes in the organs of the reproductive system and increase the potential for passing along development defects to children. An adequate evaluation of reproductive toxicity should consider the multitude of effects and how chemicals interact at the level of cells and tissues to result in dysfunction. The following discussion of male reproductive toxicology includes (1) male reproductive tract physiology, (2) methods for reproductive toxicity testing, (3) a description of specific targets, (4) examples of male reproductive toxicants, and (5) issues involving chemical regulation and safety.

Physiology of the Male Reproductive System

A series of tightly orchestrated events must occur for a male to produce viable sperm capable of fertilization and producing normal offspring (**Figure 1**). The process of spermatogenesis in the testis is subject to neuroendocrine controls via the hypothalamic-pituitary axis (I), and indirect influences arising from nutritional status, liver metabolism, and vascularization (II). Within the testis (III), endocrine, autocrine, and paracrine controls are required for the proliferation and differentiation of the stem cell spermatogonia into the mature spermatid that is released into the lumen of the seminiferous tubule. The released spermatozoa travel through the rete testis and efferent ducts to the head (caput) of the epididymis. As the spermatozoa pass through the middle (corpus) and tail (cauda) of the epididymis, they undergo maturation (IV) and gain motility as well as the ability to fertilize oocytes. Toxicants could affect any of these steps or have direct effects on sperm cell viability (V) or on the ability to penetrate and fertilize an oocyte (VI). To date, little is known about male mediated developmental toxicity, whereby the male gamete transmits inheritable defects to offspring (VII). This possibility has received more attention in recent years.

The Testis and Spermatogenesis

The testis is made up of tightly packed seminiferous tubules surrounded by a vascularized interstitium.

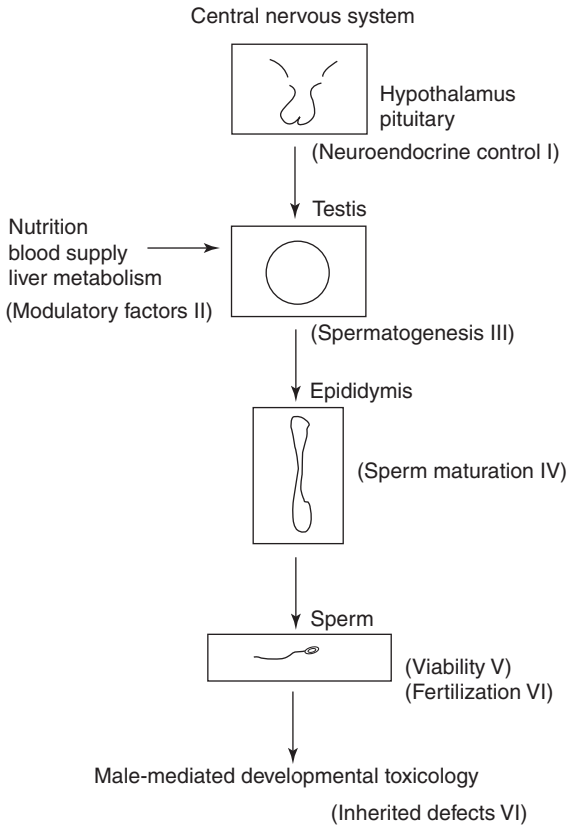

Figure 1 Overview of the male reproductive system.

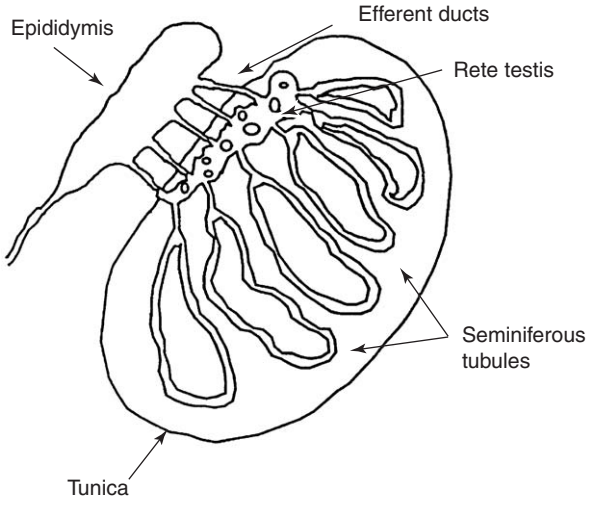

Figure 2 Schematic representation of the structure of the testis. (Reproduced from Working PK (1989) *Toxicology of the Male and Female Reproductive Systems*. New York: Hemisphere, with permission from Taylor and Francis, Inc.)

It is enclosed in a tough fibrous capsule called the tunica albuginea (**Figure 2**). Within the seminiferous tubules, germ cells develop into spermatozoa in a process called spermatogenesis. The Leydig cells located between tubules in the testis interstitium carry out the synthesis of steroids. Steroidogenesis is essential both for spermatogenesis and for developing and maintaining secondary sexual characteristics. (For more about steroids, see section 'Hypothalamic–Pituitary–Gonadal Axis'.)

The production of gametes in mammals (spermatogenesis, oogenesis) requires the process of meiosis to reduce the number of chromosomes in each cell from 46 (diploid) to 23 (haploid) so that a fertilized zygote will contain 46 chromosomes, half from each parent. In the male, the process of spermatogenesis involves mitosis and meiosis and three germ cell types: (1) spermatogonia, (2) spermatocytes in various stages of meiosis, and (3) postmeiotic spermatids undergoing elongation prior to release as spermatozoa. The first germ cell type, spermatogonia, undergo stepwise mitotic proliferation and differentiation and are classified by their stage of development (types A_1–A_4, intermediate, and type B). Type B spermatogonia ultimately divide into primary spermatocytes that enter meiosis. The process of reducing chromosomes from 46 in diploid spermatocytes to 23 in haploid spermatids starts in the preleptotene phase of meiosis I. Each primary spermatocyte enters meiotic prophase, forming distinct cell types at each phase (leptotene, zygotene, pachytene, and diplotene). At the end of meiosis I, two secondary spermatocytes are produced which enter meiosis II and rapidly divide to produce a total of four haploid round spermatids with 23 chromosomes each.

The metamorphosis of round spermatids into spermatozoa is described as spermiogenesis. Initially, the round spermatid develops an acrosome derived from the intracellular Golgi complex. The acrosome starts as a small vesicle and develops into a pronounced cap on the sperm head. The acrosome is necessary for oocyte fertilization and contains the lysosomal enzymes required to penetrate the vestments surrounding the egg. Early in spermiogenesis, a microtubule-containing flagellum begins to develop. Nuclear DNA undergoes condensation and is no longer synthesized before the nuclei elongate around a microtubule structure called the manchette. As the spermatid elongates, mitochondria collect in a sheath behind the sperm head and around the flagellum in what will form the midpiece. The mitochondria will supply energy for sperm movement. Release of mature spermatids into the tubular lumen (spermiation) is accompanied by the loss of the spermatid cytoplasm. The residual cytoplasm is endocytosed and forms residual bodies within the Sertoli cell. The final spermatozoa are ideally designed to transport DNA from the male to the oocyte, with little cytoplasmic baggage, a good mitochondrial engine, and a large tail for propulsion.

Immature germ cells develop in the basal area around the circumference of the seminiferous tubule. As spermatogenesis progresses, developing sperm advance toward the central lumen (**Figure 3**). The Sertoli cell, the 'nurse cell' of the testis, supports, nourishes, and protects the developing germ cells that it surrounds. Sertoli cell tight junctions form a 'blood-tubule' barrier that prevents the entry of blood-borne materials and maintains a specific tubular milieu necessary for germ cell development. In the rat, it takes ~56 days for spermatogonia to complete spermatogenesis and be released from the testis. Spermatogonial differentiation is initiated every ~12.9 days within the seminiferous tubules. At any given time there will be germ cells from successive generations and at different phases of development within the seminiferous tubules. Fourteen stages of spermatogenesis have been defined based on nuclear morphology and the appearance of the acrosome in the spermatid. The seminiferous epithelium cycles through the stages of spermatogenesis in a time-dependent manner. Different stages follow one another along the length of the seminiferous tubule in what is known as the 'wave of spermatogenesis'. This progression is necessary to maintain continuous sperm production. If there were no 'wave', spermatogonia throughout the testis would enter spermatogenesis at the same time and fertility would become episodic.

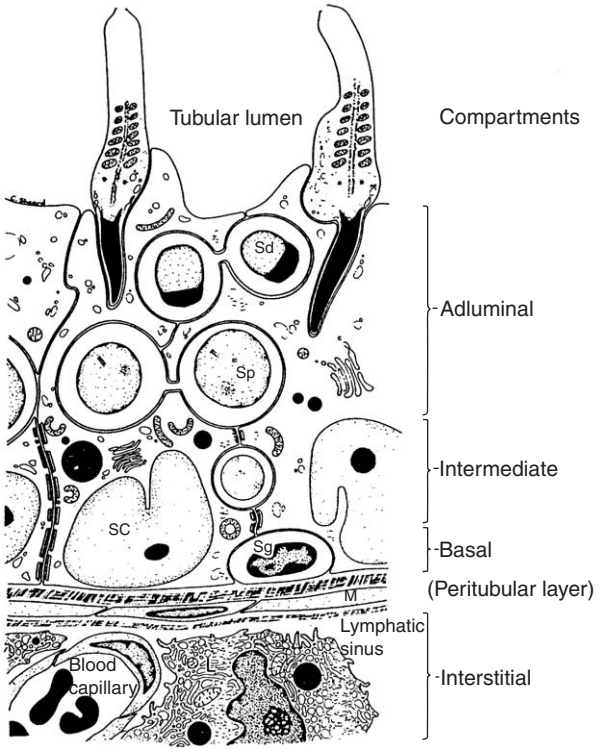

Figure 3 Diagrammatic representation of a portion of a seminiferous tubule. L, Leydig cell; M, myoepithelial peritubular cell; SC, Sertoli cell; Sg, spermatogonium; Sp, spermatocyte; Sd, spermatid. (Reproduced from Lamb JC, IV and Foster PMD (1988) *Physiology and Toxicology of Male Reproduction*. San Diego: Academic Press, with permission from Elsevier.)

The stages of spermatogenesis differ between species. Therefore, the duration and cycle length of spermatogenesis also differs between species. In the human, it is thought that there are six stages of spermatogenesis defined by specific cellular associations. This is in comparison to 14 stages in the rat. Interestingly, the human male may have no clearly defined 'wave' of spermatogenesis arranged consecutively along the length of the seminiferous tubule. While some researchers believe that human germ cell development occurs along helical and longitudinal axes, others believe that the arrangement of stages of spermatogenesis may simply be a random occurrence. From a toxicological point of view, germ cells at different stages of development and differentiation may have different susceptibility to toxicants.

The Excurrent Ducts and Sperm Maturation

Spermatozoa leave the testis by first passing through the rete testis, then flowing through the efferent ductules to the epididymis. These reproductive structures are collectively known as the excurrent ducts (**Figure 4**). In the rodent, the efferent ductules connect to the initial segment of epididymis. In humans, however, the efferent ductules are embedded within the head (caput) of the epididymis. Efferent ductules are comprised of epithelial cells that surround an open lumen. The ductule epithelial cells are specialized for reabsorption, with the portion adjacent to the testis absorbing the majority of fluid and the portion adjacent to the epididymis absorbing small proteins and other macromolecules released with sperm. From the efferent ductule, the concentrated sperm enter the epididymis.

The mammalian epididymis is a highly coiled duct where sperm undergo maturation and are stored prior to ejaculation. The epididymis is comprised of a head (caput), a body (corpus), and a tail (cauda), which can be defined by their relative location, tissue characteristics, and cell types. Within a connective tissue sheath, the epididymis is a complex of tubules lined with columnar epithelial cells attached to a basement membrane. Epithelial cell height decreases and luminal diameter increases from the initial segment to the cauda of the epididymis. There are several distinct epithelial cell types found in the mammalian epididymis, including the principal, narrow, basal, clear, and halo cells. The principal cells

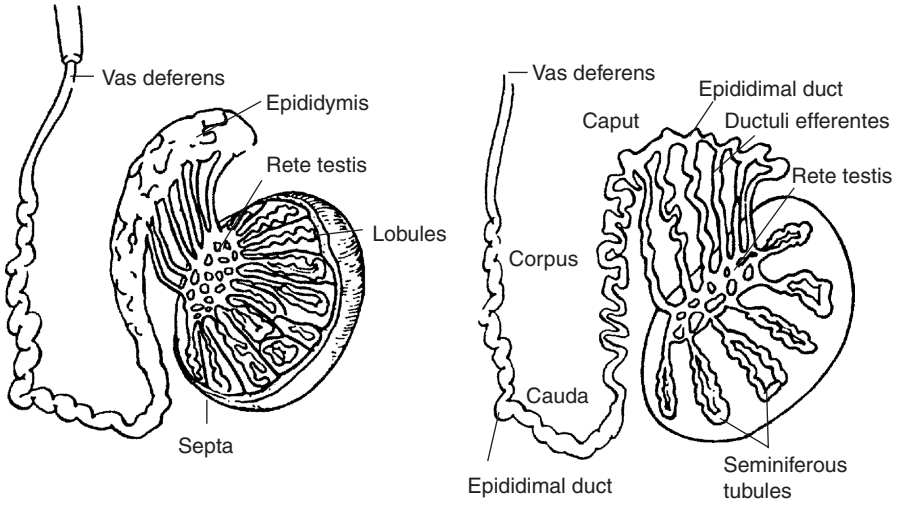

Figure 4 Structural relationships between the testis and the epididymis. (Reproduced from Zaneveld LJD and Chatterton RT (eds.) (1982) *Biochemistry of Mammalian Reproduction* © New York: Wiley. This material is used by permission of John Wiley & Sons, Inc.)

represent between 65% and 80% of the entire epithelial cell population and are involved in absorptive and secretory processes.

Sperm entering the epididymis from the testis are functionally immature and require further differentiation within the epididymis to become motile and to gain the ability to fertilize oocytes in the female reproductive tract. Sperm maturation events have not been completely elucidated. However, research indicates that sperm maturation is a complex process that involves the remodeling of the sperm plasma membrane within a changing luminal environment. The composition of the epididymal milieu is controlled in part by the epididymis–blood barrier and the active uptake and release of specific macromolecules. As sperm transit the epididymis, they are exposed to different epididymal 'microenvironments' that are important for sperm maturation. As sperm mature, they are distinguished by the loss of the cytoplasmic droplet, acrosomal and nuclear changes, and alterations to lipid and protein composition, all of which may be important to sperm gaining their fertilizing ability.

When mature spermatozoa reach the cauda of the epididymis they are stored until ejaculatory release via the vas deferens. Spermatozoa are discharged through the ejaculatory duct. The major portion of ejaculate volume is made up of products secreted by the accessory sex glands: the seminal vesicles, the prostate, and the bulbourethral glands. Rodents also have coagulating glands and preputial glands. Using mature spermatozoa from the cauda epididymis, it has been demonstrated that the secretions of the rodent accessory glands are not important for successful *in vitro* fertilization. However, there is a reduction in *in vivo* fertility when accessory gland products are not present in semen, indicating the importance of these components to successful reproduction.

Recently, it has been demonstrated that an immature human spermatid can be injected directly into an oocyte, resulting in successful pregnancy and birth. In practice, the success rates of intracytoplasmic sperm injection (ICSI) vary from 0% to 68%, depending on the number of oocytes injected, the age of the mother, and the quality of sperm. Currently there are no standardized indications for the use of ICSI for infertile couples. However, there is general agreement that ICSI should be used when male infertility (as diagnosed by semen analysis) is a factor. Some severe cases of male infertility are associated with chromosomal aberrations (e.g., aneuploidies, deletions). Therefore, the use of ICSI has raised concerns about the risk of transmission of chromosomal or genetic defects to embryos and negative consequences during development. Recent data have also suggested that the technique of ICSI, which bypasses the normal barriers of fertilization, may itself be responsible for alterations in the viability and health of fertilized embryos. Notwithstanding these concerns, the health of a majority of children delivered after ICSI has been normal.

Hypothalamic–Pituitary–Gonadal Axis

Neuroendocrine control of gonadal function is regulated through the hypothalamus in the brain and the closely associated anterior pituitary gland (**Figure 5**). Gonadotropin releasing hormone (GnRH) is released from the hypothalamus in a pulsatile

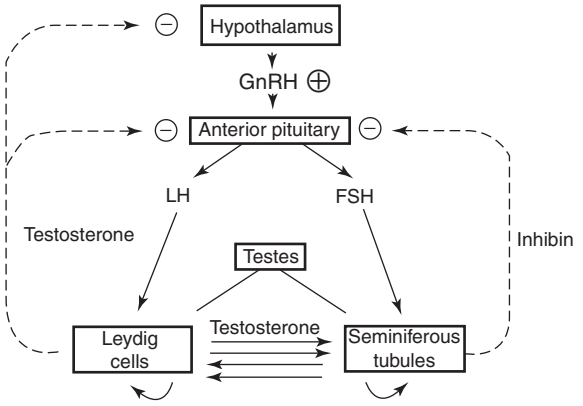

Figure 5 Neuroendocrine control of the male reproductive system. (Reproduced from Heindel JJ and Treinen KA (1989) Physiology of the male reproductive system: Endocrine, paracrine, and autocrine regulation. *Toxicology Pathology* 17 (2): 411–445, with permission from Society of Toxicologic Pathology.)

manner, and carried in the blood supply directly to the anterior pituitary. After being stimulated by GnRH, the pituitary releases the gonadotrophins luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH and FSH circulate in the blood and reach the testis where they play a central role in regulation of testicular function. Like GnRH, LH and FSH release are most likely pulsatile in nature. In the testis, LH targets the Leydig cells, where it binds to receptors and stimulates steroidogenesis. Testosterone production is episodic, coincident with the pulsatile release of LH. The Sertoli cell is the testicular target for FSH. The complete role of FSH in spermatogenesis is as yet unknown. However, FSH is necessary for spermatogenesis presumably due to its involvement in Sertoli cell function.

Hormonal regulation is through a series of feedback mechanisms taking place at both central and peripheral sites. To complete the endocrine feedback loops, testosterone regulates LH production, while inhibin and other Sertoli cell products regulate FSH secretion. These feedback loops modulate the release of GnRH from the hypothalamus as well as LH and FSH from the anterior pituitary. Within these loops, factors that perturb one component may alter regulatory influences on another. For example, if the Leydig cells were damaged, there could be a decrease in testosterone production. In response to low circulating levels of testosterone, LH release would increase in an attempt to restore testosterone production.

Approaches to Male Reproductive Toxicity Testing

A variety of tools are available to assess the male reproductive toxicity of a chemical and potential mechanisms underlying toxicity. The most important physiological endpoint is fertility. Therefore, the effect of a toxicant on fertility should be included in most assessments. However, impaired fertility is also the most severe effect. Therefore, toxicity testing should also be designed to detect subtle compromises, such as alterations in histology, testicular function, epididymal function, and sperm assessment. For humans, the most compelling reproductive toxicity data are collected from epidemiological studies and case reports. When human data are not available, toxicity testing may be conducted on experimental animals. Because experimental species such as rats, rabbits, and mice are very fecund and have far larger sperm reserves than humans, these animals generally need to be exposed to high doses of chemicals before an effect on fertility is observed.

Human Studies

Generally, it is difficult to assess the adverse effects of a chemical or exposure on the male reproductive tract unless the man is actively trying to conceive. Therefore, a chemical may be affecting fertility in a man and go unnoticed. The 'hidden' nature of male reproductive toxicity may underrepresent the effects of reproductive toxicants on the human population.

Human data may be collected from clinical studies of subfertile men or from epidemiological studies. Epidemiological case–control and historical cohort studies have successfully identified the male reproductive toxicity of several solvents and pesticides. (See section 'Examples of Male Reproductive Toxicants'.) Clinical studies might bring to light specific exposures or risk factors affecting the male's ability to conceive with his female partner. The first step is generally a detailed semen analysis, including assessment of sperm concentration, semen volume, percent motile sperm, sperm viability, and morphology. A more detailed analysis of sperm pattern and vigor can be done with computer-assisted sperm analysis. Other specialized tests, designed to assess the acrosome reaction or the fertilizability of sperm through the hamster egg-sperm penetration assay, may also be performed. Certain chemicals may affect sexual potency (the ability to achieve erection), ejaculation, and libido. Testing hormone levels, testicular size and rarely histology (through biopsy) can be important.

Animal Studies

The majority of information about male reproductive toxicants has been obtained from studies carried out in the rat, the most common animal model used for reproductive toxicity. Animal studies allow for controlled experimentation where events

underlying reproductive toxicity can be better understood. In a typical study, age- and weight-matched male animals are randomly placed into treatment and control groups. Treatment animals are dosed with a specific amount of the chemical for a given duration. The control group undergoes the same type of dosing regimen with an identical formulation minus the toxicant. Dosing is generally by oral gavage; however, other routes of dosing may be used to reflect the expected route of exposure. Various endpoints are assessed, including testis and epididymis size and weight, histology, seminiferous tubule diameter, *in vitro* fertilization, natural mating, mating behavior, and function of the accessory sex glands. Additionally, these endpoints may be studied in a group of animals that have entered a recovery phase after dosing to assess reversibility of effects.

A substantial literature exists emphasizing the use of mating trials for testing male reproductive toxicants. These studies are useful for determining not only the ability of a toxicant to affect reproductive performance, but the subsequent health and development of offspring. In a single mating trail, male animals undergo extended dosing to insure exposure throughout all phases of spermatogenesis and sperm maturation. Males are then mated with untreated females. Following successful mating, the pregnant females are necropsied and the level of gestational success is determined. Endpoints include comparing the number of live implants in females in the treated group versus the control group. Postimplantation loss, or the ratio of dead to total implants from the treated groups compared to the same ratio from the control group, can be measured along with preimplantation loss, which is based on the number of corpora lutea counts and the total implants per female in treated and control groups. Females may be mated with treated males at various times during dosing to determine which sperm cell type might be affected by the toxicant. For example, if a chemical targets the spermatocytes, fertility would decrease coincident with 4–5 weeks of dosing. This delay reflects the time required for the damaged cell population to complete spermatogenesis and pass through the epididymis. However, if the toxicant affects mature spermatozoa, the onset of fertility changes may occur very rapidly after dosing.

Multigenerational reproductive toxicity studies are established testing paradigms used by agencies such as the US Environmental Protection Agency (EPA). Typically, both males and females are dosed prior to mating, and the females are dosed throughout gestation, birth, and lactation. A group of the pregnant dams is sacrificed and the gestational success is determined, as outlined above. Another group of pregnant dams is continually dosed and allowed to deliver and nurse live pups. In order to determine if *in utero* exposure alters reproductive capability, a group of the F_1 pups is dosed from weaning to sexual maturity using the same protocol as their parents. These F_1 pups are eventually mated. Animals in each generation are necropsied and evaluated for systemic and reproductive toxicity, including the F_1 animals, which have been exposed to the toxicant through all stages of development. Endpoints include fertility and histopathology in the parent generation, litter size, and offspring weight, external abnormalities, and subsequent growth. This type of toxicity testing protocol is necessary for pesticide registrations and for other regulatory purposes, but it cannot supply detailed information about the mechanism of toxic action.

Fetal malformations are generally believed to arise *in utero*. However, there exists the possibility that adverse developmental outcomes in the fetus might arise because of paternal exposures to environmental agents. For example, exposure to a particular agent may genetically damage sperm without affecting the ability of the sperm to fertilize an oocyte. However, sperm from the exposed male may be compromised such that the fertilized oocyte will die during cell division. The dominant lethal test is designed to look for loss of the conceptus resulting from alterations in the normal chromosomal complement (e.g., aneuploidy, polyploidy, nondisjunction). The mouse is the predominant animal model for testing dominant lethality. Generally, the male is treated for an extended period and mated with an untreated female. The pregnant dam is sacrificed and pre- and postimplantation losses are calculated. In addition, the recovered embryos may be tested for the presence of chromosomal aberrations.

In addition to genetic alterations, nongenetic developmental defects might arise in the fetus because of paternal exposures to environmental agents. Male-mediated developmental toxicity (either genetic or nongenetic in origin) could result in alterations to fertilization, growth, and development in the absence of maternal exposures. Testing for male-mediated developmental toxicity in animals generally involves dosing the male animal and then mating with untreated females. The pregnant dams are typically sacrificed near the end of pregnancy and the pups are examined for teratological defects, such as bone, brain, and other organ deformities. Another group of females may be allowed to rear their young so that the effects of the paternal exposure can be evaluated on the stages of physiological and behavioral development. Exposure of the father to the anticancer drug cyclophosphamide may result in

male-mediated developmental toxicity. Studies of cyclophosphamide have shown malformations and retardation of growth in the surviving fetuses and a high frequency of fetal death, while causing only minimal alterations to fertility.

Innovative Strategies

In recent years, there has been increasing interest in toxicity tests that minimize the use of animals and allow for high throughput testing of multiple chemicals. While there is no substitute for the whole animal, integration of data from such studies is especially helpful to define mechanisms. Recently, the US EPA developed the Endocrine Disruptor Screening Program (EDSP) to determine whether pesticides, other chemical products, and environmental contaminants could affect the endocrine system. The US EPA is currently developing assays designed to detect chemical substances capable of interacting with the estrogen, androgen, and thyroid hormonal systems. One of the assays under development, the Androgen Receptor Binding assay, is designed to determine if a chemical can bind like testosterone to the androgen hormone receptor *in vitro*, and mimic the action of the natural hormone or block access of the hormone to the site, thereby affecting androgen-dependent activities of cells and tissues. Such high throughput assays may provide cost- and time-efficient ways of gathering reproductive toxicity data with minimal resources.

Specific Targets of Toxic Action

Many compounds have been implicated as male reproductive toxicants, but their sites and mechanisms of action are not well understood. The classification of male reproductive toxicants as direct or indirect is useful to help define the primary site of toxicity (**Figure 6**). A direct toxicant would primarily target the testicular cells, the excurrent duct system of the male reproductive tract, or mature spermatozoa. An indirect toxicant would cause reproductive toxicity by acting on hypothalamic/pituitary neuroendocrine controls or on extragonadal systems. Since the testis is subject to hormonal control and feedback loops, the action of indirect toxicants on endocrine homeostasis can ultimately damage testicular cell types.

Adsorption, Distribution, and Metabolism of Reproductive Toxicants

The absorption, distribution, and metabolism of a chemical can be important in determining potential reproductive toxicity. First, the amount of a chemical absorbed into the body will affect its potential to cause toxicity. Different chemicals are differentially absorbed via the skin, the gastrointestinal tract, and the lungs. Some compounds can pass through the gut or be exhaled virtually unchanged. However, once in the body, a chemical can be distributed to tissues and organs via the circulatory system. Once a toxicant reaches the liver by gastrointestinal adsorption or by circulation, it can be rapidly metabolized and detoxified. The levels of the chemical in the body would decline and there may be less opportunity for the chemical to cause reproductive toxicity. Enzymes present in the liver may not only detoxify chemicals in this manner, but also can bioactivate the parent compound into more reactive intermediates. These metabolites, if sufficiently stable, can reenter the circulation and be delivered to the reproductive tract where toxicity can result. Recent studies have also shown that the testis, efferent ductules, and epididymis contain metabolizing enzymes capable of bioactivating chemicals *in situ*. The rate of metabolism of these enzymes within the reproductive tract is far lower than metabolic rates in the liver. However, the close proximity of these testicular and epididymal enzyme activities to developing and maturing germ cells could be of particular importance for toxicity.

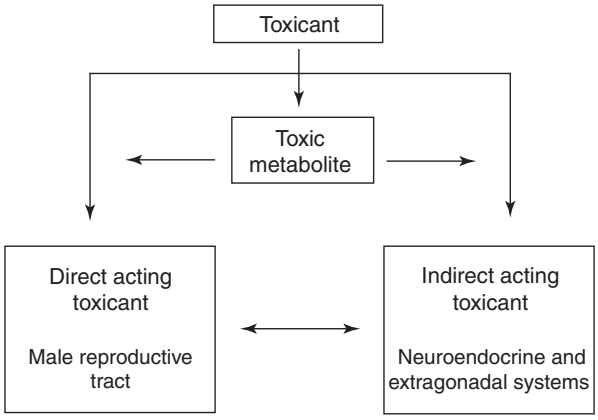

Figure 6 Classification of male reproductive toxicants as direct or indirect acting.

Another factor that may predict a chemical's ability to move about the body is its lipophilicity. The reproductive tract may be a potential target of toxicants that are fat-soluble. Like the blood barriers of the placenta and brain, the testis– and epididymal–blood barriers do not restrict the flow of compounds that are highly lipophilic. Importantly, a large fat pad surrounds the epididymis and efferent ductules. The proximity of the fat pad to these organs can potentially increase the exposure of these organs and maturing spermatozoa to lipophilic toxicants that tend to accumulate in adipose tissue. For example, the highly lipophilic organochlorine pesticides dieldrin

and aldrin have been detected in the male reproductive tract of animals after exposure.

Germ Cell Targets

The germ cells of the testis are continually undergoing renewal. Because of the diversity of events occurring in spermatogenesis, the germ cells of the testis have differing susceptibilities to the action of toxicants. However, because of the interrelationships of the developing germ cells to one another and to the supporting Sertoli cell, it is often difficult to discern which cell type was affected first.

Spermatogonia The rapidly dividing spermatogonia may be susceptible to toxicity induced by agents that affect cell division. For example, radiation and some anticancer drugs such as busulfan and procarbazine have been shown to cause genotoxicity in spermatogonia. These agents are used in cancer therapy because of their potential to damage rapidly dividing tumor cells. It is not surprising that they could also target rapidly dividing germ cells.

Spermatocytes Ethylene glycol monomethyl ether (EGME) was formerly used in the semiconductor industry and has been shown to elicit a relatively specific toxicity primarily to pachytene spermatocytes. It is not EGME, but its metabolite methoxyacetic acid, that is thought to be responsible for damaging spermatocytes. It has been found that the methoxyacetic acid disrupts protein kinase activities in dividing mitotic cells, and that cotreatment of seminiferous tubules with EGME and protein kinase inhibitors blocked the cytotoxic effects to spermatocytes.

Spermatids Few agents have been specifically implicated in spermatid toxicity. Exposure to methyl chloride (once used as a fumigant) caused a delayed release of mature spermatids from the testis. In addition, spermatids were present at much later stages than would be expected. Another discontinued fumigant, ethylene dibromide, also directly affects spermatids, although other germ cell types were also affected.

Spermatozoa α-Chlorohydrin directly affects spermatozoa with a resultant diminution in motility. Since this direct effect is reversible, α-chlorohydrin was once considered a candidate for male contraception. However, because irreversible toxicity was found in the epididymis, the potential for drug development of α-chlorohydrin was not explored further. The sulfonamide drug sulfasalazine may affect mature spermatozoa in the epididymis, resulting in decreased fertility. However, historical data on the toxicity of sulfasalazine were collected from patients being treated for inflammatory bowel disease. Therefore, it is possible that the spermatotoxicity and reproductive dysfunction may be a consequence of the disease state rather than the drug.

Testicular (Nongerm Cell) Targets

The testes are specialized for the development of germ cells into spermatozoa and the production of testosterone. The two major nongerm cell types supporting these functions are the Sertoli cell and the Leydig cell. Various agents that target the Leydig or Sertoli cells can disrupt spermatogenesis directly by affecting cell function or indirectly by interfering with the hormonal regulation of spermatogenesis. Because these somatic cells are integral to the processes of spermatogenesis, it is important to consider each cell type as a vulnerable target.

The Leydig Cell

Leydig cells have a central role in the synthesis and secretion of testosterone. LH, released from the anterior pituitary, stimulates production of testosterone by the Leydig cell. Many chemicals alter Leydig cell function and some can cause Leydig cell death. For example, ethylene dimethanesulfonate, an agent formerly used for cancer treatment, causes Leydig cell death with subsequent loss of testosterone biosynthesis. A chemical may also disrupt steroidogenesis by its action on the testosterone biosynthetic pathway without causing cell death. For example, δ-9-tetrahydrocannabinol (THC), the active ingredient in cannabis, causes a decline in the release of FSH and LH, resulting in decreased serum levels of testosterone in both experimental animals and humans. Besides disruption to the hypothalamic–pituitary–gonadal axis, THC and its water soluble metabolites can directly reduce cAMP-stimulated testosterone production in Leydig cells.

The Sertoli Cell

The Sertoli cell performs a pivotal role in spermatogenesis, orchestrating and nurturing the developing germ cells. The Sertoli cell has many functions. It plays a protective role through Sertoli–Sertoli cell tight junctions, which compartmentalize the developing germ cells away from the extratesticular milieu. This barrier means that the nutritive and hormonal requirements of germ cells must pass through or be generated within the Sertoli cell. The Sertoli cell cytoskeleton also performs specialized transport and support functions. Microtubule networks track through the cell, carrying a multitude of hormonal and nutritive factors essential for germ cell development. The

cytoskeleton also provides the scaffolding and physical support for the developing germ cell.

The Sertoli cell plays a key metabolic role in the processes of germ cell development. Compounds that disrupt Sertoli cell metabolism would be expected to cause testicular toxicity. For example, 1,3-dinitrobenzene and other nitroaromatic compounds cause testicular toxicity apparently by disruption of Sertoli cell function. These compounds can undergo reductive metabolism to toxic nitroso intermediates, which may be ultimately responsible for the Sertoli cell toxicity. As indicated above, microtubules play an important role in support and transport processes. Hexanedione has been studied extensively as an agent capable of altering testicular microtubules. Other compounds that disrupt microtubule assembly and Sertoli cell function include the fungicide benomyl and the antiinflammatory agent colchicine, both of which prevent the assembly of testicular tubulin into microtubules.

The Epididymis as a Target Organ

The potential for the epididymis to be a target organ may depend, in part, on it being unique from many other components of the male reproductive tract. The specialized processes involved in sperm maturation (i.e., ion and fluid regulation, protein secretion) and its distinct cell types may make the epididymis more or less vulnerable to the effects of toxic action. Several male reproductive toxicants target the epididymis and alter specific cell functions. For example, the immunosuppressive agent cyclosporine alters the number and size of specific epithelial cell types within the epididymis and affects epididymal sperm morphology. α-Chlorohydrin and its chlorolactaldehyde metabolite are thought to cause a reversible vacuolization of the tubular epithelium in the caput epididymis, lead to the formation of epididymal sperm granulomas, and increase the number of morphologically abnormal spermatozoa. Any agent that alters testosterone production may also perturb epididymal structure and function and the androgen-dependent processes of sperm maturation. Toxicity in the epididymis could be overshadowed by toxicity in the testis, especially if there are profound changes. It may be difficult to distinguish the direct effect of a toxicant on the epididymis from indirect effects that arise from testicular toxicity.

Another feature that may influence the epididymal toxicity of an agent is the presence of metabolizing enzymes within the epididymis. The basal cells of the epididymis contain alcohol dehydrogenases capable of oxidizing small alcohols (i.e., methanol and ethanol) to aldehydes (i.e., formaldehyde and acetaldehyde). Glutathione *S*-transferases, a family of isozymes that catalyze the detoxification of electrophilic compounds by conjugation with glutathione, have been localized in several epididymal cell types. Recently, cytochrome P450 2E1, which is important for the metabolism of chlorinated solvents, has been localized to portions of the epididymis and efferent ductules. While metabolizing enzymes in the epididymis could potentially play a protective role, these enzymes can also bioactivate chemicals, producing intermediates that in some cases are more toxic than the parent compound. Under such conditions, the presence of metabolizing enzymes within the epididymis can increase tissue toxicity.

Hypothalamic–Pituitary–Gonadal Targets

Agents that alter the central nervous system control of the hypothalamic release of GnRH have the potential to disrupt the hypothalamic–pituitary–gonadal axis. Hypothalamic release of GnRH is stimulated by α-adrenergic receptors. Therefore, agents that alter α-adrenergic function may alter GnRH release. For example, the insecticide chlordimeform may decrease GnRH release through an adrenergic mechanism. Conversely, endogenous opioids inhibit GnRH release and morphine and morphine-like drugs can suppress GnRH-mediated secretion of LH.

In recent years, scientists have suggested that certain chemicals might disrupt the endocrine system of humans and wildlife. A variety of chemicals have been found to disrupt the endocrine systems of laboratory animals. There is also compelling evidence showing that endocrine systems of some fish and wildlife species have been affected by environmental contaminants, resulting in developmental and reproductive problems. Agents that act in place of endogenous steroid hormones or disrupt receptor or enzyme action have the potential to disrupt the hypothalamic–pituitary–gonadal axis. For example, DDT, chlorodecone (Kepone), and polychlorinated biphenyls (PCBs) are classified as endocrine disruptors. Exposure of wildlife to PCBs has been associated with feminization and decreased levels of testosterone in males. The mechanism of endocrine disruption may be a change in the binding of natural hormones to their receptors or the increased binding of environmental chemicals to the hormone receptors, both of which might result in an inappropriate hormonal response. In addition, these agents might interfere with steroid biosynthesis by altering the function of enzymes along the hypothalamic–pituitary–gonadal axis. In the male, blocking the action of testosterone can result in an inappropriate release of GnRH, LH, FSH, and disruption of gonadal function.

Examples of Male Reproductive Toxicants

The following describes the male reproductive toxicity of the pesticides dibromochloropropane and carbendazim, and finasteride, a drug used for male-pattern baldness and prostate enlargement. While our knowledge is still evolving, these examples show ways in which animal and human data combine to give a broad understanding of the mechanisms underlying male reproductive tract disruption. More complete listings of male reproductive toxicants are available from a variety of sources, including the State of California Environmental Protection Agency, Office of Environmental Health Hazard Assessment (see Relevant Website).

Dibromochloropropane (DBCP)

In 1977, a group of men working as pesticide formulators and applicators in Central California noticed that few of them had recently fathered children. These men worked with DBCP, a brominated organochlorine nematocide first produced in the 1950s. Significant fertility problems came to light when the full cohort of DBCP production workers was studied. The epidemiological studies showed a significant relationship between DBCP and failed spermatogenesis. All men were exposed by inhalation and/or dermal routes, and the severity of effects increased with the length of exposure to DBCP. Testicular biopsies showed that the seminiferous tubules, and hence the site of spermatogenesis, were severely affected. Several samples showed that the cells that make up the seminiferous tubules were atrophic and contained few or no sperm cells. Several workers recovered many years after their exposure ended, and went on to father healthy children. However, some men who were initially characterized as having no sperm (azoospermic) never recovered. Ongoing epidemiological studies in California's Central Valley are considering the association between DBCP exposure via contaminated drinking water and adverse reproductive outcomes in surrounding communities; however, no clear associations have been found.

As yet, DBCP has no clearly defined mechanism of male reproductive toxicity. However, data suggest that spermatogenesis is an important target of DCBP. There is some evidence that cytochrome P450-dependent metabolism may be less important that glutathione conjugation in the toxicity of DBCP. Studies have shown that depletion of testicular glutathione with diethylmaleate protects against testicular cell damage caused by DBCP. There is also evidence that an episulfonium ion is formed following glutathione conjugation, and that this reactive intermediate may covalently bind and damage DNA. Interestingly, there are species differences in the sensitivity to the toxic effects of DBCP. The rat and the guinea pig appear to be the most sensitive test species, while the mouse and hamster are much less sensitive to the adverse effects of DBCP. If mouse or hamster data were relied upon in setting safety standards, we would have failed to properly characterize the reproductive hazards associated with human exposure to DBCP.

Benomyl

Benomyl is a benzimidazole fungicide that has been used effectively for many years on a variety of food crops and ornamental plants. Benomyl is metabolized primarily into carbendazim. It is suggested that benomyl and its metabolite carbendazim cause testicular toxicity by the same mechanism by which they act as fungicides. Benomyl and carbendazim inhibit the assembly of microtubules in fungi while leaving plant microtubules unharmed. However, they also disrupt the assembly of Sertoli cell microtubules in the testis. Microtubule organization, as mentioned before, is important to maintain the function of the Sertoli cells. Interestingly, Sertoli cell microtubules are most sensitive to the microtubule disruption caused by the fungicides, as neither microtubules involved in cell division or neuronal transport are affected at dose levels that disrupt Sertoli cell microtubules.

Early events in benomyl toxicity include the sloughing of germ cells into the seminiferous tubule lumen. This is followed by the disappearance of microtubules within the Sertoli cell. There are also reports that benomyl metabolism may affect the morphology of developing spermatids. At higher dosages, benomyl may increase fluid reabsorption in the efferent ductules and cause sloughing of ductule epithelial cells. The resulting ductule occlusion blocks passage of sperm from the testis to the epididymis. If the occlusion is severe enough, it can result in a rapid increase in testicular backpressure, leading to swelling of the testis, and, ultimately, seminiferous tubular atrophy and infertility. Both benomyl and carbendazim cause adverse effects in the testis. However, it appears that carbendazim produces more severe, longer-term damage, suggesting that the carbendazim metabolite is responsible for the adverse effects seen after benomyl exposure.

Finasteride

Finasteride is a synthetic polycyclic steroid prescribed for the systemic treatment of male pattern baldness. It is also promising in the treatment of

prostate enlargement. Finasteride selectively inhibits type II 5α-reductase that catalyzes the formation of dihydrotestosterone (DHT) from testosterone. There are two distinct isozymes of 5α-reductase, but type II is primarily expressed in the epididymis, prostate, and seminal vesicles. This enzyme is also found in the hair follicles. Over time, hair follicles produce thinner, finer hairs, and eventually senesce due to the action of DHT. Finasteride works by inhibiting DHT formation, thereby reducing serum and scalp DHT levels and slowing the progression of androgenic alopecia.

Several critical organs within the reproductive tract maintain their function because of DHT, including the epididymis, which forms DHT from testosterone by 5α-reductase. Studies in rats have shown that oral administration of finasteride causes an ~30% decrease in fertility, decreased fecundity, and related decreases in the seminal vesicle and prostate weight. The latter may actually be a beneficial effect from finasteride treatment, in that there is evidence that finasteride can help reduce prostate enlargement. In men undergoing treatment with finasteride, there are reports of slight but nonsignificant alterations in circulating levels of LH and FSH and increased circulating levels of testosterone and estradiol. In addition, men undergoing treatment with finasteride have also experienced decreased libido, erectile dysfunction, ejaculation disorders, and reductions in sperm count. However, because finasteride works by reversible competitive inhibition, several of these effects were resolved after discontinuation of therapy.

Assessing the Risks Associated with Reproductive Toxicants

Currently, there are a limited number of chemicals classified as male reproductive toxicants. As scientific, public, and regulatory interest in this field increases, the result will most certainly be an increased knowledge base of the type and number of chemicals that can adversely affect the male reproductive tract. Once a chemical is considered a potential reproductive toxicant, there are measures in place to protect humans from occupational and environmental exposure. For example, when the results from a risk assessment indicate that the potential exists for adverse reproductive effects in humans, a regulatory agency such as the US EPA may impose restrictions on the availability or uses of certain compounds. Reproductive toxicity testing is fundamental to this type of risk-based decision-making, and will hopefully lead to the development of safer chemicals and drugs.

When no human or epidemiological data exist, data from animal models can be used to predict human effects. Much of the data used for regulatory purposes is derived from animal toxicity testing. One approach is to obtain dose levels at which no-observed-adverse effect level (NOAEL) or lowest-observed-adverse-effect level (LOAEL) occurs in the animal model. To determine an exposure level acceptable for the human population a safety factor or multiple safety factors (often totaling (×) 1000) can be incorporated. The US EPA has published specific guidelines for reproductive toxicity risk assessment. Toxicokinetic and metabolic data are beginning to be incorporated into risk assessments. How a chemical enters the body, in what organs it is metabolized, and how easily the parent and reactive metabolites can reach the reproductive system are all important. The transport and metabolism of a chemical is often given considerable weight in determining the risk that a chemical poses to humans, and is generally incorporated in risk models.

While the processes of reproduction in humans and animals could be expected to have broad similarities, many species differences do exist. For example, it is well documented that rodents have sperm reserves that are much greater than man. In the rat, epididymal sperm counts can be reduced by as much as 90% without a significant affect on fertility. Since rodents have such large sperm reserves, rodent breeding studies may not detect subtle changes in reproductive capacity. To complement breeding studies, information about the effect of toxicant exposure on sperm numbers, motility, and morphology would be desirable. Mechanistic insights about causative events underlying changes in sperm parameters and their relationship to fertility would improve our ability to devise sensitive and specific toxicity tests which predict those chemicals most likely to cause reproductive harm.

See also: Benomyl; Dibromochloropropane; Glycol Ethers; Proposition 65, California; Reproductive System, Female; Toxicity Testing, Reproductive.

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Relevant Website

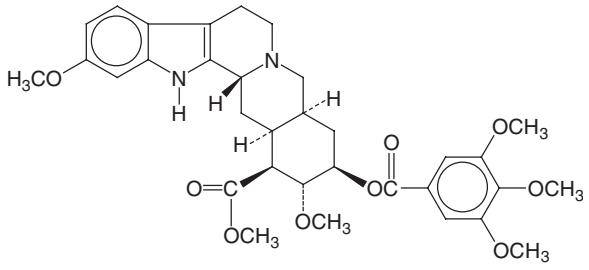
http://www.oehha.ca.gov – Proposition 65: Chemicals Known to the State of Cause Cancer or Reproductive Toxicity, State of California Environmental Protection Agency, Office of Environmental Health Hazard Assessment, Safe Drinking Water and Toxic Enforcement Act of 1986.

Reproductive Toxicity Testing *See* Toxicity Testing, Reproductive.

Reserpine

Elizabeth J Scharman

- Chemical Name: Reserpine
- Chemical Abstracts Services Registry Number: CAS 50-55-5
- Synonyms: Reserpinum; 3,4,5-Trimethoxybenzoyl methyl reserpate; (3β, 16β, 17α, 18β, 20α)-11, 17-Dimethoxy-18-[(3,4,5-trimethoxybenzoyl)oxy] yohimban-16-carboxylic acid methyl ester; Serpasil®
- Chemical/Pharmaceutical/Other Class: Rauwolfia alkaloid hypotensive agent
- Chemical Formula: $C_{33}H_{40}N_2O_9$
- Chemical Structure:



Uses

Reserpine has been used in the management of mild to moderate hypertension, the treatment of agitated psychotic states, as adjunctive therapy, second-line, for treating thyrotoxicosis, and to decrease the number and severity of vasospastic attacks caused by Raynaud's phenomenon and similar peripheral vascular disorders.

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposures to reserpine. It is available in an oral dosage form either alone or in combination with a thiazide diuretic, with or without hydralazine.

Toxicokinetics

The bioavailability of reserpine is ~40%. When given orally, peak blood levels occur within 1–3 h; however, the onset and duration of reserpine's pharmacologic effects are not related to drug concentrations in the blood or brain. The onset of action is within 3–6 days. Complete effects may be delayed by 2–3 weeks. Over 90% of the drug is metabolized in the liver to inactive metabolites. Sixty per cent of an oral dose is recovered in the feces in 4 days when given orally; 30% is recovered in the feces during the same time period when given intramuscularly. Reserpine is highly distributed into tissues, especially adipose tissue. The volume of distribution has not been determined. Reserpine crosses the blood–brain barrier, the placenta, and appears in breast milk. Protein binding is 96%. The elimination of reserpine is biphasic; the half-life is 50–100 h. The half-life may be longer in obese patients and is significantly longer if the creatinine clearance is $<10\,\text{ml}\,\text{min}^{-1}$. Pharmacodynamic effects may last from days to weeks after chronic use is stopped.

Mechanism of Toxicity

The exact mechanism of this peripheral adrenergic neuron blocking agent is not well defined. Reserpine administration results in depleted stores of norepinephrine, dopamine, and serotonin in multiple organs. The decreased peripheral resistance and cardiac output that results is manifested as a decrease in blood pressure. A central nervous system (CNS) effect may also play a role in decreasing blood

pressure. Depletion of catecholamines in the brain may explain the drug's adverse effects on the CNS.

Acute and Short-Term Toxicity (or Exposure)

Animal

Reserpine is used in horses as a tranquilizer but its use is not well studied. Toxic effects in horses are extensions of side effects and include colic and drowsiness. Dogs are extremely sensitive to the effects of reserpine. A single $10\,mg\,kg^{-1}$ dose can be fatal.

Human

Little experience exists to define a minimum toxic dose. Most of the reported cases have been in the pediatric population; amounts ingested in overdoses substantially larger than adult therapeutic doses have not resulted in fatalities. Manifestations of toxicity may include hypertension and tachycardia followed by hypotension and bradycardia. Ataxia, drowsiness, lethargy, or coma may be noted. Pupils may be pinpoint and not reactive to light. Diarrhea may occur. Extrapyramidal symptoms and cardiac dysrhythmias have been documented.

Chronic Toxicity (or Exposure)

Animal

Dogs administered reserpine daily for 1 year showed signs of CNS depression, muscle tremors, and parkinsonian symptoms. Lower doses administered to dogs resulted in prolapsed nictitating membranes, miosis, diarrhea, CNS depression, and changes in hematocrit.

Human

Side effects seen with chronic therapy may include drowsiness, depression (which can be severe), headache, dizziness, flushing, anxiety, nasal congestion, dry mouth, gastrointestinal upset, sodium and water retention, and an increase in appetite, dreaming, and nightmares. Depression is most likely with doses $>0.25\,mg\,day^{-1}$ and usually occurs 2–8 months after starting therapy.

In Vitro Toxicity Data

Mutagenicity studies in Ames Salmonella, *Escherichia coli*, and rat hepatocyte assays have been negative.

Clinical Management

Reserpine is adsorbed by activated charcoal. Treatment is largely symptomatic and supportive. Standard supportive therapies, such as vasopressors, should be utilized as clinically indicated. Because symptoms may be delayed, observation for up to 72 h may be indicated.

See also: Charcoal; *E. coli* (*Escherichia coli*).

Further Reading

Dick HLH, McCawley EL, and Fisher WA (1962) Reserpine-digitalis toxicity. *Archives of Internal Medicine* 109: 503–506.

Resistance to Toxicants

Stephen R Clough

In toxicology, the term resistance may be defined as an inherent genetic capability of an organism to oppose any adverse effects, manifest in either potency or dose, of a toxicant. Others have defined resistance as the ability of an organism to tolerate toxic doses of a substance that would be lethal to most in a normal population of the same species. It is important to distinguish the phenomenon of resistance from tolerance, which is the ability of an organism to adapt to the adverse effects of a toxicant with each successive dose of that toxicant. Resistance can also be a relative term with regard to the population or species that may oppose a toxic effect. For example, in a typical toxicology study the number of animals responding to a range of doses of a chemical usually reveals a small percentage of the population showing adverse effects in the lower dose range and a small percentage of the population showing no adverse effects in the higher dose range. The animals responding at the lower doses are typically categorized as susceptible individuals, whereas the animals showing little or no response at the higher doses are categorized as resistant individuals. Similarly, some species of bacteria are resistant to penicillin, whereas others are susceptible.

Microorganisms probably provide the best examples of the phenomenon of resistance. Although the science of toxicology generally addresses higher levels of organisms, such as fish or mammals, bacteria may serve as a good illustration of resistance to toxic effects because antibiotics, generally derived from microorganisms, evolved in nature as a form of 'toxic warfare' allowing one microorganism to gain a competitive advantage over another. Humans have taken advantage of this by developing drugs, based on the structures of these natural antibiotics, which are effective in curing infectious diseases. Bacteria may be resistant to certain antimicrobial agents because (1) the drug fails to reach its target, (2) the drug is detoxified, or (3) the intended target is changed in a way that the drug cannot affect it. Some bacteria have cell walls that will not allow a drug to cross it, thus providing resistance. Other species or strains have enzymes on or within the cell wall that are capable of inactivating the drug. The physical and/or chemical composition of the cell wall may also resist the diffusion of a drug that may be dependent on certain environmental conditions such as a certain pH or the presence of oxygen.

Because bacteria can produce hundreds to thousands of generations within a very small time frame, they can acquire resistance through natural selection, that is, a small mutation may change a cellular process to allow resistance to a drug, and the subpopulation, cloned from the cell that acquired the mutation, now has an advantage in the presence of drug treatment and can cause infection even in the presence of the drug. Bacteria may also acquire resistance through a transfer of a resistant gene to another strain or even a different species. This can occur through conjugation (direct transfer of genes through a sex pilus or bridge), transduction (transfer via a bacteriophage), or transformation (envelopments and incorporation into the bacteria of resistant-encoded DNA that is free in the environment into the bacteria).

Resistance is a phenomenon that can also be observed in the higher animals, although whether or not it applies to a specific situation is a matter of dispute (i.e., whether an animal is simply less sensitive versus more resistant). Factors that may impart resistance include age, sex, species, and/or strain; of these, species is probably the most common factor imparting resistance to a toxicant. For example, a human cannot eat acorns because of the presence of toxic alkaloids present in the meat of the nut. Squirrels, however, are resistant to the toxic effects of these alkaloids because they possess liver enzymes capable of detoxifying these natural toxins. Another example is the classic resistance of certain strains of mice to oppose the effects of cadmium on the male reproductive system. It has been known for decades that most strains of mice will show severe testicular hemorrhage, followed by necrosis and sterility, after the parenteral injection of small amounts of cadmium chloride. Some strains, however, are remarkably resistant to this toxic phenomenon, being able to endure lethal doses with little or no effect on the testis. This resistance is also seen in some species of animals that have testis that are located within the abdominal cavity.

An age-related effect of resistance to metal toxicity can be seen following exposure to lead in humans. Although adults usually have higher blood lead concentrations than children, they are apparently more resistant to the neurotoxic effects of lead poisoning than a child. This is probably due to age-related differences in neurological development, as well as the permeability of the gastrointestinal tract and the blood–brain barrier to lead. Some may argue that children are simply more susceptible to lead poisoning than adults, but the change in resistance with age deserves some attention.

See also: Immune System; Modifying Factors of Toxicity.

Further Reading

Taylor M and Feyereisen R (1996) Molecular biology and evolution of resistance of toxicants. *Molecular Biology and Evolution* 13: 719–734.

Resource Conservation and Recovery Act, US

Mario Mangino

- Title: RCRA
- Agency: US Environmental Protection Agency (EPA)
- Year Passed: 1976
- Groups Regulated: Chemical industry

Synopsis of Law

Several statutes administered by the US EPA regulate the treatment, storage, and disposal of hazardous

waste and hazardous materials. The principal law is the Resource Conservation and Recovery Act (RCRA), enacted in 1976. The term RCRA is often used interchangeably to refer to the original statute, the implementing of the US EPA regulations, and EPA policy and guidance. RCRA regulations have a wide reaching authority affecting thousands of commercial facilities that generate or handle hazardous chemicals and chemical wastes. The goals of RCRA are: to protect human health and the environment from the hazards posed by waste disposal; to conserve energy and natural resources through waste recycling and recovery; to reduce or eliminate, as expeditiously as possible, the amount of waste generated, including hazardous waste; and to ensure that wastes are managed in a manner that is protective of human health and the environment.

To achieve these goals, RCRA established three distinct yet interrelated programs. RCRA Subtitle D, the 'solid waste program', encourages all the US states to develop comprehensive plans to manage nonhazardous industrial solid waste and municipal solid waste, establishes criteria for municipal solid waste landfills and other solid waste disposal facilities, and prohibits the open dumping of solid waste; RCRA Subtitle C, the 'hazardous waste program', establishes a system for controlling hazardous waste from the time it is generated until ultimate disposal – essentially from 'cradle to grave'; RCRA Subtitle I, the 'underground storage tank (UST) program', regulates underground tanks storing nonwaste materials, mainly gasoline and other petroleum products. (Although RCRA creates the framework for the proper management of hazardous waste and nonhazardous solid waste, it does not address the problems of hazardous waste found at inactive or abandoned sites or those resulting from spills that require emergency response. These problems are addressed by a different act, the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), commonly called Superfund, which was enacted in 1980.)

Under Subtitle C, RCRA established a comprehensive Federal scheme for identifying and managing hazardous waste. Directed to promulgate criteria for identifying hazardous wastes, the US EPA has specified these criteria as ignitability, corrosivity, reactivity, and toxicity. The agency has identified acceptable protocols for determining these characteristics and established a list of chemical substances whose presence will make a waste hazardous.

RCRA directs the US EPA to regulate the activities of generators, transporters, and those who treat, store, or dispose of hazardous wastes. Standards applicable to generators, transporters, and handlers of hazardous wastes must 'protect human health and the environment'. The US EPA's regulations applicable to generators and transporters establish a manifest system that is designed to create a paper trail for every shipment of waste, from generator to the final destination, to ensure proper authority over persons who own or operate hazardous waste treatment, storage, or disposal facilities. Pursuant to RCRA, the US EPA issued regulations prescribing methods of treating, storing, and disposing of waste; governing the location, design, and construction of facilities; mandating contingency plans to minimize negative impacts from such facilities; setting qualifications for ownership, training, and financial responsibility; and requiring permits for all such facilities. The permits cover a myriad of different operations including wastewater treatment plants, solvent recyclers, hazardous waste landfills, and hazardous waste combustors (e.g., incinerators, cement kilns, boilers, industrial furnaces). In addition, the Subtitle C program contains provisions that allow the US EPA to authorize state governments to implement and enforce the hazardous waste regulatory program. State programs must be at least as stringent as the federal program. Because the state authorization process normally takes place in a piecemeal fashion, a specific state may be authorized to implement and enforce some RCRA regulations and not others. Currently, the US EPA has authorized 48 states to implement their own regulatory programs in place of all or substantial portions of the RCRA federal hazardous waste program.

RCRA has been amended several times since 1976, and continues to evolve as Congress revises it to reflect changing waste management needs and concerns. The Act was amended significantly in 1984, by the Hazardous and Solid Waste Amendments (HSWA), which expanded the scope and requirements of RCRA. HSWA was created largely in response to citizen concerns that the historical methods of hazardous waste disposal, particularly land disposal, were not safe as a long-term solution. The Congress also revised RCRA in 1992 by passing the Federal Facility Compliance Act, which strengthened the US EPA's authority to enforce RCRA at federal facilities. In addition, the Land Disposal Program Flexibility Act of 1996 amended RCRA to provide regulatory flexibility for the land disposal of certain wastes. Of these amendments, HSWA is the most significant because it directed EPA to establish the Land Disposal Restrictions (LDR) program and the Corrective Action program.

The LDR program requires that protective treatment standards must be met before hazardous waste is land disposed. As soon as a waste is generated, it is

subject to three LDR prohibitions: (1) The Disposal Prohibition – before a hazardous waste can be land disposed, treatment standards designed for that specific waste material must be met. An operator may meet such standards by either treating hazardous chemical constituents in the waste to meet required treatment levels by any available method other than dilution, or treating hazardous waste using a treatment technology specified by EPA. Once the waste is treated with the technology required under LDR, it can be land disposed. (2) The Dilution Prohibition – waste must be properly treated and not simply diluted in concentration by adding large amounts of water, soil, or nonhazardous waste. (3) The Storage Prohibition – waste must be treated and cannot be stored indefinitely. This prevents generators and treatment facilities from storing hazardous waste for long periods merely to avoid treatment. Waste may be stored only for the purpose of accumulating quantities necessary to facilitate proper recovery, treatment, or disposal.

The Corrective Action program requires corrective action (e.g., removal, stabilization, engineering controls) for all releases of hazardous waste or chemical constituents from any solid waste management unit at a facility seeking a permit under Subtitle C, regardless of the time at which the waste was placed in the unit. If such corrective action cannot be completed prior to issuance of the permit, the permit must contain a schedule for completion of the corrective action and provisions for financial assurance. In addition, corrective action can be required through the US EPA or state 'enforcement order' if an agency finds evidence that chemical constituent releases occurred from a waste management unit that is not identified under an existing RCRA permit. When releases of hazardous constituents are documented, the corrective requirements could apply to several environmental media, including soil, groundwater, surface water, and sediments. The facility must also take corrective action measures beyond facility boundaries to protect human health and the environment when necessary.

See also: Clean Air Act (CAA), US; Clean Water Act (CWA), US; Hazardous Waste; Toxic Substances Control Act, US.

Relevant Website

http://www.epa.gov – US Environmental Protection Agency.

Respiratory Tract

Donald E Gardner and Daniel T Kirkpatrick

Introduction

The route by which a chemical enters the body is a major factor in determining whether a substance is toxic. More than 100 years ago it was noted that the air we exhaled was less dusty than the air we inhaled, demonstrating that airborne substances were removed from the inhaled air and deposited in the respiratory tract. When toxic chemicals are inhaled and deposited on sensitive tissues, normal respiratory functions required to maintain the morphological and physiological viability of the respiratory system may be significantly impaired, increasing an individual's risk of disease. With each breath, our body is potentially exposed to numerous gases, vapors, and airborne viable and nonviable particles that could adversely affect the vital function of this system. The lung is a most vulnerable target organ since it has nearly four times the total surface area interfacing with the environment as does the total combined surface area of the gastrointestinal tract and the skin. Because of this large surface area ($70\,m^2$), inhalation becomes a major route for entry into the body of toxic substances from occupational and environmental exposure. It has been calculated that at rest, the average adult breathes $\sim 15\,kg$ of air each day. This is significantly more than the daily intake of food and water, which is ~ 1.5 and $2.0\,kg\,day^{-1}$, respectively. Breathing is a function that must be continuous on a minute-by-minute basis, whereas extended intervals without exposure occur between periods of water and food intake. In addition, the dose of polluted air reaching the respiratory tract is dependent on the state of exercise with minute ventilation varying by up to a factor of 30 between sleep and exercise.

To maintain its primary function as an organ of gas exchange, the mammalian respiratory system must be able to defend itself from constant assault of hazardous agents that enter the body by this route of exposure. When these normal pulmonary defenses are compromised, inhaled toxic substances have the potential for initiating or aggravating existing lung disease. The health effects associated with airborne

contaminants are not limited to the respiratory tract. This route of exposure may also be the portal of entry for substances that can then be translocated from the respiratory tract to systemic sites. Because the blood leaving the lung is rapidly distributed to all parts of the body, deposited contaminants may be transported to the entire body. To produce an effect that is beyond the pulmonary system, it is necessary that the chemical, its metabolite(s), or a reactive product(s) be transported to some specific susceptible target site. There is also evidence that lung tissue can be damaged when toxic chemicals enter the body by other routes and are then transported by the bloodstream to the lung. For example, interperitoneal injection of butylated hydroxytoluene or ingestion of the pesticide, paraquat, produces acute lung damage.

This entry presents a discussion of the principles of respiratory toxicology including (1) an historical perspective, (2) approaches used to evaluate respiratory responses to inhaled chemicals, (3) classification of airborne chemicals, (4) concepts of dose–time relationships, (5) factors influencing toxicity of airborne substances, (6) the basic biology of the respiratory system with emphasis on those structures and functions that are involved in toxicological responses, (7) biomarkers of pulmonary effects, (8) toxicological response associated with inhaled chemicals, and (9) assessing the human risk of airborne chemicals.

Historical Perspective: Respiratory Morbidity and Mortality

The consequences of breathing contaminated air have long been known. The public awareness of and concern for the nature and degree of health and welfare risk associated with exposure to airborne chemicals have varied considerably over history. Concern for air pollution may have begun with human's first use of fire for heating and cooking and was accelerated following the wide use of coal as an energy source. As early as the thirteenth century the public began to complain of impaired visibility, soiling, odor, and health effects associated with coal smoke. As a consequence of the need for more energy to support the industrial revolution, a number of serious air pollution episodes began to occur. In 1930, pollution in the Meuse Valley of Belgium reached levels sufficient to cause over 60 deaths and hundreds of illnesses. In 1952, the high levels of air pollution in London combined with fog, resulting in an atmosphere that caused over 4000 deaths. Other serious air pollution problems occurring in Donora, Pennsylvania, in 1948, in Tokyo in 1970, and in New York in 1953 and 1963, brought public attention to the hazards associated with uncontrolled emissions. These most serious life-threatening episodes usually were of an acute nature and produced the most serious effects among the old, infirm, and those with respiratory disease. Meterologic conditions (inversions) over the polluted area typically led to an increase in mortality and morbidity.

The 1950s and 1960s were periods during which the public became increasingly aware of environmental pollution with industrial chemicals. Rachel Carson's book *Silent Spring* was a milestone in arousing public concern over environmental contaminants that produced human health effects. Increased health risk from accidental release of massive amounts of extremely hazardous substances into the environment from chemical spills, industrial explosions, fires, or accidents involving railroad cars and trucks transporting these chemicals have recently become a major concern. People living in communities surrounding these spills are at considerable risk of being exposed. The possibility of such sudden exposure at sites where hazardous substances are produced, stored, or used became very evident following the release of methyl isocyanate from a pesticide manufacturing plant in Bhopal, India, in 1984. The incident caused over 2000 deaths and $\sim$20 000 more suffered irreversible damage to their eyes and lungs. In another case in which the exposure was chronic rather than acute, levels of beryllium released from a manufacturing plant were sufficient to cause beryllium disease in people residing near the plant. These most serious incidents brought an increased public and scientific awareness of air pollution and its sources, the health and welfare effects associated with such exposure, and the need to develop sound strategies for elimination of such risk. Guidelines have now been developed by the National Research Council to be used to develop community emergency exposure levels for such extremely hazardous airborne substances.

While today's health effects due to such air pollution episodes may be less dramatic, the scientific community is greatly concerned that other natural and man-made substances may be released into the environment at very low levels that may still result in serious long-term effects. For some chemicals, a threshold level for effects might not exist. Four of the leading causes of death (cancer, pneumonia and flu, chronic obstructive pulmonary disease (COPD), and emphysema) involve the respiratory system and may be related to exposure to airborne chemicals. It has been estimated that at least 11%, and possibly as much as 21%, of the lung cancers may be attributed to air pollution. Air monitoring studies have revealed a large number of contaminants, including carcinogens such as benzene, vinyl chloride, and chloroform. Such airborne pollutants may be associated

with the occurrence of a higher incidence of lung cancer in urban populations. In the United States, as in most other developed countries, regulations have been established to control pollutant concentrations in outdoor air and in the workplace. New pollutants are regularly introduced into the environment and identifying and understanding the association between such contaminants and any resulting disease states remains a challenge for the toxicologist.

Exposure to airborne contaminants is not only limited to the outdoor air or to the working environment, but may also occur in the home. In addition voluntary exposures may occur through personal activities such as cigarette smoking and certain hobbies. The health effects associated with the indoor environment, where an individual may spend as much as 90% of his/her time, have become a major concern. Families of workers have developed documented illness associated with contact with clothing that is contaminated with industrial dusts. The US Environmental Protection Agency (US EPA) has ranked the American home as fourth on its list of serious health hazards. New building materials, emissions from wood and gas stoves, heaters, furnishings, air-conditioning systems, insulation, tobacco smoke, and household products such as pesticides that are used indoors have been linked to serious health risk. Nonspecific symptoms in occupants of modern office buildings, often referred as sick building syndrome (SBS), have been widely reported but have not been clearly linked to a specific air contaminant. SBS symptoms include (1) eye, nose, and throat irritation; (2) sensation of dry mucous membranes; (3) erythema (skin irritation and redness); (4) mental fatigue and headaches; (5) high frequency of airway infections and cough; (6) hoarseness and wheezing; (7) itching and unspecific hypersensitivity; and (8) nausea and dizziness. Young children may be unusually sensitive to the toxic effects of these chemicals.

Approaches Used to Evaluate Respiratory System Response to Airborne Chemicals

Supporting data for evaluation of adverse biological responses to chemical exposure and subsequent prediction of human health risk of a particular level and pattern of exposure are generated using epidemiology, studies of controlled clinical exposures, laboratory animal toxicology, and *in vitro* studies. Each category of study has certain intrinsic advantages and limitations and, in general, a database including results from multiple study categories is required to overcome the individual shortcomings (**Table 1**).

Epidemiological studies may show an association between exposure and mortality, morbidity, or a specific disease and may allow direct inference of human risks since actual human exposure conditions, such as the presence of appropriate chemical mixtures, are involved. Examples of strong associations include lung cancer with cigarette smoking or with inhalation of asbestos or metallic compounds of arsenic, chromium, or nickel; liver tumors with occupational exposure to vinyl chloride; and leukemia with occupational exposure to benzene. Studies involving environmental exposures of the general population have the advantage of including sensitive subpopulations. For example, the elderly and individuals with preexisting cardiopulmonary disease were a sensitive cohort during the previously described London air pollution episode of 1952 and, recently, children were found to be more sensitive than adults to NO_2 emissions from gas cooking stoves. Epidemiologic studies of urban regions in the United States and Europe have demonstrated associations between ambient particulate matter (PM) levels and acute increases in cardiovascular and respiratory morbidity (e.g., increased hospital admissions, respiratory symptoms, and cardiovascular episodes) and mortality or decrements in lung function. (See section on 'Classification of Airborne Chemicals' for definitions of PM fractions.) Statistical relationships have tended to be stronger when the fine particle ($PM_{2.5}$) or ultrafine fractions of ambient PM were used as the exposure parameter or susceptible subpopulations were considered (the elderly, young and those with pre-existing pulmonary or cardiovascular disease). An important example is the Harvard Six Cities Studies, in which PM and mortality data from six eastern US cities were analyzed using complex multivariant statistical methods. The strongest associations with mortality were for the concentrations of PM_{10}, $PM_{2.5}$, and sulfate particles, but not aerosol acidity. Studies of asthmatics in the United States, Germany, and the Czech Republic produced significant associations between concentrations of fine or ultrafine particles and peak expiratory flow, respiratory symptoms and/or use of medication.

A prominent shortcoming of most such studies is limited or incomplete exposure information, including both the exact chemicals involved and the airborne concentrations. As a result, evaluation of dose–response relationships and determination of acceptable exposure limits is difficult. Even when strong associations can be demonstrated for high levels of exposure, as in the case of benzene exposure, the low statistical sensitivity of epidemiological methods makes it difficult to assess the risk to individuals with a history of long-term exposure at lower levels. Confounding variable bias is usually a significant problem since exposure histories typically

Table 1 Advantages and limitations of study approaches used to assess pulmonary response to inhaled toxicants

	Epidemiological studies	*Controlled clinical studies*	*Animal studies*
Exposure conditions	+ Realistic concentrations + Real chemical interactions − Definition of exposure difficult − Confounding agents interfere	+ Well-defined exposures − Limited to low concentrations	+ Well-defined exposures + Wide concentration range possible + Easy exposure manipulation
Exposure time frame	+ Realistic, acute to chronic	− Short-term only	+ Acute to chronic − Relevance of pattern/length of exposure is questionable
Toxicologic effects	− Limited to severe or crude effects (mortality, morbidity) − Insensitive, twofold change required to detect effect − Disease present, prevention not addressed	+ Subtle, less severe effects measurable − Only mild, reversible effects, questionable toxicological significance	+ Wide range of responses may be evaluated − Relevance of subtle effects to human is uncertain
Population characteristics	+ Measured in humans + Large population size possible + Full range of sensitive subpopulations possible	+ Measured in humans − Limited number of subjects + Possible to study sensitive subpopulations	− Extrapolation to humans + Large group size possible − Homogeneity of animal model population and environmental factors-relevance to human?
Utility	− Assessment of dose response is difficult − No information on mechanism of action − Costly and time consuming	+ Dose response may be tested (limited range) − Limited information on mechanism of action − High cost	+ Dose response may be tested over a wide range + Possible to investigate mechanism of action + Relatively lower cost

+, Advantage; −, limitation.

include multiple chemicals. This is particularly important for both cancer and nonneoplastic disease of the respiratory tract. For example, inaccurately reported cigarette smoking or work history can greatly distort findings. Such bias may be involved in the highly controversial finding of an association between environmental tobacco smoke exposures and lung cancer. Studies of worker populations in the synthetic rubber, plastic resin, and coating industries provide additional examples of confounding exposures. Synthetic rubber and thermoplastic workers may have complex exposure histories with prominent exposures to styrene, butadiene, and, in some cases, acrylonitrile. Coating industry workers typically have histories of exposure to a broad spectrum of chemicals including styrene, epoxy compounds, acrylic monomers, isocyanates, and anhydrides. Finally, exposure to wood dust may have been a confounding factor in studies reporting an association between formaldehyde exposure and sinonasal tumors.

Epidemiology also suffers from the fact that effects are generally counted when significant disease, morbidity, or mortality has occurred and, thus, protection from disease is not addressed. The use of validated biomarkers for early effects may improve the utility of these studies. As an example, studies have found a clear link between occupational beryllium exposure and the presence of a sensitization-dependent, progressive, and incurable granulomatous disease of the lung (chronic beryllium disease). Development of beryllium-dependent transformation tests for bronchoalveolar lavage and peripheral blood lymphocytes may lead to early diagnosis of sensitization and subsequent removal of workers from further exposure and/or corticosteroid treatment to limit progression of the disease.

Studies showing associations between ambient PM levels and parameters of human health illustrate many of the weaknesses of epidemiological studies. Statistical methods are inconsistent and result in high levels of uncertainty. Air pollution is typically a complex mixture of particles and gaseous materials for which concentrations are often correlated. Eliminating the effects of gaseous components to show an association for PM is difficult.

Controlled clinical studies using volunteers have most frequently been used to evaluate human effects of exposure to low levels of air pollutants, including sulfur dioxide, nitrogen dioxide, ozone, carbon monoxide, PM fractions, and acid aerosols of sulfates and nitrates. Major advantages of this approach are that humans make up the exposure population and that it is possible to closely define and control the exposure concentration. To a limited extent, sensitive subpopulations may be tested. For example, airway hyperreactivity to sulfur dioxide and sulfuric acid aerosols have been demonstrated in asthmatics (asymptomatic at the time of testing). Individuals with heart disease are especially at risk when exposed to carbon monoxide. When patients with histories of angina pectoris were exposed to low levels of carbon monoxide, they experienced reduced time to onset of chest pain as a result of insufficient oxygen supply to the heart muscle. Since the safety of the experimental subjects must be a primary concern, only short-term exposures to low concentrations that produce only mild and transient responses may be used. The effects of chronic exposures cannot be tested and the range of end points that can be assessed is often limited to pulmonary function measurements and blood clinical chemistry assays. In some university hospital settings, additional evaluation procedures are used, including examination of bronchoalveolar or nasal lavage fluids and evaluation of effects on mucociliary clearance and epithelial permeability using inhalation of radiolabeled aerosols. In general, the reversible changes that are observed following single human exposures are of uncertain clinical significance in predicting long-term effects.

Assessments of chemicals or chemical mixtures for risk to workers and/or the general population clearly require a database obtained from intact, living organisms. *In vitro* methods cannot be used to model the complex interactions and feedback processes between cells, tissues, and organ systems of a functioning mammalian organism or the complex deposition, uptake, and clearance processes of the respiratory system. Alternatively, *in vitro* studies can be very useful for screening a large number of chemicals for a specific effect, for example, genotoxicity or cytotoxicity, and for development of information on mechanism of action. In terms of risk assessment, *in vitro* study data may provide information that aids in the interpretation of the database derived from animal and human exposure.

In regulatory decisions, the primary standard *in vitro* methods are the genotoxicity assays, including the Ames test and the mouse lymphoma cell mutagenesis assay. Animal and human respiratory tract cells or tissues in culture are frequently used for screening and mechanistic studies. For example, alveolar type II cells in culture have been used to evaluate xenobiotic chemical metabolism; alveolar macrophages in culture have been used to test for cytotoxicity, macrophage activation; and the effects of exposure on macrophage function (e.g., phagocytosis and bacterial or virus inactivation), and tracheal explant cultures have been used to model the preneoplastic action of airway carcinogens.

The driving philosophy behind an aggressive strategy of toxicity testing in laboratory animals is the conviction that human beings should not have to suffer from avoidable, debilitating, or lethal chemical-induced toxicity or cancer when the effect of the chemical can be demonstrated in a test animal species. Historical examples such as benzene, asbestos, and vinyl chloride for which animal models were developed after the association of disease with exposure was demonstrated in humans, show the need for well-designed safety testing in animals. Animal studies allow maximal flexibility in choice of chemical agents, exposure concentrations and regimens, biological end points, and test species. Exposure conditions can be tightly controlled and readily manipulated and exposures can be acute, subchronic, or chronic. Studies can be designed to help elucidate the mechanism of action and the existence and basis for species differences in response. A broad range of biological responses can be evaluated, including target organ histopathology, changes in hematological and blood chemistry parameters, changes in organ system function, changes in immunological responses, effects on neurobehavioral parameters, and reproductive/developmental effects. Large chemical testing programs, such as the hazardous air pollutant (HAPs) and high production volume (HPV) testing programs in the United States frequently include studies of neurotoxic and reproductive/developmental effects following inhalation exposure since available information regarding such effects is insufficient to evaluate human risk. Of particular importance to evaluate respiratory tract toxicity are histopathology, lung function, and bronchoalveolar lavage fluid cytology and chemistry.

Many examples of the use of animal exposures to study the respiratory tract toxicity of inhaled chemicals are discussed in portions of this entry describing indicators of respiratory tract response. Examples cited here demonstrate ways in which animal studies are used to help protect human populations and guide assessment of human risk. For most chemicals that pose a potential inhalation risk to workers, there are insufficient human data to set safe occupational exposure limits. Using inorganic nickel compounds as an example, epidemiological data indicate an

increased risk for nasal and lung cancer in workers involved in nickel sulfide ore smelting and refining processes, and lung tumors have been found in rodents chronically exposed to nickel compounds. However, the current occupational threshold limit value (TLV) for soluble nickel salts has been set based on studies in which nonneoplastic lesions, including epithelial hyperplasia, inflammation, and fibrosis, have been evaluated in laboratory animals.

Animal models have been developed for chronic pulmonary effects of asbestos fibers and silica. The insolubility and cytotoxicity of the chemicals and the inability of alveolar macrophages to normally phagocytize and clear the chemicals are important features of the models of asbestos-induced fibrosis and cancer and silica-induced fibrosis. These validated animal models have been used to evaluate the potential for man-made fibers to cause cancer or other crystalline materials to cause fibrosis. Tested using this approach, exposure to glass fibers has produced both positive and negative results. Using interpleural and intraperitoneal instillation of very thin glass fibers, researchers in Germany produced tumors in rats and hamsters. In a recent article supporting the need for inhalation testing, data cited indicated that chronic whole body inhalation of glass fibers failed to induce lung cancer in rats, even at very high fiber loads in the lung. In parallel studies using chrysotile asbestos, 18.9% of the animals had tumors and about half of those were malignant or carcinomas. From these studies, the author concluded that even with levels of exposure 1000 times higher than seen in a typical exposure situation, there was no evidence of tumors. Such information indicates the importance of using a natural route of exposure when assessing the risk of inhaled substances.

Effects of long-term exposure to air pollutants are difficult to evaluate using human data since, in epidemiology, exposure history and confounding factors cannot be controlled or, in clinical studies, only short-term exposures are possible. Long-term exposure studies using laboratory animals provide information that can be used to predict human effects with several models suggesting changes that correspond to well-documented human disease states. Long-term exposure of rats to sulfur dioxide produces thickening of the tracheal mucous layer and hypertrophy of goblet cells, both features of human chronic bronchitis. Repeated sulfur dioxide exposure of rats also interferes with clearance of inert particles. Rabbits that have been exposed to sulfuric acid aerosols have a slowing of mucociliary clearance, goblet cell hyperplasia, decreased pH of intracellular mucous, decreased airway diameter, and increased airway reactivity to acetylcholine. This pattern of response is similar to the pathology observed in patients with chronic bronchitis and asthma. US EPA scientists have exposed rats to ozone using a diurnal concentration pattern (range 0.06–0.25 ppm), producing alveolar epithelial hyperplasia within 12 weeks, which resulted in a slowing of the clearance rate of asbestos after 6 weeks and functional changes indicative of a stiffer lung after 12 months. Use of such an exposure regimen considered to be realistic (for an urban area of high pollution) suggests possible relevance to human toxicity.

The use of data derived from laboratory animal exposures to assess human risk is complicated by issues concerning extrapolation from animals to humans. Differences between animal and human biochemical and pharmacokinetic processes may diminish or negate the relevance of a particular animal model. Xenobiotic metabolizing capacities and patterns of distribution of these activities within the respiratory tract may differ between species. A biochemical that is specific for male rats (and is not found in humans), α_{2u}-globular protein, appears to be required for susceptibility to renal tubular nephropathy and tumors induced by inhalation of unleaded gasoline vapors. In the respiratory tract, species differences in three-dimensional airway structure may result in differences in toxic effects. For example, the complexity and relative surface area of the nasal turbinates are very different in rodents and humans. Respiratory tract detoxification processes may also differ between species. In addition, the genetic homogeneity of laboratory animal strains and the closely controlled environmental conditions (e.g., diet) used in laboratory animal studies may affect the relevance of such studies to humans. In laboratory rodent carcinogenicity studies, high background incidence rates for certain tumors appear to be related to unrestricted food availability. There is concern that this rodent model might also have heightened susceptibility to chemically induced tumors or that resultant life-shortening for the model might interfere with detection of tumors. All of these potential differences highlight the importance of animal and *in vitro* studies to provide pharmacokinetic data and information on the mechanism of action.

Extrapolation from high-dose exposures in animals to realistic human exposure levels is also a serious concern for risk assessment. For example, metabolic and detoxification processes may be dependent on exposure level. A commonly cited example involves the increased incidence of lung tumors in rats following particulate exposure regimens that produce high lung particle loads (e.g., chronic, high level diesel exposure). Macrophage-based clearance mechanisms become overwhelmed with chronic

exposure at high concentrations and this may be associated with tumor development that would not be seen at ambient levels. The relevance of tumor incidence under these conditions to prediction of human risk has been questioned.

Classification of Airborne Chemicals

Airborne substances that are of interest to inhalation toxicology include gases, vapors, aerosols, and complex mixtures in various combinations. Aerosols may exist as mists, fogs, smokes, fumes, or dusts. Physical properties of airborne chemicals are most frequently used by the inhalation toxicologist for primary classification, with the first division based on whether the material is a gas, vapor, or aerosol (particulate material). For materials that are not highly reactive, movement and behavior in the respiratory airstream, the sites of deposition and/or uptake, the fraction retained, and the rate of interaction with airway tissues and cells are highly dependent on the physical state (see section on Factors Affecting Toxicity). In addition, this approach provides the inhalation toxicologist with valuable information on the nature of the material to which a population at risk is exposed, information that can be used to decide on the best methodology for generation of test atmospheres of a material for toxicological studies. Although classification by chemical type is used for applications such as industrial hygiene, this approach has important limitations. Materials with very different chemical structures may have similar toxic effects and materials with similar chemical properties or even a single chemical may have different toxic effects depending on whether the form inhaled is a gas or an aerosol/gas mixture.

Gases and vapors are usually grouped together since a vapor is the gaseous fraction of a chemical that is a liquid at ambient temperature and atmospheric pressure. Two properties, solubility and chemical reactivity, are particularly important determinants of the toxic actions of inhaled gases and are also used for classification. In general, the solubility of a gas/vapor is important in determining the primary sites of deposition and injury (see section on Factors Influencing Toxicity). Reactive gases interact chemically with components of cells at the site of deposition, producing direct injury that is typically followed quickly by inflammation and edema but can progress to cause a variety of toxic effects. Following deposition, nonreactive materials may undergo activation to a reactive intermediate or may interact with the cellular oxidation–reduction machinery to be activated or to deplete critical cellular reducing substances or antioxidants (e.g., NADPH and glutathione).

An aerosol may be defined as a suspension in air of solid particles, as in dusts, fumes, and smokes, or liquid droplets, as in fogs, mists, and liquid aerosols of organic materials. Dusts are formed by milling or grinding of larger masses of a parent material, while fumes and smokes are formed by combustion, sublimation, or condensation usually with a chemical change in the material. In fibrous aerosols, the solid particles have a length along one axis that is at least three times greater than that along either of the other two axes (i.e., an aspect ratio of greater than 3:1). Examples include asbestos, glass and plastic fibers, and mineral wool. Mists and fogs are typically formed by condensation of water on microscopic particles. Liquid aerosols are also produced by nebulization or spraying in the use of man-made products (e.g., pesticides and paints). Particles in aerosols may also consist of viable agents, including bacteria and viruses, as well as fungal spores and pollen. Thus, inhaled biological aerosols may produce infectious diseases such as influenza, viral and bacterial pneumonia and tuberculosis, and allergic reactions. Other properties of inhaled aerosols that are used for classification include particle size, which is the primary determinant of regional airway deposition, electrical charge, solubility, and rate of dissolution in aqueous media, and hygroscopicity.

Ambient air PM is one of the seven air pollutants regulated under the US EPA National Ambient Air Quality Standards (NAAQS) and has become a significant focus for air pollution research. PM_{10} is defined as PM with a mass median aerodynamic diameter (aerodynamic particle size) of $<10\,\mu m$ and is appropriately used to represent all ambient particles that are inhalable and, therefore, are a potential human health concern. $PM_{2.5}$ and $PM_{10–2.5}$ represent fine particles with aerodynamic diameters $\leqslant 2.5\,\mu m$ and coarse particles with aerodynamic diameters between 10 and $2.5\,\mu m$, respectively. The $PM_{2.5}$ class includes the ultrafine particle subclass made up of particles with diameters $<0.1\,\mu m$. Fine particles result from combustion of fuels and atmospheric reactions of primary pollutants, tend to have greater deposition in the pulmonary region of the airways (deep lung) and represent a greater health concern than coarse particles. $PM_{2.5}$ includes a broad range of particle types, including sulfuric and nitric acid aerosols, ammonium salts, organic and elemental carbon, metals and metal salts, and biological material. In addition, PM may include carbonaceous particles with adsorbed gaseous pollutants or constituent transition metal compounds.

Most human inhalation exposures in the workplace, home, or outdoor environment involve airborne mixtures of chemicals, which frequently

include both gaseous and particulate material. In addition, gases and vapors may be adsorbed onto the surface of aerosol particles and be carried to potential sites of injury in the lungs by the respirable particles. Three environmental mixtures of continuing concern for air pollution are photochemical smog, diesel exhaust, and environmental tobacco smoke. Smog is typically a complex mixture of gaseous combustion products, including oxides of carbon, sulfur and nitrogen, ozone, hydrocarbons, reaction products of ozone, and other pollutants and particulate aerosols of carbon and various metal oxides. Diesel exhaust and environmental tobacco smoke are also mixtures of particulate and gaseous combustion products. Of particular concern are potential carcinogens, including polycyclic aromatic hydrocarbons and tobacco-specific nitrosamines, that may reach sensitive regions of the airways adsorbed to particles.

Exposure, Concentration, and Dose–Time Concepts

Confusion often occurs with the use of the terms 'exposure', 'concentration', and 'dose'. Dose is the amount of contaminant that is deposited or absorbed in the body of an exposed individual over a specific duration. Dose occurs as a result of exposure. Concentration is that level of contaminant present in the air potentially available to be inhaled. The atmospheric concentration of a chemical by itself does not define the total dose of a chemical delivered or the specific sites of potential injury. For a substance present in inhaled air to be toxic, a significant dose must first be removed from the inhaled air and be deposited on sensitive tissue. Knowledge of the dose to initial target sites provides a critical link between exposure and the subsequent biological response. Understanding the disposition of inhaled xenobiotics is complex and, due to space limitations, cannot be described in detail here. However, certain basic concepts need to be presented to provide information on the various factors related to exposure, dose, and response that are fundamental to understanding the potential human risk from inhaled chemical agents.

The prediction of biologic effects from inhaled pollutants is often based on the study of concentration–time. However, in inhalation toxicology, the concept of dose is most important to the understanding of the relationship between exposure concentration and the body's response. Actually dose can be apportioned into two components: internal dose and biological effective dose. Internal dose is the amount of a contaminant that is absorbed into the body over a given time. Biological effective dose is the amount of contaminant or its metabolites that has interacted with a target site over a given period of time so as to alter a physiological function. The consequence of the chemical reaching the target tissue is governed by its pharmacokinetic behavior, which includes the processes of absorption, distribution, metabolism, and elimination. The effective dose to the respiratory tract, for example, for inhaled particles, is proportional to particle retention and integrated particle retention is derived from the balance of two processes: deposition and clearance.

Because of the difficulties in determining actual dose in inhalation studies, the toxicologist must assess the extent to which the concentration (C) of a given chemical and the duration of exposure (T) interrelate to determine the magnitude of the biological response (K). Often the formula, $C \times T = K$ will be used to relate the toxic effect of certain inhaled substances to its concentration and time of exposure. This formula, referred to as Haber's law, is valid only for certain combinations of concentrations and exposure time and for only a limited number of substances. While it may be necessary to use Haber's formula in certain conditions, caution must be exercised in using the general expression, $C \times T = K$, when comparing exposure conditions that are to be used in extrapolating from effects seen in laboratory animals to humans. A more appropriate general expression for estimating $C \times T = K$ would be given by $C^a \times T^b = K$, where the exponents a and b are estimated from the data. Such a formula allows for the fact that C and T do not always contribute equally to the observed toxicity. Haber's law may be inappropriate for certain materials such as ammonia and nitrogen dioxide which are more toxic with high concentration over shorter exposure periods.

Factors Influencing Toxicity

Scientists who seek an understanding of the toxicological hazards associated with inhalation of airborne substances need a basic knowledge of the structure and normal functioning of the respiratory system. This information is essential to understanding how this system responds to inhaled substances and the possible health consequences resulting from exposure to toxic substances. Various regions of the respiratory system can be sensitive target sites for inhaled xenobiotics. However, the potential hazard associated with such exposure will depend on many interacting factors that will be discussed in the following section. In each region of the respiratory tract there are certain defense systems capable of coping with an insult. However, when these defenses are compromised or overwhelmed, the potential for disease is significantly increased. A number of factors

can significantly influence the deposition, retention, and redistribution of these inhaled substances, which in turn can directly affect the toxicity of the inhaled substance.

The factors and processes affecting the deposition of airborne substances in all regions of the respiratory tract can be broadly categorized as those related to the (1) structure of the respiratory system, (2) chemical and physical properties of the airborne substance, and (3) ventilatory functions including route of breathing (nasal, oral, and oronasal).

The morphology of the specific respiratory tract region at both the gross anatomical and the microscopic levels is an important factor. In extrapolating animal effects to the human, one must be aware that the respiratory tract structure will vary both within individuals and between species at each level of anatomy.

In all regions of the respiratory tract, the specific anatomy, dimensions, composition, flow, and thickness of the mucous or fluid lining layers and regional differences in tissue types and metabolic capabilities all have a major effect on that region's dosimetry. Dosimetry refers to estimating or measuring the amount of a compound or its metabolite or reactive product that reaches a specific target site after exposure to a given concentration.

Whenever the airborne substance is deposited on the linings of the respiratory tract, its new biological environment will react to it. For inhaled particles, a major factor that influences deposition is size. A particle's characteristics may alter its size; for example, if the particle is hygroscopic, it can be expected to grow substantially while still airborne within the respiratory tract and will be deposited based on its hydrated size. The deposition probability for particles with geometric diameter $\geqslant 0.5\,\mu m$ is governed largely by their equivalent aerodynamic diameter. Smaller particles are deposited based on their actual diameter. Since particles are generally inhaled as aerosols rather than as a single particle, the mass median aerodynamic diameter is the most appropriate parameter to use with aerosols in which the particles have actual diameter $\geqslant 0.5\,\mu m$. Aerosols containing particles with diameters less than this should be expressed in terms of diffusion diameter or geometric size. **Figure 1** shows the range of deposition variations in the various respiratory regions. Particle deposition at various sites within the respiratory tract is dependent on several mechanisms. These include impaction, sedimentation, Brownian diffusion, interception, and electrostatic precipitation (**Figure 2**). The most important are impaction, sedimentation, and diffusion. Impaction is the inertial deposition of a particle onto an airway surface. It is the main mechanism by which particles having a diameter $\geqslant 0.5\,\mu m$ are deposited in the upper respiratory tract. The probability of impaction increases with increasing air velocity, rate of breathing, and particle size and density. Sedimentation is deposition due to gravity and is an important mechanism for particles with a diameter $\geqslant 0.5\,\mu m$ that penetrate to those airways when air velocity is relatively low. Submicrometer-size particles are deposited due to a random motion owning to their bombardment by surrounding air molecules (Browning diffusion) that results in particle contact with the nearest airway wall. This is a major mechanism in airways where the airflow is very low (e.g., bronchioles and alveoli).

Physical and chemical properties of the ultrafine ($< 100\,nm$) fraction of ambient PM give this fraction the highest potential for toxic effects. These particles readily reach the alveolar region, have a high deposited fraction, are present in high numbers with high

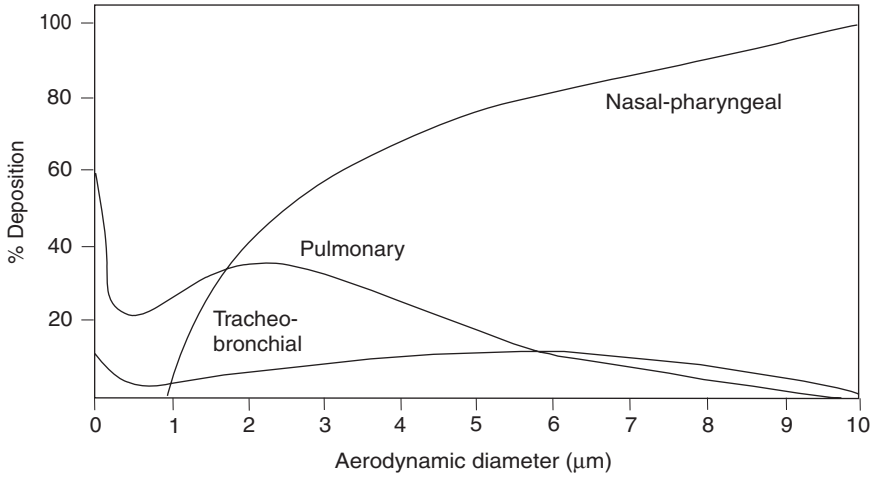


Figure 1 Regional deposition of inhaled aerosols as a function of particle size. (Reproduced from Hayes AW (ed.) (1989) *Principles and Methods of Toxicology*, 2nd edn., p. 364. New York: Raven Press.)

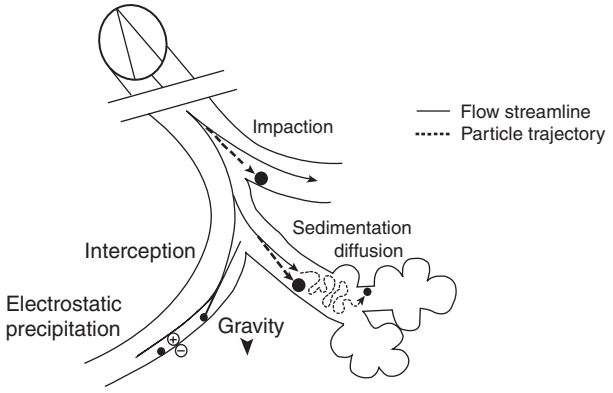


Figure 2 Mechanisms by which particles may be deposited in the respiratory tract. (Adapted from Schlesinger RB (1989) Deposition and clearance of inhaled particles. In: McClellan RO and Henderson RF (eds.) *Concepts in Inhalation Toxicology*, p. 164. New York: Hemisphere, with permission from Taylor and Francis, Inc.)

relative surface area and often include biologically active transition metal components.

Respirable fibers may be quite long and extend beyond 50 μm. However, the most important factor in the deposition of fibers, such as asbestos, is the diameter of the fiber, not the length. Fibers of small diameter (0.5 μm) will remain suspended in the airway and drift with the airflow to be deposited in the airspace.

Particle size, lengths, and configurations not only influence the site of deposition, which in turn affects the mode by which the particle is cleared, but also influence the metabolic fate of the chemical. All segments of the respiratory tract, from the nasal cavity to the periphery of the pulmonary compartment, contain enzymes that are capable of metabolizing xenobiotic compounds. These enzymes are capable of metabolizing some compounds to products that are less toxic, while other metabolites may be more toxic than the original inhaled chemical. There are significant differences in rates of metabolism at the different sites in the respiratory tract. In general, the nasal and the pulmonary regions have a higher metabolic activity than other regions. Metabolic capability is an important factor that plays a crucial role in defining species susceptibility to toxicants.

The solubility of an inhaled contaminant influences the disposition of gases, vapors, and particulates. In general, those substances that are highly water soluble, such as ammonia, formaldehyde, and hydrogen chloride, will be removed by the upper respiratory tract. Formaldehyde is concentrated in the nasal mucosa and is a nasal carcinogen in the rat. Chemicals with intermediate solubility, such as halogens and ozone, deposit in both the upper respiratory tract and the lung, while chemicals with low solubility, such as phosgene and nitrogen dioxide, deposit in and affect mainly the lung. To understand the kinetics related to solubility and to predict the toxic response one must be able to establish the solubility of the chemical not only in water but also in other media including mucus, blood, or tissue. Some particles, such as fogs, mists, and therapeutic aerosols, are aqueous droplets that rapidly merge with the mucus or liquid lining layer, greatly increasing their bioavailability for absorption.

The absorption of gases is dependent on the solubility of the gas in the blood. For example, chloroform has high solubility and is nearly completely absorbed. Respiration rate is the limiting factor. However, ethylene has low solubility and only a small percentage is absorbed – blood flow limited absorption. It is of interest to note that as a generalization, there is a pattern of relative absorption rates that extends between the different routes of exposure. This order of absorption (by rate from fastest to slowest and in degree of absorption from most to least) is intravenous $\geqslant$ inhalation $\geqslant$ intramuscular $\geqslant$ intraperitoneal $\geqslant$ subcutaneous $\geqslant$ oral intradermal $\geqslant$ other dermal. It should be remembered that because of the arrangement of the body's circulatory system, compounds inhaled and absorbed initially enter the systemic circulation without any 'first-pass' metabolism by the liver.

The depth and rate of breathing influences the dose and site of deposition of airborne substances. The process of ventilation is controlled by a variety of internal and external physical and chemical stimuli which can be affected by airborne chemicals. For many inhaled agents, deviation from normal breathing pattern serves as the earliest indicator of response. Assessing the breathing patterns, lung volumes, and lung mechanical properties are frequently used techniques in evaluating the toxicology of inhaled materials. These tests can provide useful information on whether or not pulmonary function has been impaired, the type of impairment, and the extent or magnitude of the function loss. These are not only excellent methods for assessing toxicity but are also useful in characterizing the pathogenesis of lung disease and for extrapolation of such data from animal to humans. There have been numerous studies documenting the importance of measuring respiratory parameters such as respiratory rate and tidal volume in animals exposed to inhaled toxicants. Significant alterations (depression) in these functions have been associated with exposure to methyl chloride, methylene chloride, methyl bromide, and formaldehyde. In such cases, the predicted delivered dose would have been overestimated had respiratory measurements not been recorded.

Structural Factors Influencing Toxicity

Because of the complexity of the respiratory system, it is frequently described by dividing the system into three general regions or compartments based on the anatomical structure and the corresponding physiological functions attributed to that region. **Figure 3** is a schematic showing these various compartmental areas.

Nasopharyngeal Region

The nasopharyngeal (NP) region is the most proximal region of the respiratory system and is the first potential target for airborne substances. The specific structures making up this region include the anterior nares, the turbinates, the epiglottis, the glottis, the pharynx, and the larynx. The nose is the normal portal of entry for all inhaled material. In addition to being an organ for smell, the nose has other functions including the conditioning and transporting of inhaled air and providing an effective filtering system that serves to protect the upper respiratory system against toxic chemicals and biological agents. This mechanical barrier, while being fundamentally nonspecific, can be quite effective. For example, under normal conditions ~100% of the sulfur dioxide, 20–80% of the ozone, and 73% of the nitrogen dioxide drawn through the nose are trapped in this region, preventing the pollutant from reaching the lower areas of the lung. However, under certain conditions, such as at times of high physical activity, individuals resort to mouth breathing and, as a result, the inhaled air bypasses these defenses. This significantly changes the deposition pattern of the inhaled gases or particulates and possibly their toxicity. The concentration of inhaled pollutants at the NP region can be expected to be higher than the level delivered to the lower respiratory airways and is most similar to the ambient concentration.

While the removal of airborne contaminants by the nose is effective, this action also renders this organ susceptible to toxic damage. The behavior of the inhaled substances in the NP airways and the ultimate determination of whether they are deposited or exhaled depends on numerous factors; for example, breathing patterns that influence nasal airflow rates and the chemical and physical properties of the airborne material, such as size, shape, water solubility, and reactivity. Soluble particles may, once deposited, rapidly enter the blood circulation and be transported systemically. Thus, the effective dose of toxicant delivered to the target tissue depends on factors other than the environmental concentration.

In the anterior one-third of the nose, where particles larger than 5 μm are deposited, the principal means of clearance is by blowing, sneezing, or wiping. However, following some exposures, certain

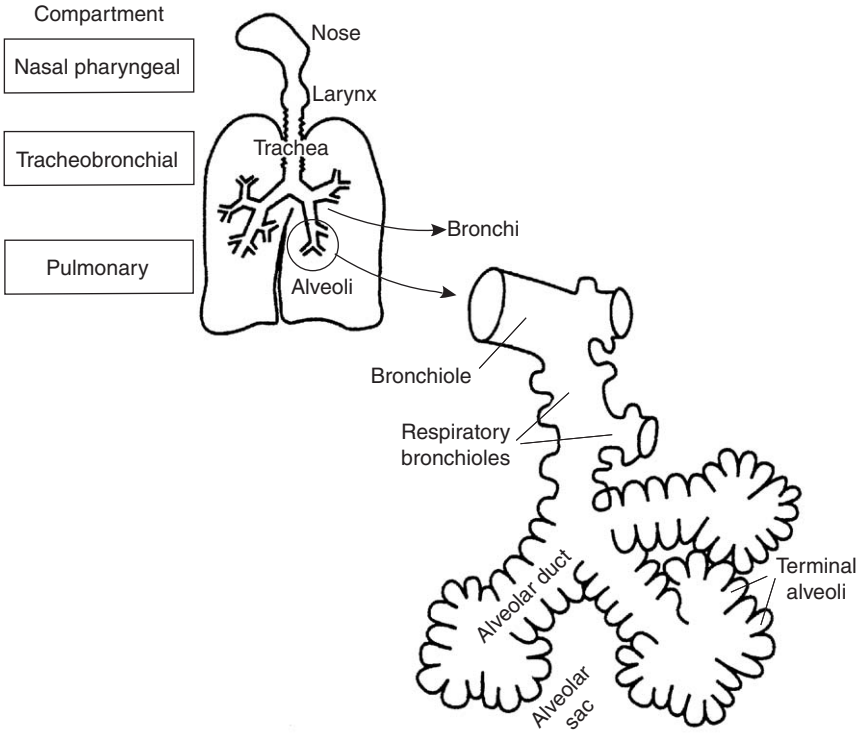


Figure 3 Compartment model of the respiratory tract. (Reproduced from Witorsch P and Spagnolo S (eds.) (1994) *Air Pollution and Lung Disease in Adults*, 1st edn., p. 22. Boca Raton, FL: CRC Press, with permission from CRC Press.)

particles may actually remain in this area for several days after deposition. Such retention patterns may be responsible for serious health effects. For example, nasal cancer identified in machinists was shown to be related to the high nasal collection efficiency for those particles above 10 μm in diameter. Such large-size particles are made airborne by various grinding and sandblasting activities. Particles approximating 3 μm in diameter are deposited in the NP region primarily through inertial impaction that occurs at airway branching. Breathing patterns involving higher air flow rates tend to increase deposition of particles as small as 1 or 2 μm in diameter. In all cases, deposition can be expected to increase with duration of breathing and depth of breathing. Deposition occurs during both inhalation and expiration. Chemical and biological agents deposited in this region may lead to inflammation (rhinitis), congestion, impairment of the sense of smell, ulceration, and cancer. To prevent a buildup or uptake of these deposited substances and possible long-term health effects, it is important that they are removed quickly. The mucus layer lining the NP epithelium plays an important role in clearance of deposited material by providing a moist, sticky surface that entraps inhaled particles and gases. Particles are then transported mouthward, due to the beating of the underlying cilia, where they are swallowed or expectorated. Various disease states, such as chronic sinusitis, bronchiolectasis, rhinitis, and cystic fibrosis, adversely alter mucociliary clearance from this region. It has been estimated that normal physical clearance from the NP region has a half-life of $\sim$4 min.

Nasal metabolism also plays a role in the response of this organ to xenobiotics. The nose contains a large number of enzymes including cytochromes P450, dehydrogenases, esterases, transferases, and hydrolases. Nasal metabolism can be responsible for both the protection of and the damage to the nose. For example, the breakdown of formaldehyde by dehydrogenases may be protective, whereas the toxicity of certain nitrosamines has been attributed to metabolic activation to toxic metabolites by nasal cytochromes P450.

Because the nose is directly exposed to a wide variety of infectious and antigenic agents, the nasal immune system also plays an important role in defending this region from such agents. Nasal secretions contain locally produced antibodies that can be monitored in both humans and test animals by nasal lavage. This is a technique that permits the detection of both immune mediators and the influx of inflammatory cells in this region, providing useful biomarkers of effects.

Tracheobronchial Region

Inhaled air together with all of the airborne substances not removed in the NP region enter the tracheobronchial (TB) region. The TB region of the respiratory system consists of the trachea and the bronchial tree down to and including the terminal bronchioles. In the mammalian TB trees, there are two forms of branching, monopodial and regular dichotomous. In most nonprimate species the branching is monopodial; that is, there are long, tapering airways with small lateral branches that come off of the main airway at an angle of $\sim 60^{\circ}$. In a human lung the branching is symmetric (dichotomous) involving the division of a tube into two daughters, each with nearly equal diameters and nearly equal angles of branching with respect to its parent tube. The major function of this TB region is to transport the inhaled air into the pulmonary region for gas exchange and to remove it during exhalation. At the entry into this region, the proximal end of the trachea is continuous with the larynx and extends distally into the thoracic cavity to the carina where it bifurcates to form two primary branches called bronchi. The tracheal airway is maintained during breathing by cartilaginous rings that prevent it from collapsing. As the bronchi continue to divide into smaller and smaller diameters there is a point where the cartilage is no longer present and the airways are composed of smooth muscle and loose connective tissue. At this region they are referred to as nonrespiratory bronchioles. There are several generations of such bronchioles. The most distal conducting and nonrespiratory airways in the respiratory tract are the terminal bronchioles. It is this distal end of the conducting airways that connects to the respiratory bronchioles.

Since the TB region contains both very large and very small conductive airways, substances of all sizes and chemical compositions can be expected to be deposited in this region. The many bifurcations in the TB region are vulnerable sites of high regional deposition. For example, the centriacinus, which is the site of the junction between the most distal conducting airways and the gas exchange area, is a common site of injury from a variety of airborne chemicals including diesel exhaust, oxidant air pollutants, and asbestos fibers. With physical exertion and mouth breathing, the beneficial defenses of the NP region are significantly reduced and a greater number of larger particles can be expected to be deposited in this region. Toxic substances deposited in this region in sufficient quantities may lead to bronchospasm, allergic reactions, congestion, bronchitis, and cancer. The more rapidly these materials are cleared, the less time for injury.

The primary clearance mechanism for this region is similar to that of the NP region; that is, by mucociliary transport to the glottis with subsequent expectoration or swallowing. The TB region is

equipped with both ciliated and secreting cells for removal of deposited material. The airway epithelium has secretory capabilities for synthesis and release of mucus, ions, and water. In certain diseases (e.g., bronchitis, cystic fibrosis, and asthma) an excess of secretions may result, causing airflow obstruction, increasing the residence time of inhaled substances, and thus increasing the dose to the airways. The ciliated cell is one of the major cell types and is probably a nonproliferative, terminally differentiated cell. The ciliated cells have on their mucosal surface ~200 cilia per cell. Their length is ~6 μm in the large airways and 5 μm in the smaller airways. Groups of cilia beat spontaneously with metachromal waves in a coordinated fashion independent of nervous control. The nonciliated cells consist of various secretory cells (mucous, clara, and serous) depending on species and a nonsecretory basal cell. The mucous and clara cells may differentiate into ciliated cells. The ciliated cells can actually retract their cilia and become a secretory cell. Because of the difference in the airway size, the clearance rate differs significantly within the region. Since smaller sized particles are deposited deeper in the airways, the clearance of such particles from the TB tree is slower when compared with those larger sized particles deposited higher up in the airways. Within the large airways, the half-time for clearance is ~0.5 h. For the intermediate airways the half-time is 2.5 h and in the finer airways the removal half-time is 5 h. Exposure to inhaled toxicants and viral and bacterial microorganisms can significantly decrease the efficiency and speed of TB clearance and hence increase the potential health risk. Certain human disease states are also associated with alteration of clearance from this region. Bronchial mucus transport may be impaired in people with bronchial carcinoma, chronic bronchitis, asthma, and acute infections.

Pulmonary Region

Ultimately, the air with its remaining contaminants reaches the pulmonary region, which is the most distal and includes the respiratory bronchioles, alveolar ducts, alveolar sacs, and alveoli. The main purpose of the alveolar architecture of the mammalian lung is to expose blood to gas over a large surface area within a comparatively small volume.

The pulmonary region includes the functional gas exchange sites of the lung. The terminal bronchioles of the TB tree branch to form the respiratory bronchioles. The major structural elements of the parenchyma of this region of the lung include the alveolar ducts, alveolar sacs, alveolar capillaries, and pulmonary lymphatics. The walls of the tubular alveolar ducts are covered with alveoli. As these branch, they exhibit increasing number of alveoli opening into their lumina. The human alveolus (which number ~300 million) is a polyhedral structure ~250–300 μm in diameter. These alveoli are thin walled and surrounded by blood capillaries for ease of gas exchange. The total thickness of this air-to-blood interface has been demonstrated by electron microscopy to range from 0.36 to 2.5 μm in the human. Gas exchange in the lung must be very efficient since the average human consumes ~2.5 l of O_2 per minute. To be transported through this air–blood barrier and reach a red blood cell, a molecule such as oxygen must penetrate this tissue at the alveolar surface, transverse the tissue barrier, enter the capillary blood at the capillary surface, move through the blood to a red blood cell, and finally penetrate the red blood cell to bind with hemoglobin. The greater the exchange surface, the more area available for oxygen to diffuse. In addition, the thinner the air–blood barrier, the smaller the resistance to the oxygen diffusion, and the more blood flow, the greater the amount of oxygen that can be bound to hemoglobin. The air–blood tissue barrier is sealed toward both blood and the air space by continuous cell layers, the blood capillary endothelial cell layer and the alveolar epithelium. The endothelial cells function to control the passage of fluid, proteins, and other blood components from the vessel lumen into the interstitium and the air spaces of the lung. These endothelial cells also function in a wide variety of specific metabolic activities important in the pulmonary processing of vasoactive substances – for example, enzyme inhibitors, receptors, and transport systems of these endothelial cells can determine the level of biogenic amines, kinin, angiotensins, and prostaglandins entering the circulation. Airborne toxic substances are capable of causing many types of injury to this gas exchange region, possibly increasing the thickness of the epithelial lining or changing the permeability and resulting in an influx of cellular and acellular fluids into the alveolar spaces. Such changes can have an adverse effect on normal gas exchange.

The most prominent cell making up the epithelial lining layer in the pulmonary region is the type I cell. It makes up more than 93% of the alveolar surface area. These squamous epithelial cells line the alveolar surface and are only ~0.1–0.3 μm or less in thickness, minimizing the barrier for gas exchange. The thicker type II cell is cuboidal and covers only ~7% of the alveolar surface area. The airway surfaces of these cells are covered with microvilli, greatly increasing the surface area. Together, these two cell types function as a permeability barrier to limit the movement of molecules between the alveolar space and the interstitium. The type II cells are the progenitors of the

type I cell and proliferate to reestablish the epithelial surface when the type I cells are injured. The type II cells also function as a source of an essential alveolar lining fluid, surfactant. A deficiency of surfactant may lead to alveolar collapse, resulting in hypoxemia and decrease in lung compliance. It is speculated that the alveolar macrophages release certain factors that may promote growth of the type II cells.

Due to its anatomic location, the alveolar epithelial surface is often directly exposed to inhaled gases and particulates. Injury to alveolar cells has been associated with a number of pulmonary toxicants, for example, silica, ozone, NO_2 herbicides, trace metals, and a number of organic vapors. It is of interest that the chemicals paraquat and diquat both cause type I cell damage but only paraquat damages type II cells, indicating that these two cells may differ in their sensitivity to chemical insults. This alveolar region is the site of several pathological lesions including centrilobular emphysema, fibrosis, and a variety of cellular injuries due to oxidant gases. Loss of or damage to alveolar tissue adversely affects the efficiency of gas exchange in this region.

The alveolar macrophages are large, nucleated cells that are found on the surface of the alveoli. These cells are not a fixed part of the alveolar epithelial wall but are mobile and possess the ability to engulf (phagocytize) and remove foreign material from the region. Macrophages, in addition to being responsible for clearing the lung of debris, play a major role in initiating and modulating the primary immune response in this area of the lung and are also effective in maintaining the sterility of the lung by killing or inactivating viable microorganisms. These cells locate the material by either random motion or are directed to the site by certain chemotactic substances. Under normal conditions the number of macrophages is estimated to be $\sim$3% of the total alveolar cells, but this number can be significantly increased with an increase in deposition of particles in the lung. These cells can ingest more than 10 times their weight in particles without any measurable loss in mobility or phagocytic ability. A number of host and environmental factors can modify the rates of pulmonary clearance by this mechanism. Individuals with chronic obstructive lung disease, viral infections, asthma, interstitial fibrosis, and inflammation, as well as individuals exposed to numerous inhaled gases and particulates, have reduced numbers and impaired function of these cells, resulting in a concomitant increased risk of pulmonary disease. Certain kinds of particles may be difficult to clear due to their particular shape. Long fibers such as asbestos may be cleared more slowly and may induce biochemical changes that can ultimately be toxic to the macrophage.

Once loaded with particles the macrophage may be cleared from this region by a number of pathways. The primary route out of the alveoli is via the mucociliary escalator. The macrophages reach the distal terminus of the mucus blanket and then are swept distally by ciliary beating within the airways. Macrophages may also migrate within the interstitium to the lymphatic system. There is also evidence that macrophages may enter the blood directly where they, together with their engulfed particles, can travel to extrapulmonary sites. If the ingested substance is toxic to the macrophage, it may be lysed while still in the alveolar region and the particle released to be taken up by another macrophage. Such cytotoxic substances may thus remain in the lung for considerable time. Clearance kinetics indicates that the successful removal of insoluble particles by macrophages consists of two phases. The first has a half-life measured in days and the second in hundreds of days. Clearance routes and kinetics are a function of lung burden, the physicochemical and toxicological properties of the material to be transported, and the health of the individual.

An actively functioning pulmonary immune system is critical for defense of the lung. Immune activity has been shown in both the conducting airways and in the lung parenchyma. All major immunoglobulins – IgA, IgG, IgM, and IgE – are present in the bronchial secretions. These are derived from local synthesis and by transudation from serum. In the parenchyma of the lung, the pulmonary macrophage participates in the generation, expression, and regulation of the immune response. These cells serve as antigen-presenting cells, as effector cells for T-cell immunity, and as regulatory cells that serve either as promoters or suppressors of pulmonary immune response. A detailed description of the mechanism of the immune response in the lung is complex and beyond the scope of this entry.

Briefly, antigenic materials deposited on pulmonary tissue initiate and stimulate the immune process. Antigens are taken up by and processed by the macrophage. Antigens in alveolar spaces that escape this phagocytic action and other clearance mechanisms may still gain access to the pulmonary interstitium, where they may be subsequently transported to nearby lymphoid tissue where immune stimulation can occur. The pulmonary macrophage presents the antigen to local lymphatic tissue that ultimately produces cell-mediated or humoral immune response. Lymphatic tissue and lymphocytes are present at or near the air–tissue interface at all levels of the respiratory tract from the nasopharynx to the alveolar spaces of the pulmonary racemus. These tissues are important in ensuring pulmonary immune response

Table 2 Examples of immunomodulation by various inhaled chemicals

Classification	*Symptoms*	*Chemical agents*
Immediate (type I) hypersensitivity	Bronchial asthma, asthmatic bronchitis, urticara, rhinitis, atopy	Beryllium, chloramine, ethylenediamine, ethylene oxide formaldehyde, isocyanates, platinum, nickel
Cytolytic (type II) hypersensitivity	Chemically induced hemolytic anemia, bone marrow depression, thrombocytopenia	Trimellitic anhydride, mercury
Arthus-immune complex (type III) hypersensitivity	Hypersensitivity pneumonitis, rheumatoid disease, sarcoidosis, vasculitis	Trimellitic anhydride, mercury
Cell-mediated (type IV) hypersensitivity	Contact dermatitis, sarcoidosis, anergy, delayed hypersensitivity	Beryllium, chromium, isocyanates, mercury, phthalic anhydride, trimellitic anhydride
Immunosuppression	Altered immune responses and host resistance following inhalation exposure	Asbestos, silica, metals, toluene, oxidant gases, tobacco smoke, benzene, toluene
Irritancy or nonimmunological	Pseudoallergic symptoms of bronchial asthma and asthmatic bronchitis	Formaldehyde, isocyanates, ethylenediamine

since they contain antigen-presenting cells and the full repertoire of antigen-reactive T and B lymphocytes needed to react with the antigen.

Cell-mediated response begins with the macrophages but is then mediated through the thymus-derived lymphocytes (T cells). These T cells regulate the immune system. T cells interact with B cells for antibody production. T cells can not only kill cells presenting antigen but can also release cytokines that modulate the immune response. Humoral immune responses are the end result of antigen interacting with marrow-derived or bursal cell-equivalent lymphocytes (B cell). B cells secrete antibodies that inactivate antigens in the body. The B-cell function is regulated by two subpopulations of T cells: helper T cells that are required for optimal production of antibody and suppressor T cells that are active in modulating the humoral response once initiated. A wide array of substances that can affect the immune response is discussed in the section on effects on pulmonary defenses. **Table 2** lists examples of immunomodulation by various inhaled chemicals.

Macrophages also release substantial amounts of diverse substances that exhibit a broad range of biological activities. Examples of such mediators include (1) interleukins, which play an important role as mediators of inflammation, are chemotactic for neutrophils, promote the differentiation of natural killer (NK) cells, and function in the maturation of helper T cells; (2) monokines, which regulate the growth and activation of other cells such as fibroblasts and endothelial cells; and (3) interferon, which represents a group of antiviral proteins that function to inhibit the intracellular replication of many viruses and the proliferation of malignant cells and promote NK cell functions. These NK cells do not play a role in the antigen-specific antibody response but are critical components of the general, nonspecific immune defenses.

From this brief description it is evident that the immune system is very complex and proper functioning depends on the interaction of several components. Each of these steps is a potential target for a toxic chemical.

Biomarkers of Pulmonary Effects

There is a growing need for development of sensitive assays that can be used in inhalation toxicology as biological markers of adverse health effects associated with pulmonary injury. A pulmonary biomarker should be able to reflect a change in a biological system that can be related to a specific effect or an exposure to a specific toxic substance. Such a marker should be an indicator of early biological response of the respiratory system, indicating alterations in cellular, biochemical, or immunological processes or functional or structural changes. An ideal biomarker of an effect should be unique to a specific disease, capable of quantitatively relating to a particular stage of the disease, reproducible, sensitive to small changes due to an exposure, and specific to a particular test substance. A number of such markers have been developed and are discussed in the 1989 National Research Council monograph, *Biological Markers of Pulmonary Toxicity*. The major focus of this section is to provide examples of biomarkers of pulmonary response and to discuss how these indicators can help to improve our understanding of the respiratory system in normal and disease states.

Markers of physiological effects can be useful in identifying early changes in respiratory functions of the lung due to inhaled material. Biomarkers are available to measure lung mechanical properties, ventilation, expiratory flow, intrapulmonary gas distribution, alveolar–capillary gas exchange, and perfusion. Such measurements have been used to test the effects of exposure to an array of inhaled toxicants. These assays can reveal functional manifestation of structural changes in the respiratory system, whether

these changes are transient, resulting from bronchoconstriction, inflammation, or edema, or irreversible, such as from fibrosis, emphysema, or chronic obstructive lung disease. While the current functional tests are useful in evaluating clinical lung disease, they, by themselves, are not sensitive markers of the lung injury. The lung responds to air contaminants in much the same way regardless of the specific toxic nature of the toxicant. Increased efforts are being devoted to the development of functional tests that will be better indicators of specific alteration, focusing on certain regions of the respiratory system, such as the terminal bronchioles and respiratory bronchioles. Alterations at these sites, which are likely targets of several types of airborne toxicants, would be indicative of small airway disease.

Airway hyperreactivity is a useful marker that can be assessed by measuring increased bronchoconstriction (i.e., contraction of airway smooth muscle). Hyperreactivity can be measured in the pollutant-exposed subject by following exposure with a challenge of (1) a variety of pharmacological chemicals such as methacholine, carbachol, histamine; (2) a physical stimuli such as cold or dry air or exercise; or (3) air pollutants such as sulfur dioxide. An exposed individual may develop bronchoconstriction after inhaling a lower concentration of a provoking agent than is needed to cause a similar degree of change in the airway in a normal subject. Airway hyperreactivity has proved to be useful in assessing airway responsiveness following exposure to a low concentration of pollutants, such as ozone, nitrogen dioxide, sulfuric acid aerosols, allergens, and certain irritant gases. Evidence indicates that these tests constitute markers that are useful for detecting risk of accelerated loss of lung function, which may be indicative of the development of chronic lung disease.

Since the mechanisms of clearance of particles from the respiratory tract are similar in most mammals, markers measuring alterations in the effectiveness of these defenses have been used to predict respiratory tract disease and for extrapolating animal data to humans. Both human and animal studies have shown that exposure to certain gases and PM may significantly alter bronchial mucociliary clearance rate. Relating these changes to specific health effects remains speculative. However, there is a predisposition to respiratory infections (e.g., chronic bronchitis), with retarded clearance from the airways. By increasing the residence time of carcinogens, altered mucociliary clearance may also be a factor in the development of bronchial cancer.

More noninvasive markers are needed for assessing early alterations in lung structure. The cells of the nasal, tracheal, and bronchial regions can be relatively accessible with bronchoscopy, brushing, and biopsy. In the TB region, markers of differentiated phenotypes are useful in providing a direct indication of cellular damage. Mucous glycoproteins are markers for alterations of mucous cells and specific histochemical staining techniques are used to characterize secretory cell products. A low-molecular-weight protein appears to be a specific marker of clara cell secretory products. The presence of dynein appears to be a good marker for structural changes in the ciliated cells. Other biochemical and immunological markers (keratin expression, transglutaminase, and sulfotransferase) may reflect differentiation of TB epithelial cells. Measuring such changes could provide early indication of pathologic changes in easily accessible airway lining cells.

Inhalation of many types of toxic chemicals can cause selective injury to the more proximal portions of the gas exchange region of the lung. Markers focusing on specific focal patterns of injury that may be caused by different pollutants would be useful. For the alveolar region, specific markers of injury or disease are even less developed. Because an early response to cell injury from airborne pollutants is likely to result in proliferation of airway cells (e.g., epithelial, fibroblast, and macrophages) markers have been used to measure these responses following exposure to cigarette smoke, asbestos fibers, and oxidant gases. Using morphometrics, the total number of cells in the lung and the distribution of cells among the various types of alveolar cells have been determined in both humans and animals. Such techniques, although difficult to apply, offer promise for the development of sensitive markers of early structural changes.

Cellular and biochemical markers have been widely used to detect changes in the acellular and cellular content of nasal, bronchial, or bronchoalveolar lavages. The response of these regions to several inhaled substances, such as ozone, nitrogen dioxide, ambient PM, fibrogenic material, and several trace metals, can be measured by examining the lavage fluid to assess any variation from normal. Indicators being used include the presence of blood neutrophils and mast cells (markers of permeability changes and influx of inflammatory cells), influx of eosinophils and basophils (indicators of allergic reaction), serum protein (marker of increased permeability of alveolar–capillary barrier), lactate dehydrogenase (marker of cytotoxicity), and lysosomal enzymes (markers of activation or lysis of macrophages). Other markers of effect have also been measured in lavage fluid, including growth factor, interleukins, arachidonate metabolites, and increase in prostaglandins. There is still a need to develop reliable markers to detect specific cell responses at the molecular level. Molecular type

markers to characterize changes in DNA and RNA, changes in DNA sequences, and changes in the extent or pattern of gene expression would be of most value since they might aid the scientist in identifying individual susceptibility to pulmonary disease.

Toxicological Response to Inhaled Chemicals

The toxicology literature is extensive in the documentation of many human and animal studies that have been conducted to detect the health effects associated with airborne pollutants. Causal relationships between exposure to an agent and various forms of toxicity can be readily established using controlled animal studies. Animal studies suffer the obvious drawback of requiring extrapolation of these responses to humans. Such studies are nevertheless commonly used to identify toxic properties of chemical agents because of the shortcomings of human epidemiological and clinical studies. Unfortunately, many of the available animal studies were designed and conducted to study the responses at relatively high concentrations, making it difficult to directly relate such responses to the relatively low levels found in the ambient environment. It is not the intent of this entry to provide a complete overview of all treatment-induced effects associated with inhaling airborne chemicals; instead, this entry provides a toxicity profile that is focused on an array of health effects caused by exposure and relates these observed responses to the potential health risk of the population.

The objective of any toxicological study is to determine the relationship between an appropriate exposure and a measured biological response in a susceptible species by the most valid and sensitive technique. Current research continues to focus on identifying that portion of the respiratory system that experiences the greatest effect of an inhaled toxicant. However, the point of maximal injury can be expected to vary with the nature of the toxicant, its concentration and duration of the exposure, the effectiveness of local defense mechanisms, and the inherent susceptibility to damage of the cells at risk. The size and complexity of the respiratory system in humans and animals provides numerous sites of potential damage caused by inhaled gases and particulates. To fully understand the toxicological consequences resulting from exposures, testing procedures must apply multiple end points and varying durations of exposure and must evaluate a variety of target tissues for injury. In evaluating the significance of the available database, the toxicologist needs to understand the various relationships that may exist between the measured response and the exposure. With multiple end points of toxicity and a given concentration of the agent, an infinite number of linear and curvilinear relationships could be generated. The dose–effect relationship may be steep, indicating that a small increase in concentration (dosage) elicits a dramatic increase in the effect, or the slope may be shallow, indicative of only a small change in the altered state accompanying a large increase in the dosage of the toxicant. Frequently, a toxicant may elicit effects on more than one target organ, giving rise to dose–effect curves of different configurations. Such information is vital in predicting dose–response relationships.

Irritation and Inflammatory Response

Although irritation often suggests a relatively mild, transient effect, respiratory tract irritation is one of the most significant airway responses for the inhalation toxicologist. Irritation is frequently the first observable adverse response of the airways following exposure to airborne materials. In addition, irritation often occurs at relatively low concentrations that may be realistic for typical human exposures. The number of chemicals and common mixtures that are known to be respiratory irritants is far greater than that for any other respiratory system response. Many common components of air pollution, including sulfur dioxide, H_2SO_4, nitrogen dioxide, ozone, and various metal oxides, are respiratory irritants. This, along with the fact that many people have personal experience with such irritation, for example, by household ammonia, cigarette smoke, or photochemical smog, produces a high public awareness and concern for the irritancy of airborne chemicals. Agents that produce an irritant response on contact with airway tissues are termed direct irritants. The responses may be mild to severe, with typical concentration dependence, and they are usually reversible. Many organic vapors that are potential workplace hazards are sufficiently reactive to produce irritant injury to the airways. Examples include aldehydes (e.g., acrolein), epoxy compounds (e.g., ethylene oxide and propylene oxide), halogenated alkanes (e.g., bromotrichloromethane), aliphatic isocyanates (e.g., methyl isocyanate), and aliphatic nitro compounds (e.g., tetranitromethane). Many of these chemicals are also capable of producing respiratory tract neoplasms in laboratory animals. Respiratory irritancy is the most frequently used basis for setting occupational exposure limits, such as American Conference of Governmental Industrial Hygienists TLVs.

The mouse respiratory depression model of Alarie, which is described in more detail in the section on physiological assessment, provides a lung function-based

system for classifying and describing the relative potency of respiratory tract irritants. Upper respiratory tract irritants, the 'sensory' irritants of the Alarie model, are usually water-soluble chemicals, such as formaldehyde, ammonia, sulfur dioxide, and acrolein. The early effects produced by such chemicals, including burning sensations of the eyes and upper airways and the cough and bronchoconstriction caused by irritation of conducting airways including the larynx, as well as the decreased respiratory frequency in mice, are neurally mediated reflex responses. Irritant receptors in the conducting airways also respond to mediators, such as histamine, serotonin, and prostaglandins, and produce bronchoconstriction via a reflex increase in vagal efferent activity. Some human populations, such as asthmatics and the young, may be especially sensitive to the effects of upper airway irritants, responding at lower concentrations than the general population. Irritants that penetrate to the deeper regions of the lung, the pulmonary irritants of the Alarie model, are generally less water soluble or, in the case of aerosols, have small particle diameters. Examples include ozone, nitrogen dioxide, phosgene, and oxides of metals such as cadmium and beryllium. Again, the early responses – cough, chest tightness, and substernal soreness in humans, rapid, shallow breathing in rats, and respiratory depression in mice – appear to be neurally mediated reflexes.

Although the initial responses to irritants are reflexes mediated by irritant nerve endings, prolonged and/or repeated exposures result in cellular and tissue injury, edema, and inflammation. Such irritant-induced structural effects have been demonstrated for most sensory and pulmonary irritants, including chlorine, sulfuric acid, methyl isocyanate, formaldehyde, ozone, and nitrogen dioxide. It is generally believed that materials that produce primary respiratory irritation have the potential to produce long-term effects following repeated exposure. An important question concerns the potential role of the irritant response in the pathogenesis of chronic disease and cancer.

Under normal conditions, the alveolar epithelial and endothelial cell layers that make up the air–blood barrier control the passage of fluids and cells between the air spaces of the lung and the interstitium. Damage to this delicate barrier can cause an inflammatory response and the impairment of lung function. Changes in the permeability of the alveolar–capillary barrier lead to an infusion of proteinaceous serous fluid (edema) and blood cells (neutrophils, macrophages, and eosinophils). This influx of cells usually peaks within the first 3–7 days of the inflammatory response. If the inflammation is sustained it is usually accompanied by a specific immune response mediated by pulmonary lymphocytes.

This is the normal reaction and may be the lung's first response against the insult. However, after entering the lung, inflammatory cells can actually enhance the effect of the original insult and may be causally related to certain chronic lung diseases. These cells respond to injury by producing a number of potent chemicals, such as cytokines, chemotactic factors, prostaglandins, lysosomal enzymes, active oxygen radical species, and leukotaxines. Involvement of oxygen radicals has been hypothesized for a number of pulmonary diseases related to exposures to numerous agents, including asbestos, paraquat, cigarette smoke, ozone, nitrogen dioxide, and ionizing radiation. In normal circumstances, the generation of oxidants by defense cells is essential for effective host defense against invading microorganisms. If the inhaled substance causes subsequent lysis of these cells, these highly active cellular products would be released into the lung where they could act directly on the pulmonary tissue. Macrophages, for example, release proteolytic enzymes that can degrade intercellular components of lung connective tissue and also interact with certain constituents of serum such as complement. These agents may, alone or in combination, cause functional impairment of epithelial cells, mesothelial cells, and fibroblasts, resulting in disease.

The analysis of isolated bronchoalveolar lavage fluid is an effective means for the detection of inflammatory responses in the lung. In both animals and humans, cell counts and cell distributions can be determined, along with measures of protein and bioactive mediators.

Asphyxiation

By definition, asphyxiants are chemicals that deprive the tissues of oxygen when inhaled. Any physiologically inert gas, including hydrogen, nitrogen, helium, and methane, that is inhaled at a high enough concentration to exclude an adequate concentration of oxygen acts as a simple asphyxiant. Chemical asphyxiants such as carbon monoxide, cyanide, hydrogen sulfide, and nitrites block the use of oxygen, causing asphyxiation when inhaled along with an adequate concentration of oxygen. Carbon monoxide is an odorless and tasteless by-product of incomplete combustion of carbonaceous materials. Carbon monoxide poisoning continues to be a significant public health concern both because of its use in suicides and because of accidental poisonings caused by faulty ventilation of home-heating devices. Since the binding affinity of red blood cell hemoglobin is 200 times

greater for carbon monoxide than for oxygen, carboxyhemoglobin formed at a relatively low concentration of this gas can block oxygen transport by a large proportion of hemoglobin. Full dissociation of carbon monoxide from hemoglobin occurs following removal from the carbon monoxide-containing environment. Therefore, poisoning is not cumulative. Carbon monoxide is an air pollutant and component of cigarette smoke, and smokers, parking garage workers, and traffic policemen are repeatedly exposed at low levels. Although asphyxiation is not a concern with such exposures, transient neurobehavioral deficits may develop and there may be an increased risk to individuals with heart disease. Other chemicals, such as sodium nitrite, interfere with transport of oxygen by oxidizing the iron moiety of hemoglobin, producing methemoglobin. Cyanide does not block oxygen transport but is a classic tissue-level poison, inhibiting cytochrome oxidase and blocking energy production.

Morphological and Structural Effects

Morphological studies are often the cornerstones of toxicity experiments. Pathological evaluation of exposed tissue permits the identification and characterization of structural damage to the respiratory system. Animal studies have been effective in improving our understanding of the pathologic sequelae of chemical deposition at specific sites in the respiratory system. The difference in the structure of the respiratory system of humans and experimental animals may complicate but does not necessarily prevent qualitative extrapolation of risk to humans. Since the lesions resulting from a particular exposure can be similar in several mammalian species of test animals, it would appear likely that the biological processes responsible for the lesions in animals could also occur in humans. However, it should be understood that different exposure levels may be required to produce a similar response in humans. The concentration at which effects become evident in humans can be influenced by a number of factors such as preexisting disease, dietary factors, combination with other pollutants, and the presence of other stresses.

A wide variety of morphological changes have been associated with inhalation of airborne contaminants. Both acute and chronic exposures directly affect the structural integrity of the respiratory system. Acute studies are conducted primarily to define the intrinsic toxicity of the chemical, to identify the target organs, and provide information for the design and selection of doses for long-term studies.

The epithelium of the conducting airways represents a tissue that is uniquely sensitive to a number of inhaled toxicants and that shows early histopathological damage when injured. Such injury in turn often elicits a variety of acute inflammatory responses. The ciliated cells, which are distributed throughout much of the length of the conducting airways, often exhibit morphological damage causing ciliary dysfunction, slowing of transport rate, and excessive mucus production. It appears that these ciliated conducting airway cells are the most sensitive to direct-acting toxicants and that cells with the most secretory capacity are less sensitive (i.e., mucous and clara cells). Cilia may be reduced in length or diameter and exhibit reduced density, and the cells may exhibit a variety of cytoplasmic changes including dilated endoplasmic reticulum, swollen mitochondria, and condensed nuclei. Tests for clearance of marker substances have been used to demonstrate that morphological effects on the cilia can result in a significant reduction in mucociliary clearance. Cigarette smoke, sulfur dioxide, alcohol, H_2SO_4, ozone, nitrogen dioxide, trace metals, and certain bacterial infections are toxic to the cilia and lead to impairment of mucociliary clearance. Individuals with bronchial carcinomas, cystic fibrosis, chronic bronchitis, and certain infectious diseases, such as influenza, atypical pneumonia, and tuberculosis, have impairment of lung clearance. Disruption or impairment of this defense system may result in greater accumulation of and potential injury by various airborne substances and increase the susceptibility to bacterial and viral infections. Continued chemical exposure can cause necrosis and the subsequent sloughing off of ciliated epithelial cells. The epithelial tissue may be repaired by the proliferation of the secretory cells. In areas of repair, the nonciliated cells often appear to be more numerous. In this type of injury, the repair process is initiated soon after the test animals are removed from the exposure atmosphere.

The respiratory alveolar epithelial response to toxic injury can be rapid, resulting in necrosis and subsequently sloughing of the sensitive type I cells. This type of response is seen with exposure to such toxicants as ozone, nitrogen dioxide, and butylated hydroxytoluene. This injury stimulates the proliferation of the more resistant type II cells. This proliferative response typically peaks at $\sim$48 h after onset of the initial injury to the type I cells. The increase in number of type II cells can be expected to alter the diffusion capacity of the pulmonary region through populating this membrane with these thicker cells.

Following lung injury, recovery depends on prompt and orderly repair. The type and extent of the injury determine whether cell replication results in the restoration of the normal structure or in

abnormal remodeling that may lead to profound anatomic distortion due to an exuberant fibroproliferation response. Reepithelization of any damaged respiratory area is critical to maintenance of normal lung function. For example, shortly after an injury, the alveolar surface may be denuded with only type II cells remaining. These type II cells begin to replicate, resulting in the repopulation of alveolar basement membrane. Eventually, the replacement cells flatten and begin to acquire the morphological features of the type I cells as the air–lung interface is reconstituted. However, it is also possible that in this repair process, a rapid migration of fibroblasts into the damaged area may occur. When this happens, these cells begin to replicate and deposit connective tissue. This obliterates the air space architecture, resulting in alveolar fibrosis. Pulmonary fibrosis results in decrease in diffusion capacity and a decrease in lung volume and compliance. Inhaled agents causing such fibrosis in humans include silica, asbestos, organic dust, cadmium fumes, paraquat, and some infectious microorganisms. The proliferation of epithelial cells, fibroblasts, and other lung cells following exposure can be measured *in vivo* and is useful in studying the pathogenesis of pulmonary disease. Cell proliferation assays are designed to quantify the relative rates of cell division within such target tissues using specialized immunohistochemical staining techniques to detect proliferating cells.

Alterations in capillary permeability are often associated with structural injury to endothelial cells. The endothelial defects are less evident at low concentrations but include cell swelling and disruption of the basement membrane. The difference in the extent of epithelial and endothelial damage can be explained by the different repair potential of these two lining layers rather than the dissimilar reaction to the injury. The pulmonary endothelium is susceptible to injury by oxygen-based free radicals. It is especially sensitive to the effects of high oxygen tension. Numerous studies have shown that such lung oxygen damage is the result of a direct toxic effect through intracellularly generated O_2 intermediates and not solely by the recruited polymorphonuclear cells. Paraquat, nitrofurantoin, cyclophosphamide, and bleomycin are among substances known to injure endothelial cells.

Three-dimensional reconstruction of cells and tissues is now being used to study subtle changes in intracellular organelles and cell-to-cell relationships that are affected by exposure. Developing such techniques has required advances in computer processing power to supply the memory and appropriate algorithms necessary to make this process technically feasible. Together with time-lapse photography and high-voltage electron microscopy, computer-time reconstructions can be used to study the effects of chemicals on cell function and cell regulation.

The main types of noncarcinogenic response of lung cells to chronic exposure are hyperplasia, hypertrophy, and metaplasia. In human studies, it is difficult to identify the chemical(s) causing a chronic pulmonary disease that is associated with morphological alterations due to the long latency period involved. In many cases, the disease symptoms may fail to be evident until after 20 or more years of exposure. Chronic lung disease can be conveniently classified into three broad groups: restrictive lung disease, chronic obstructive lung disease, and cancer.

Both restrictive and obstructive lung disease are associated with serious impairment of the flow of gases into the gas exchange regions of the lung. Pulmonary function tests are used to distinguish between these two diseases. Chronic COPD includes three major types – asthma, chronic bronchitis, and emphysema. Existing chronic bronchitis and asthma result in greater susceptibility to the effects of air pollutants, including SO_2, acid aerosols, and other PM_{10} components. Forced peak expiratory flow is reduced by greater bronchoconstriction and respiratory symptoms increase during episodes of high ambient PM_{10}. In humans, a clear distinction between emphysema and bronchitis is not possible. Most patients who have chronic bronchitis also have emphysema. The resulting gas trapping and persistent slowing of airflow make expiration difficult. The bronchial wall thickness may be 50–100% greater than normal. Individuals with COPD can be recognized by their difficulty in performing more than light to moderate exercise and nonuniform distribution of ventilation. They frequently have associated cardiovascular disease, chronic cough, and recurrent expectoration.

Chronic bronchitis is a major health problem that is associated with long-term cigarette smoking, dusty environments such as grain elevators and coal mines, trace metal exposure (vanadium, arsenic, and iron oxide), phosgene exposure, and the exposure to ambient air heavily polluted with sulfur oxides and combustion products. Chronic bronchitis is clinically evident as excessive bronchial mucus production. Histological examination of human bronchial airways shows hypertrophy of mucus glands in the large bronchi; chronic inflammatory changes, including cellular infiltration and an accumulation of fibroblasts and connective tissue; edema; and possibly increases in smooth muscle in the airways. In the early stages, these effects are potentially reversible, but in advanced stages they are irreversible. Additional features of chronic bronchitis include inflammation of

the mucous membranes of the bronchial airways and a reduction in the number of ciliated cells together with increased secretions having abnormal physicochemical properties. These effects ultimately result in grossly impaired mucociliary transport. Cough aids as the clearance mechanism for excess mucus.

Asthma is defined clinically by recurrent episodes of airway obstruction that reverse either spontaneously or with bronchodilator therapy. The airway obstruction is accompanied by increase in airway resistance due to bronchospasm, inflammation, and excessive mucus production. Bronchoconstriction, airway closure, and gas trapping may eventually lead to respiratory failure. Hyperresponsiveness is considered a hallmark of asthma, making these individuals uniquely sensitive to exposure to airborne chemicals such as isocyanates.

Emphysema differs from the other two conditions in that there is evidence of anatomic alterations of the lung characterized by abnormal, uneven, permanent enlargement of the air spaces distal to the terminal bronchioles, resulting from the destruction/distension of the alveolar walls. Airway restriction or collapse results from loss of supporting tissue that normally maintains airway patency. Such structural changes are associated with various pulmonary functional abnormalities related to loss in lung elasticity and decreases in normal diffusion capacity and forced expiratory volume. Emphysema has been associated with long-term exposure to coal dusts, cigarette smoke, osmium tetroxide, cadmium oxide, and some common atmospheric pollutants (e.g., ozone). When such chemicals are inhaled they cause cell injury and an inflammatory response. During this process, proteases, lysosomal enzymes, and oxidants are released during phagocytosis, cell injury, and cell death. To maintain structural integrity under such conditions, the lung can respond with biochemical modifiers such as antiproteases. A balance between these two responses must be maintained since these reactive substances can degrade pulmonary elastin and collagen, resulting in a destruction of the supporting structure of the alveoli. With this destruction of lung tissue, there is a subsequent loss in total lung surface area and reduction in the ability of the lung to meet gas exchange demands.

Restrictive lung disease occurs when the elastic properties of the lung are so impaired that the lung becomes stiff as in fibrotic diseases related to silicosis, pneumonia, and asbestosis. This disease condition is characterized by increased lung recoil and a decrease in lung volumes, such as vital and total lung capacity. Such restriction decreases the normal ability of the lung to expand, making inflation of the lung more difficult.

When the lung is chronically exposed to a contaminant that is not easily removed or degraded, the lung may undergo a process referred to as granuloma formation. This lesion is characterized by accumulation of mononuclear cells (macrophages, lymphocytes, and giant cells) into a relatively discrete structure. These granulomas may distort the interstitial architecture, interfering with the normal process of gas exchange, and can cause tissue damage and fibrosis that may result in permanent dysfunction and morbidity. Granulomas are dynamic structures in that freshly recruited monocytes are continually entering the lesion and replacing mature cells. Ultimately, these granulomas may resolve or become fibrotic due to the influx and proliferation of fibroblasts. These lesions may be initiated by infectious agents (mycobacteria, fungi, and viruses) and inorganic substances like beryllium. A common property of all such agents is their low biodegradability and persistence, often within the macrophage. Individuals exposed to beryllium fumes may develop acute pulmonary edema and pneumonia. While most of these individuals recover, some develop chronic granulomatous lesions appearing years after the initial exposure. Generally, such chronic disease results from prolonged exposure to low concentrations of beryllium.

Fibrotic lung disease is directly associated with chronic inflammation in which the inflammatory process in the lower respiratory tract injures the lung and modulates the proliferation of mesenchymal cells to form a fibrotic scar. Practically any chronic injury that is capable of sustaining a continued inflammation will produce some degree of interstitial fibrosis. Such effects reflect a chronic, ongoing process and may ultimately involve the entire organ. The fibrotic process involves damage to the normal alveolar architecture, which in turn leads to activation of the macrophage and release of potent growth factors. These factors cause the mesenchymal cells to proliferate and produce large amounts of collagen that then accumulates in the interstitial space. This excess collagen deposition leads to pulmonary fibrosis. It is interesting to note that in the postexposure period, the fibrotic process tends to continue. Once fibrosis occurs within a group of alveoli, it is unlikely that those alveoli will ever recover. Fibrosis-producing agents include inorganic particulates (silica, beryllium, coal dust, iron oxide, chromium, and asbestos), toxic gases (ozone, nitrogen dioxide, and high concentrations of oxygen), cigarette smoke, paraquat, and a variety of immunotoxicants.

Pulmonary Function: Physiological Assessment

Although pulmonary injury by a toxic agent and/or disease process is normally defined by morphological

Table 3 Common measurements for assessment of changes in pulmonary function

Test category	*Individual test/parameter*	*Definition/functional significance*
Ventilatory pattern	Respiration rate	Breathing frequency (breaths min^{-1})
	Tidal volume	Volume of breath
	Minute volume	Total volume inspired/expired per minute
Static lung volumes	Vital capacity	Maximum volume that can be expelled from the lungs by forced effort following maximum inspiration
	Total lung capacity	Volume of gas in lungs at end of maximum inspiration
	Residual volume (RV)	Volume of gas in lungs at end of maximum expiration
	Functional residual capacity (FRC)	Volume of gas remaining in lungs at end of tidal expiration
	Inspiratory capacity	Maximum volume of gas that can be inhaled from FRC level
	Expiratory reserve volume	Maximum volume of gas that can be expired below FRC level
Respiratory Mechanics	Air flow resistance	
	Total lung flow resistance	Flow resistance of airways
	Static lung compliance	
	Dynamic lung compliance	Stiffness (elasticity) of the lung
	Maximum forced expiratory maneuver	'Stress' test for obstruction of airflow in peripheral airways
	Forced vital capacity (FVC)	
	Forced expiratory volume	
	Peak expiratory flow rate	
	Expiratory flow at 50%, 25%, and 10% of FVC	
Distribution of ventilation	Single and multiple breath nitrogen washout	Homogeneity of ventilation in lungs – airflow obstruction and gas trapping causes greater variability of ventilation
	Closing volume	Volume difference from RV representing onset of closure of small airways; increases with air flow obstruction
Diffusion	Carbon monoxide diffusing capacity	Measurement of efficiency of alveolar gas exchange; decreases with thickening of alveolar blood–air barrier
Blood gases	Measurements of arterial pO_2, pCO_2, and pH	Evaluates adequacy of ventilation; changes typically require severe functional deficits
Pulmonary circulation	Edema: marker radioisotope movement to airways; wet/dry lung weight ratios	Evaluation for transudation of fluid into airways
	Cardiovascular pressures	Hyper- or hypotension in vascular system
	Cardiovascular volumes, flow resistance	Cardiovascular function

change, the functional manifestations of these structural effects have proved to be sensitive indicators of toxic response and lung disease. Pulmonary function testing provides a safe, noninvasive approach for clinical evaluation of the presence, type, and severity of pulmonary impairment. When workplace conditions include a risk of inhalation exposure to toxicants, preemployment and periodic, repeated lung function testing can be a key element in health effect screening and disease prevention. For many lung function tests, repeated testing is also possible in laboratory animals and progression of or recovery from disease may be evaluated in animals and individuals. Evaluation of pulmonary function in both humans and animals complements evaluation of structural changes caused by inhaled chemicals. In addition, specific lung function tests may detect significant respiratory tract effects or disease states that do not produce lasting or detectable structural changes. Finally, a large body of experimental evidence from animal models of specific pulmonary diseases and animal toxicology studies suggests that similar lung insults and/or structural changes produce similar functional effects in humans and animals. Therefore, effects observed in animals may be used to predict human pulmonary effects. **Table 3** provides examples of pulmonary function measurements that have been used for evaluation of impairment by airborne toxicants.

In interpreting pulmonary function data, several key points should be understood. (1) A specific functional effect is not diagnostic for a single structural change. For example, reduced vital capacity or compliance may be caused by several structural changes, including fibrosis, edema, hemorrhage, cellular hyperplasia, and heavy particle loading. (2) The respiratory system has a large functional reserve. Therefore, a relatively diffuse and extensive lung lesion may be required to produce a detectable effect on lung function. (3) A useful approach to pulmonary function testing is the use of a battery of measurements to develop patterns of functional change that are consistent with a particular disease such as fibrosis or emphysema. (4) Restrictive lesions (e.g., fibrosis) are characterized by a lung that is less elastic, while obstructive lesions (e.g., emphysema) are characterized by changes that obstruct the movement of air in the airways. (5) Methods used in animals often require the use of anesthesia or restraint and the potential impact of such procedures on measurements must be considered.

The lung function tests that are most frequently used in animals evaluate breathing, patterns, lung volumes, lung mechanical properties (including compliance, airway resistance, and flow rates), and diffusing capacity. Breathing pattern measures include respiratory frequency, tidal volume (the volume of a single, normal breath), and minute ventilation. A useful screening approach for evaluation of acute respiratory irritancy of inhaled chemicals has been developed by Alarie and co-workers. Using a head-only exposure system for mice, the effect of chemical exposure on respiratory frequency is monitored and, to allow comparison of irritancy between chemicals, the concentration that depresses the frequency by 50% is calculated. Two patterns of irritancy have been described. 'Sensory' or upper airway irritants are usually highly water-soluble chemicals, such as formaldehyde and ammonia, and cause a reflex depression in respiratory frequency with a slow expiratory phase. Pulmonary or peripheral airway irritants are typically less soluble chemicals, such as phosgene, ozone, and nitrogen dioxide, and cause a respiratory depression marked by pauses between breaths. In rats, pulmonary irritants produce tachypnea (rapid shallow breathing).

Measures of breathing pattern tend to be relatively insensitive to early restrictive or obstructive lesions, but with advanced chronic disease, restrictive lesions produce rapid, shallow breathing, and obstructive lesions cause slow, deeper breathing. Fibrosis produced by subchronic inhalation exposures to metal oxides of cadmium or vanadium produce tachypnea. 'Stress test' methods developed to enhance the sensitivity of these ventilatory end points in unanesthetized animals employ exercise-induced or carbon dioxide-induced hyperventilation. The latter approach, which has been used in guinea pigs and restrained rats, has been used to detect lung injury by several agents, including methyl isocyanate, sulfuric acid, quartz dust, cotton dust, and wood smoke.

Figure 4 depicts the physiologically defined lung volumes of humans and animals. Inhalation exposures that cause restrictive lung lesions, including exposure to silica, cadmium compounds, ozone, and diesel exhaust, produce decreases in total lung capacity and vital capacity. Obstructive lesions lead to breathing at higher lung inflation (due to gas trapping), with increased total lung capacity, residual volume, and functional residual capacity. Inhalation

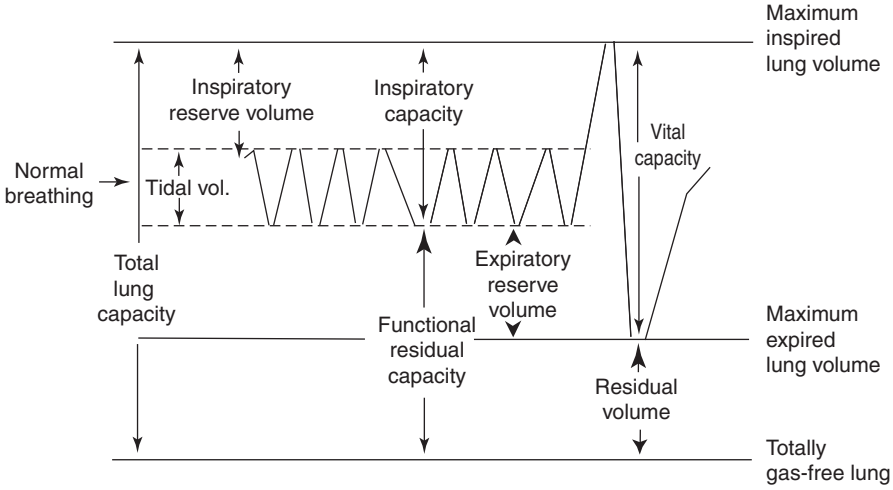


Figure 4 Capacities and lung volumes of the lung. (Adapted from McClellan RO and Henderson RF (eds.) (1989) *Concepts in Inhalation Toxicology*, p. 364. New York: Hemisphere, with permission.)

exposure to ozone and acrolein may produce this type of response.

Mechanical properties of the lung may be tested using static or dynamic testing modes. The static test can be conducted in living animals or using excised lungs and is based on information derived from the pressure–volume curve produced during lung deflation. Static compliance, a measure of lung elasticity derived from this curve, is decreased following exposure to fibrogenic agents, such as mineral dusts, or agents that cause edema, inflammation, and cellular hyperplasia, such as oxidants and irritants. It is increased following subchronic exposures to agents that cause emphysema-like lesions, such as ozone and nitrogen dioxide. Tests for dynamic lung mechanics require monitoring of flow, volume, and pressure and provide measures of total lung resistance, which is most dependent on large airway obstruction, and dynamic compliance, which is a measure of elasticity that is also sensitive to peripheral airway obstruction. Because of their sensitivity to bronchoconstrictors, guinea pigs are frequently used for evaluation of irritant effects, nonspecific airway hyperreactivity to bronchoconstrictors (e.g., histamine), and immunologically determined airway hypersensitivity. Many irritants that cause decreased dynamic compliance and/or lung resistance, such as ozone, sulfur dioxide, sulfuric acid, acrolein, and toluene diisocyanate, also cause nonspecific increased airway reactivity. Specific immunoglobulin-dependent sensitization to inhaled proteinaceous materials (e.g., ragweed pollen, animal dander, and grain dust) has been demonstrated in animals and humans using lung function measurements. Asthmatic responses with pulmonary sensitization to low-molecular-weight chemicals (haptens), such as isocyanates and anhydrides, have been observed in workers and have been modeled in guinea pigs using lung function tests. The role of the immune system in responses to many haptens remains in question since it has not been possible to consistently demonstrate the presence of an antigen-specific antibody.

The approach most commonly used to evaluate effects on distal airways in clinical and occupational medicine is the maximum forced expiratory maneuver, which allows measurement of airflows as a function of lung volume from total lung capacity to residual volume. Typically, the forced vital capacity (FVC) and the forced expiratory volume at 1 s (as a % of FVC) (FEV_1) are measured. Peak expiratory flow is a frequently used measure since simple portable devices permit self-evaluation by patients with obstructive disease. Decreased airflow rates are seen with emphysema, chronic bronchitis, and following acute exposures to bronchoconstricting irritants or agents that produce asthmatic responses following previous sensitization. When exercise is superimposed on certain pollutants, for example, with an exposure of humans to certain pollutants (e.g., ozone), decrements for FVC and FEV_1 have been observed at realistic air pollution levels (0.08–0.12 ppm). Animal models of forced expiratory maneuvers require the use of anesthetics and/or paralytic agents. The forced expiration produced in an apneic animal by applying a steady negative airway pressure following inflation to total lung capacity is measured. FVC, peak expiratory flow rate, mean mid-expiratory flow, and expiratory flow at 50%, 25%, and 10% of FVC may be derived from the maximum expiratory flow–volume curve. Using such methods, it has been demonstrated that the qualitative patterns of flow–volume curves are similar in humans and small laboratory animals when values are normalized for lung volume. As expected, decrements in airflow rates are seen in rats with elastase-induced emphysema. However, for demonstration of effects of more realistic exposures to toxicants and/or more subtle injury, the utility of measurements from forced expiratory tests in small animals may be limited.

Since the efficiency of the diffusion of O_2 and carbon dioxide at the alveoli is directly related to the primary function of the lung, tests of diffusing capacity are important components of a lung function testing battery. Diffusing capacity for carbon monoxide (DL_{CO}) is usually measured and normalized for lung volume. Diffusing capacity can be reduced as a result of structural changes in the alveolar region, as in thickening of the air–blood barrier (restrictive disease/fibrosis) or destruction of alveolar epithelium, or to an effective reduction in alveolar surface, as in obstructive disease. Thus, decrements in DL_{CO} may be difficult to interpret without correlation with other functional end points or histopathological evidence. In addition, rodents appear to compensate with hypertrophic or hyperplastic lung changes resulting in unexpectedly normal DL_{CO} values.

Pulmonary Carcinogenicity

Testing for the potential carcinogenic effects of airborne chemicals has received high priority in an effort to protect human health. Lung cancers are the most rapidly increasing cancers in Western Europe and North America. In the United States, cancers of the lung or bronchus were diagnosed in more than 170 000 people in 1993. Because of the long latency period associated with chemical carcinogenesis, it is

often difficult to identify, in the human population, the specific causative agent. Although there are a number of short-term tests for determining genotoxicity, these are generally used in screening and have not replaced the need for long-term animal testing. The basic premise of carcinogenesis research is that a substance that affects animal cells in such a way as to cause cancer is highly likely to affect human cells in the same way. Positive results from such testing are useful in that they demonstrate that a specific chemical is carcinogenic to animals under the conditions of the test. This information can be used as an indicator of the potential carcinogenic hazard to humans. Pulmonary cancers, like other forms, can be caused by both external factors (chemicals, radiation, viruses, and diet) or internal factors (hormones, inherited genes, and immune conditions) or the interaction of these factors. Evidence of pulmonary carcinogenicity in animals can be based on (1) an increase in the incidence of a specific tumor type, (2) the development of a specific tumor type earlier than seen in controls, (3) the presence of types of tumors normally not seen in control groups, and (4) an increase in multiplicity of tumors. In the absence of adequate human data, it appears reasonable and appropriate to predict that, with sufficient evidence of carcinogenicity in animals, the chemical presents a similar risk to humans. Nearly all known human carcinogens have been shown to be carcinogenic in some animal model. Recent reviews of chemical carcinogenesis, mechanisms of carcinogenesis, and pathophysiology of induced tumors provide current information on all aspects of carcinogenesis in each organ system.

Carcinogens can be divided into two general types: those that act directly and those that act indirectly. Direct-acting carcinogens are those that interact with cellular constituents such as protein, lipids, and nucleic acids. There are relatively few direct-acting carcinogens (e.g., bis(chloromethyl)ether, ethylene oxide, and nitrogen mustard).

The indirect-acting carcinogens are all substances that require metabolic activation before they interact with cellular macromolecules. These agents are often referred to as pro- or precarcinogens and include certain cyclic and polycyclic aromatic hydrocarbons (benzene and benzo(*a*)pyrene), aliphatic hydrocarbons (methylene chloride and pesticides), nitrosamines, and other chemicals such as urethane, formaldehyde, and acrylonitrile. Both the indirect- and direct-acting carcinogens can ultimately react with the genetic material of the cell. Such substances are referred to as genotoxic carcinogens, which differ from nongenotoxic or epigenetic carcinogens that do not appear to bind with the DNA of the cell but have other mechanisms of action. Agents included in this latter group include fibers, trichloracetic acid, and certain plasticizers. These chemicals do not damage DNA nor are they mutagenic in the standard short-term screening assays.

Examples of inorganic carcinogens include arsenic, asbestos, chromium, and nickel. The chemical form is important in determining the dose response. For example, beryllium sulfate is more carcinogenic than beryllium oxide. The difference may relate to the solubility of these compounds in the lung and the actual dose of the chemical to the target tissue. Although beryllium, lead, cadmium, and silica are carcinogenic in animals, the evidence in humans is less substantial. Epidemiological studies have implicated nickel as a carcinogen for cancer of the nasal cavity, lung, and possibly larynx. Carcinogenicity of chromium is associated with slightly soluble chromates (CR^{+6}), while the insoluble and very soluble salts of chromic acid or trivalent forms show little or no carcinogenicity. Mesotheliomas, tumors of the pleural lining of the lung and thoracic cavity, result from inhaled asbestos. Quartz and silica are generally not considered to be carcinogenic in humans, but some recent studies have demonstrated that these substances may cause lung cancer in rats. Multiple mechanisms may be involved in the carcinogenicity of asbestos. Asbestos is considered to be a cocarcinogen or tumor promoter. The hypothesis is that asbestos may cause generation of active oxygen species that lead to lipid peroxidation and DNA strand breakage. High levels of arsenic increase the risk of lung cancer. Trivalent arsenic appears to be the most active form and affects DNA synthesis and repair.

The induction of nasal carcinomas following inhalation exposure to several chemicals, including benzene, acetaldehyde, diallylnitrosamine, formaldehyde, hydrazine, and vinyl chloride has been reported. It is of interest that some chemicals, such as epichlorohydrin and bis(chloromethyl)ether, that produce nasal carcinomas in rats also produce lung cancer in humans. However, there is no epidemiological evidence indicating an increased incidence of nasal cancer in workers exposed to these industrial chemicals. Moreover, agents such as nitrosamines that are delivered by other routes of exposure can also induce tumors in the nose. For example, the consumption of salted fish having high concentrations of volatile nitrosamines may cause nasal tumors in experimental animals. Consumption of alcohol has also been associated with laryngeal cancers in humans.

Tumors of the nasal passages can vary from small papillomas and adenomas to large carcinomas that have the potential to metastasize to other parts of the body. While most of these neoplasms arise within the

epithelium of the nose, chemical carcinogens have also induced mesenchymal and neuroectodermal neoplasms. Nasal tumors are encountered most frequently. Laryngeal tumors are relatively rare but have been reported following exposure to smoke and acetaldehyde vapors.

Numerous chemicals have been identified as capable of causing pulmonary cancer in both animals and humans. The International Agency for Research on Cancer (IARC) states that there are adequate experimental inhalation studies in animals for several chemicals. **Table 4** lists chemicals that cause lung

Table 4 Carcinogenic agents associated with lung or pleural cancer in laboratory animals and humans

Agents causing lung tumors in animal models	*Agents associated with human lung cancer*
Organic chemicals	Industrial processes
Gases	Aluminum production
Benzene[a]	Coal gasification
Bis(chloromethyl)ether[a]	Coke production
Bromomethane (ethyl bromide)[a]	Hermatite mining, underground with exposure to radon
1,3-Butadiene[a]	Iron and steel founding
1,2-Dibromo-3-chloropropane	Painter, occupational exposure
1,2-Dibromoethane	Rubber industry
Dimethyl sulfate[a]	Chemicals for which exposure has been occupational
1,2-Epoxybutane	Asbestos[a]
Ethylene oxide[a]	Bis(chloromethyl)ether[a]
Formaldehyde[a]	Chromium compounds, hexavalent[a]
Methylene chloride	Coal tars[a]
3-Nitro-3-hexene	Coal tar pitches
1,2-Propylene oxide[a]	Mustard gas[a]
Tetrachloroethylene	Nickel and nickel compounds[a]
Tetranitromethane	Soots[a]
Urethan	Talc containing asbestiform fibers[a]
Vinyl chloride[a]	Vinyl chloride[a]
Particles	Environmental agents and cultural risk factors
Benzo(a)pyrene[a]	Erionite
Polyurethan dust	Radon and its decay products[a]
Inorganic compounds	Tobacco smoke[a]
Metallic	
Antimony compounds	
Beryllium compounds[a]	
Cadmium chloride[a]	
Chromium dioxide[a]	
Nickel compounds[a]	
Titanium compounds	
Nonmetallic	
Asbestos fibers[a]	
Zeolite fibers	
Ceramic aluminosilicate fibers	
Kelvar aramid fibers	
Silica[a]	
Oil shale dust[a]	
Quartz	
Volcanic ash	
Radionuclides	
α-emitting radionuclide particles	
β-emitting radionuclide particles	
Radon and its decay products[a]	
Complex mixture	
Cigarette smoke[a]	
Diesel engine exhaust	
Gasoline engine exhaust	
Coal tar aerosols[a]	
Artificial smog	

[a] Identified by IARC as chemical causing lung cancer in humans and respiratory cancers in animals.

Reproduced from Gardner DE, Crapo JD, and McClellan RO (eds.) (1993) *Toxicology of the Lung*. New York, NY: Raven Press.

neoplasia in laboratory animals and humans following inhalation. Studies investigating the carcinogenic potential of airborne chemicals usually focus on the morphological examination of tissue to determine the number of various types of tumors, the number of tumor-bearing animals, the number of tumors per animal, and the time of onset of the tumor. In such studies, few biochemical or physiological assessments are performed except for periodic hematological assays.

Inhalation of certain durable natural mineral fibers of amphibole asbestos, such as amosite and crocidolite, can lead to the development of inflammation, fibroproliferation, pulmonary neoplasms, and cancer of the serosal lining of the body cavities or mesothelioma. Administration of fibrous particles to laboratory animals has included, in addition to inhalation, intratracheal instillation and intracavitary implantation and instillation. Inhalation studies are difficult to conduct due to the problems associated with the generation and characterization of the fibers during all phases of the assay.

While many studies have been conducted using rats, there has been concern that the rat may not be an appropriate model for studying particulate-induced pulmonary tumorigenesis. One problem involves the finding that tumors can be induced under conditions of so-called 'pulmonary overload' even with 'inert' particles. These overload tumors may arise via mechanisms distinct from those normally associated with pulmonary carcinogenesis.

Another problem with inhalation studies conducted with rats is a lack of sensitivity for detecting the induction of fiber-inducted neoplasms, particularly mesotheliomas. Rats develop a low incidence of such tumors following exposure to amosite or crocidolite, which are known to cause tumors in humans.

In an effort to increase sensitivity, investigators have used other exposure methods including intratracheal, intrapleural, and intraperitoneal methods. There is significant concern over the induction of cancers by these nonphysiological exposure routes. Cancers induced by intracavity instillation may be due more to chronic inflammation and fibrosis from the 'bolus effect' rather than to the mechanisms of fiber-induced proliferative disease that normally occurs following the inhalation route of exposure. With intratracheal instillation, the distribution in the lung is not uniform and the resulting lesions differ from those reported in inhalation studies. It is generally agreed that long-term rodent inhalation studies provide the most definitive animal data for extrapolation to human assessment.

While many carcinogenicity studies on individual chemicals have been conducted, the study of complex mixtures presents a formidable scientific challenge for the toxicologist. One of the most difficult tasks is related to finding the primary causative agents of the effects. Examples of complex mixtures that have been studied for carcinogenicity include tobacco smoke and diesel engine emissions.

Cigarette smoke has been extensively studied due to its association with human lung cancer. However, it is not an impressive inducer of lung tumors in experimental animals. Lung tumors have been observed following long-term exposure using special strains of mice. Strains of mice such as A strain are known to have a high incidence ($\geqslant 70\%$) of spontaneous tumors. While there have been many studies of tobacco smoke using the laboratory rat, only one study showed an increase in lung tumors. Syrian hamsters exposed to whole smoke have developed laryngeal cancer but not lung tumors. This may be the result of an unusual increase in deposition of the inhaled smoke at this site in the hamster.

Several studies have shown that diesel exhaust is carcinogenic to the rat following long-term exposure. In these studies two basic types of tumors were found – bronchoalveolar tumors and squamous cell tumors – both arising from the alveolar parenchyma. Diesel exhaust represents complex mixtures of numerous organic and inorganic chemicals as well as various gases that may be toxic or carcinogenic. The complex interaction of organic hydrocarbons and carbon particles may be responsible for the tumors seen in these studies. The organic hydrocarbons present initiate the process and the particles, with adsorbed hydrocarbons, promote the initiated cells. A number of epidemiologic studies in London, the United States, and Canada did not detect significant health risk or indicated only a small increase in lung cancer incidence among workers exposed chronically to diesel exhaust. However, the animal studies together with supporting *in vitro* (e.g., mutagenic to bacteria and mammalian cells) data taken in aggregate led to the conclusion that diesel engine exhaust is a potential human carcinogen but probably represents a low level of risk.

While various types of lung cancer have been noted in humans, all known human pulmonary carcinogens are taken into the body by inhalation. It is equally important that inhaled chemicals can cause neoplasms at sites elsewhere in the body. For example, exposure to vinyl chloride, butadiene, acrylonitrile, and ethylene oxide by inhalation may produce a significant increase in cancer incidence in other organs (liver, brain, and blood) as well as in the

lung. Often, the neoplasms in other organs have a higher incidence and are a more serious health risk than the lung tumors.

Effects on Normal Pulmonary Defenses

The host defense system is one of the prime targets for which function is adversely affected by exposure to a wide range of environmental chemicals. During the air pollution episodes of this century (Meuse Valley, Belgium, London, and Donora, Pennsylvania) excess deaths were recorded from lower respiratory tract infections. The American Thoracic Society has published guidelines on what constitutes an adverse respiratory health effect. Among the five most important adverse respiratory effects are a greater incidence of lower respiratory infections. Because of the importance and the complexity of this system many *in vivo* and *in vitro* assay systems have been used to assess the integrity and biological activity of both the cellular and acellular components of the lung defenses. Any breach in these defenses should be considered as a possible indicator of an increased risk of pulmonary disease. This section is intended to familiarize the reader with the various defense system responses that have been studied, the measurements made, and the gaps in the information database. The host defense parameters which have been used most widely to examine the association between airborne toxicants and lung disease include mucociliary clearance dysfunction, functional and biochemical activity of the alveolar macrophages, immunological competency, and susceptibility to infectious disease. Increases in respiratory morbidity and impairment of lung clearance occur at ambient levels of air pollution and are associated with susceptibility to pathogenic microorganisms.

As discussed earlier, a major component of the respiratory defense system is the mucociliary clearance mechanism of the conducting airways. Mechanisms for clearance of deposited substances appear to be quite similar in most mammals, including humans. The effectiveness of this defense has been determined by measuring the rate of transport of deposited particles, the frequency of ciliary beating, the integrity of the ciliated cells, the physical–chemical properties of the mucus blanket, and the rate of mucus production and transport. Exposure to a variety of inhaled agents, such as formaldehyde, cigarette smoke, ozone, nitrogen dioxide, airborne PM, including trace metals (cadmium and nickel), and sulfuric acid, causes ciliary damage and dysfunction, such as slowing of the frequency of ciliary beating, resulting in a significant reduction in transport rates.

Impairment of alveolar macrophage function alters the ability of the cell and/or the lung to (1) maintain sterility within the gas exchange regions of the lung, (2) provide an effective clearance mechanism from the lung for inhaled particles and cellular debris phagocytized by these cells, (3) interact with lymphocytes, and (4) release immunologically active soluble mediators. To fully meet these functional responsibilities, these cells must maintain mobility, a high degree of phagocytic activity, an integrated membrane structure, and a well-developed functional enzyme system.

As the first line of defense, the resident macrophage must (1) isolate ingested particles by phagocytosis, (2) act as a vehicle for physical movement from the lung, and (3) inactivate or detoxify inhaled and ingested microbes or chemicals. The sequence of events that must take place for this defense system to function is complex and involves a number of intricate and interrelated biological functions. Chemicals can interfere with this function at many sites. Alterations in the ability of any of these functions could be expected to significantly increase the host's risk of pulmonary disease. A number of assays have been developed to identify functional changes in alveolar macrophages and have been used to demonstrate effects following exposure to agents such as carbon, diesel exhaust, PbO, nickel chloride, CO, Pb_2O_3, cigarette smoke, cotton dust, and quartz. These chemicals also promote the influx of new macrophages into the lung. These new cells may be derived from (1) an influx of interstitial macrophages, (2) proliferation of interstitial macrophages with subsequent migration of the progeny into the airspace, (3) migration of blood monocytes, or (4) division of free lung macrophages. While such an accumulation of macrophages may appear to be a necessary response to the immediate insult, a possible consequence of this mass recruitment may be the development of chronic pulmonary disease, as was discussed earlier.

Not just macrophages migrate into the lung during pulmonary insults. Polymorphonuclear leukocytes (PMN) also accumulate following exposure to such agents as diesel exhaust, ozone, nitrogen dioxide, iron oxide, cotton dust, cigarette smoke, HCl, and cadmium chloride. A large pool of PMN normally remains within the microvessels and few are found in the air spaces. However, following injury these migrate out of the vascular space. These cells migrate through the endothelium to the inflammatory site, where they attempt to phagocytose and destroy foreign material and may sometimes damage the host tissue in the process. While in the lung, powerful oxidants (oxygen radicals) and enzymes can be

released and produce tissue injury. For example, there is evidence that cigarette smoke can activate the PMNs and produce a shift in favor of proteolysis by the release of elastase from its lysosomal granules and by the generation of oxidants with the NADPH oxidase system and myeloperoxidase. Such a proteolytic imbalance may cause lung tissue destruction leading to emphysema.

Not all exposure to chemicals results in an increase in the number of available macrophages in the lung. Exposure to lead sequioxide, silica, asbestos, Pb_2O_3, cadmium fumes, MnO_2, Mn_3O_4, ozone, crysotile, amosite, cadmium oxide, acrolein, and nickel chloride actually causes a reduction in the number of these defense cells. These chemicals are cytotoxic and result in a lysis of the macrophage upon exposure. Some chemicals, such as fly ash, carbon monoxide, and certain trace metals, may affect cellular viability but not cause lysis. In some cases, the same chemical may, at different concentrations, elicit a variety of measurable and significant effects. For this reason, the most appropriate approach is to use a battery of functional assays. Parameters such as total number, stability, viability, morphology, phagocytic and bacterial function, and biochemical metabolism are useful measurements of total functional capacity of the macrophage.

The efficiency of the phagocytic and lytic system of the macrophage determines the sterility and health of the lung. Marked changes in phagocytic efficiency of these cells are found following exposure to nickel chloride, nitrogen dioxide, ozone, sulfur dioxide, CH_2O, cadmium chloride, and cigarette smoke. Depressed bactericidal function of macrophages has been reported following exposure to many of the previous chemicals and to H_2SO_4, ethanol, and lead chloride.

The activity of a number of macrophage enzymes (e.g., acid phosphatase, β-glucuronidase, β-*N*-acetylglucosaminidase, peroxidase, and lysozyme), which function to combat infectious disease, has been significantly depressed following exposure to a number of toxicants. Depression in the ability of the macrophage to produce interferon, a substance that is involved in host defense against viral infection, has been identified following exposure to ozone, irradiated auto exhaust, and nitrogen dioxide.

Alterations in the previous respiratory defenses would be expected to make the lung more vulnerable to infectious disease. Animal models have served to demonstrate the effects of airborne chemicals and to establish associations between these effects and actual increases in susceptibility to respiratory disease. These *in vivo* models combine the adverse effects of the toxicant with the added stress induced by an infectious microorganism to measure the effectiveness of the host defenses after exposure to the toxicant. The test animals, and in some cases humans, are challenged with a laboratory-induced respiratory infection (bacterial, viral, or mycoplasma) following exposure to the test chemical. If the host defense mechanisms are functioning normally, there is a rapid inactivation of inhaled organisms that have been deposited in the lung. However, if the pollutant exposure has caused a dysfunction(s) in these defenses, the microbes will proliferate rapidly and a measurable increase in pulmonary infection can be identified. While exposure to a test substance alone may not be life-threatening, association with other environmental stresses, such as infection, could prove critical in the promotion or exacerbation of a particular disease. In a recent study by New York University researchers of the effects of ambient PM on host resistance in aged rats, $PM_{2.5}$ exposure did not appear to increase the susceptibility to a post-PM exposure bacterial challenge. However, a $PM_{2.5}$ exposure of previously infected rats at levels close to the NAAQS of $65\,\mu g\,m^{-3}$ resulted in higher bacterial burdens and lower lavageable neutrophils (%) and inflammatory cytokine levels compared to filtered-air-exposed, infected controls. This may model ambient PM-induced increased mortality and morbidity in elderly humans and an association of PM with pre-existing pneumonia.

The lung is an active immunologic organ which, when exposed to toxicants, can exhibit specific local immunologic effects as well as play a role in systemic alterations, such as changes in circulating immunoglobulins. Wheezing, chest tightness, rhinitis, and asthma are symptoms of a sensitization response to a foreign material by the pulmonary immune system. Immunity can be defined as all of the physiological mechanisms that enable an individual's body to recognize materials as foreign and to neutralize, eliminate, or metabolize them without injury to its own tissue. Over the past decade, data have been accumulated to clearly substantiate cases in which lung immunoregulatory functions of humoral and/or cell-mediated immunity have been compromised by inhaled chemicals.

While the immune system is highly regulated by complex interactions, both between components of the system and between immune and nonimmune organ systems, xenobiotics can modulate the immune system effecting either 'up'- or 'down'-regulation of the process. Inhaled chemicals may provoke a variety of different responses, including (1) reduction of normal immune response – immunosuppression resulting in an increased incidence of

infection or tumors (e.g., benzene, malathion, lead, cadmium, nickel, and nitrogen dioxide); (2) overactivation of the immune system or exaggeration of the response causing hypersensitivity reactions (beryllium, mercaptans, chromates, diiocyanates); and (3) promotion of an autoimmune reaction, a pathological condition, in which there is a failure of the body to distinguish between 'self' and 'nonself' and resultant production of structural and/or functional damage to tissues and organs (e.g., mercury, cadmium, vinyl chloride, and methyl cholanthrene). Over 100 xenobiotics have been associated with such autoimmunological effects.

Inhaled substances exacerbate various immune-mediated disorders including asthma, hypersensitivity, pneumonitis, allergic rhinitis, and workers' pneumoconiosis. **Table 5** gives examples of the chemical agents that, when inhaled, are capable of eliciting an immunotoxic effect.

It is of interest that the same person may have an immediate-onset response on one occasion, a delayed-onset reaction on another, and, under other exposure conditions, exhibit a dual response starting with immediate-onset symptoms that resolve within an hour and followed several hours later by a second set of symptoms. The underlying mechanisms for such effects are not known. However, clinical and experimental evidence has indicated that this process is like many other toxicologic effects in that the response is related to concentration, duration, and frequency of exposure.

Table 5 Examples of immunotoxins

Halogenated aromatic hydrocarbons
Polychlorinated biphenyls
Polybrominated biphenyls
Dioxins
Pesticides
Organophosphates
Organochlorides
Carbamates
Polycyclic aromatic hydrocarbons
Benzo(*a*)pyrenes
Methylcholanthrene
Dimethylbenz(*a*)anthracene
Solvents
Benzene
Heavy metals
Beryllium
Manganese
Nickel
Cadmium
Platinum
Air pollutants
Ozone
Nitrogen dioxide
Cigarette smoke

Assessing the Risk of Airborne Chemicals

Risk assessment has been defined as the process whereby the most relevant biological, dose–response, and exposure data are used to identify and characterize risk. This information is used to produce a qualitative and/or quantitative estimate of the probable hazard to human health resulting from exposure to the airborne chemical(s). This process was significantly improved when the National Research Council evaluated the role of risk assessment as it relates to toxicology and developed uniform guidelines for federal agencies to use in assessing risk. They categorized the process into four steps: hazard identification, dose–response assessment, exposure assessment, and risk characterization. The process, summarized in **Figure 5**, is now widely used in evaluating the risk of inhaled chemicals. The important question in risk analysis is not simply what is the specific toxic response to some chemical, but rather what is the likelihood that the chemical may actually produce, under conditions of human exposure, significant health effects. There are multiple reasons for conducting risk assessment for airborne material. In addition to using the information for establishing federal, state, and local governmental regulations necessary to protect the worker or the general public, the risk assessment is also of value in identifying data gaps and planning future research. It also provides a useful integration of our existing knowledge database. Since risk analysis is being used to establish air standards by policymakers, the toxicologist must play a key role in this process. Because of the public health and economic implications of risk assessment, the US Congress has requested a survey and analysis of the risk literature and a federally funded program on health risk assessment.

While each assessment is unique, there are certain basic principles that are common to all. This review concentrates on those that are appropriate for airborne chemicals. In utilizing data for risk assessment, certain assumptions have to be made. For noncarcinogenic effects, it is assumed that adverse effects will not occur below a certain level of exposure, even if the exposure continues over a lifetime. This threshold assumption is supported by the fact that the toxicity of many chemicals, including airborne materials, is manifested only after the depletion of a physiological reserve and that the various host biological repair and defense capabilities can accommodate a certain degree of damage. In such cases, the objective of the toxicological risk assessment is to establish, with best scientific certainty, a threshold dose below which adverse health effects are not expected to occur. For carcinogenic effects, especially

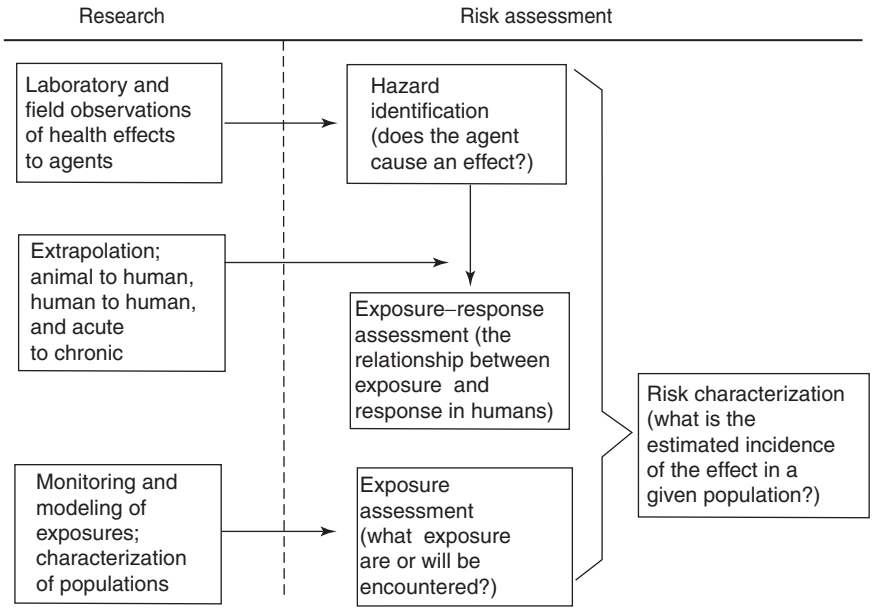


Figure 5 Summary of the process used in evaluating the risk of inhaled chemicals.

those considered to be due to genotoxic events (e.g., mutations), a threshold may not exist. Regulatory agencies consider that exposure to carcinogens pose a finite risk at all doses and that the probability of developing cancer increases with increased dose. The US EPA, in assessing the risk of carcinogens, assumes that the same total daily body burden will give the same tumor incidence, regardless of the route of exposure. This approach does not consider that some tumors at the site of contact (e.g., following inhalation) may be site specific or that the dose to a target organ may be modulated by the route of exposure. An important implication of this is that all levels of exposure, however small, add to the background risk and thus the experimental data are usually never adequate to exclude the possibility of added risk for the exposed population.

Effectively predicting the human health risk from exposure to airborne contaminants is complex and requires reliable data for hazard identification, an understanding of the dose–response relationships, and an analysis of the human exposure. Significant advances have been made in inhalation toxicology in accumulating useful data to support hazard identification and dose–response assessment. The development of sensitive analytical instrumentation permits the exposure to and monitoring of chemicals at increasingly lower concentrations. This is important not only in conducting laboratory studies but also is of value in developing techniques useful for assessing total human exposure of individuals or populations. Exposure assessment includes the identification of the contaminant, contaminant sources, environmental media of exposure, chemical and physical properties of the airborne substance, and the intensity and frequency of the exposure. Important improvements have been made in developing and validating reliable biomarkers of health effects and of exposure. Traditional analysis of body samples, such as urine, blood, exhaled air, and tissue, is useful for determination of dose. Detailed toxicokinetic studies in animals have provided information on the dose–response relationship. Physiology-based mathematical modeling of toxicokinetic parameters measured in animal studies has allowed easier interpretation and extrapolation of these animal data to human exposures.

The first step in risk assessment is to identify the potential deleterious effects of the substance. Frequently, risk assessment is conducted based on limited data. In cases in which the most relevant data are not available, attempts are often made to extrapolate from other toxicological information regardless of the route of administration or the concentration/dose used to produce a certain effect. Using such information may not ensure the protection of human health and may result in either overregulating or underregulating the risk of the chemical in question. The most reliable and scientifically defensible risk assessment analysis should be based on toxicological data collected under exposure conditions that are realistic and relevant to the human exposure; that is, by the same route of exposure, for similar duration, and in quantities that mimic the expected human exposure. All these factors are known to modulate the dose of the inhaled pollutant and/or its metabolites and hence the toxicological effects resulting

from the exposure. Currently, scientists are attempting to develop mathematical models that would permit appropriate extrapolation of high-dose studies to low-dose studies, short-term effects to lifetime health risk, and the ability to make appropriate route-to-route and species-to-species extrapolation from one compound to another and from *in vitro* to *in vivo*.

Developing reliable dose–response data from well-conducted inhalation studies is an essential step in the overall risk assessment process. With inhalation exposure, more so than with other routes of exposure, special attention needs to be paid to the difference between exposure and dose. In assessing health effects, exposure is often used as a surrogate for dose. However, when this is done important factors that may significantly modify the predicted effect (these factors include physical–chemical characteristics of the material, protective mechanisms, metabolism, and biological characteristics of the subject) are ignored. Defining the appropriate dose resulting from a certain exposure becomes a more difficult task when the exposure involves complex mixtures of airborne material such as automobile emissions, cigarette smoke, and atmospheric pollutants.

Being able to determine whether people exposed to airborne chemicals are at significant risk and the magnitude of the risk requires meaningful exposure assessment analysis. This analysis should include all exposures a person has to a specific contaminant, regardless of environmental medium (air, water, food, or soil) or the route of entry (inhalation, ingestion, or dermal contact). Exposure assessment can provide information on the distribution of the contaminant exposure within a population, the dose received, and routes of entry into the body. Exposure can be assessed using personal monitors near the breathing zone of the individuals. Passive samplers for air contaminants frequently use diffusion or permeation to concentrate the airborne material on a collecting medium, which is then returned to the laboratory for analysis. These samplers have been used for volatile organics, nicotine, formaldehyde, nitrogen dioxide, and carbon monoxide. Active samplers use small pumps to draw contaminated air through some collecting medium for analysis or through some form of direct-reading detector. When appropriate biomarkers are used in combination with the personal exposure data, an indication of internal dose can be estimated. For example, blood and urinary cotinine levels can be linked to air nicotine concentration and blood carboxyhemoglobin levels can be related to air carbon monoxide concentrations. The National Academy of Science has published an extensive discussion of the various approaches being used for assessing human exposure to airborne pollutants.

The final step, risk characterization, involves the integration and analysis of the existing database to provide a numerical estimate of the incidence of the adverse effect in a given population, assuming specific conditions of exposure.

The existing methods available for scientifically defensible risk characterization are not yet ideal since each step has an associated uncertainty resulting from data limitation and incomplete knowledge on exact mechanism of action of the toxic chemical on the human body. For noncancer end points, safety factors or uncertainty factors are applied since these effects are assumed to have a threshold below which no adverse effect is expected to be observed. US EPA has used the concept of a reference concentration (RfC) to estimate acceptable daily human exposure from HAPs. The RfC was adapted for inhalation studies based on a reference dose (RfD) method previously used for oral exposure assessment. The derivation of the RfC differs from that for the RfD in the use of dosimetric adjustment to extrapolate the exposure concentration for animals to a human equivalent concentration. Both are estimates, with uncertainty spanning perhaps an order of magnitude, of a daily exposure to the human population, including sensitive subgroups, which would be without appreciable risk of deleterious effects over a lifetime.

The RfC is estimated based on available knowledge of the toxic response of both humans and animals. Appropriate uncertainty factors (UF) and modifying factors (MF) are incorporated into the equation:

$$\mathrm{RfC} = \mathrm{NOAEL}/(\mathrm{UF}) \times (\mathrm{MF})$$

The NOAEL is the no-observed-adverse-effect level. **Table 6** indicates how these factors are used in deriving appropriate risk characterization.

For carcinogens, risk is estimated based on human and experimental animal data and other supporting evidence of carcinogenicity (e.g., structure–activity correlations, kinetics, and *in vitro* data). Decisions on the carcinogenicity of chemicals in humans need to be based on considerations of all relevant data, whether they are indicative of a positive or negative response, and should embody sound biological and statistical principles.

However, because animal carcinogens are not the same with respect to potency, target organs, mechanism, and so forth, and thus are not equally relevant to humans, hazard evaluation is on a weight-of-evidence basis. The weight-of-evidence evaluation of carcinogenic hazard to humans provides a basis for

Table 6 Application of uncertainty factors in deriving RfC

Type	*Magnitude*	*Purpose*
Interindividual	10	Intended to account for the variation in sensitivity among the human population
Interspecies	10	Used to account for uncertainty in extrapolating results from animals to average human population
Subchronic to chronic	5–10	Used to account for uncertainty in extrapolating less than chronic exposure results on animals or humans when no long-term human data are available
LOAEL to NOAEL	5–10	Accounts for the uncertainty inherent in extrapolation downward from LOAEL to a NOAEL
Incomplete to complete data	10	Used when experimental data are incomplete. This factor is intended to account for the inability of any single study to adequately address all possible adverse effects in humans. Depends on scientific judgment of the uncertainties of the study and data base

carcinogen classification and potency estimation. These assessments involve fitting mathematical models to experimental data and extrapolating from these models to predict risk at doses well below the experimental range. A range of risks can be produced using different models and assumptions about dose–response curves and the susceptibility of humans and animals to the test agent. Both IARC and US EPA have established a weight-of-evidence classification for carcinogens. They are similar but the IARC method does not address the potency of carcinogens, whereas the US EPA approach offers a means for developing quantitative estimate of carcinogen potency.

Space Flight and Respiratory Toxicology

Being able to predict the human health risk from exposure to airborne chemicals can be complex, requiring reliable analysis of human exposure. While the basic principles of risk assessment are applicable to various conditions of exposure, characterizing how an individual's health status can significantly influence the threshold for effects can be a most challenging component of the risk assessment process. One needs to consider the overall scientific weight-of-evidence to predict whether or not an individual may be uniquely susceptible to certain exposures in a specific exposure environment. This can be illustrated by examining the many factors facing individuals who have the responsibility for establishing safe levels of exposure necessary for ensuring the health and welfare of astronauts during space travel. The successful exploration of space not only depends on a high degree of excellence in engineering technology but also on maintaining a healthful environment for the space explorers. More than 400 air contaminants have been identified and their concentrations measured in the spacecraft atmosphere. Atmospheric chemicals detected during space missions include both gaseous (alcohols, aldehydes, aromatic hydrocarbons, ketenes, organic nitrogens, ammonia, carbon monoxide, etc.) and PM including microbes, cabin materials that have become airborne, ultra-fine particles, and pyrolysis products from small fires that are known to occur during flight.

Using the available toxicological database developed for exposure on earth may not be appropriate for assessing health risk in the unique living environment of outer space.

Several decades of human space flight have shown numerous changes in the health status of astronauts during and following space travel. As a consequence of the altered physiological status caused by the body's adaptation to microgravity, confinement, and stress, the human's response to airborne contaminants may be quite different from what has been learned from earth-based studies. Changes that occur from being in weightlessness include alterations in normal functioning of the human respiratory, cardiovascular, muscoskeletal, neurophysiological, renal, endocrine, hematological, and immune systems. Alterations in pulmonary function include changes in gas exchange, regional differences in blood flow, ventilation, diffusion capacity, residual volume, and intrapleural pressure.

Although the mechanisms of space flight-induced changes are not well understood, such changes during space flight can undoubtedly alter the normal physiological response to inhaled contaminants. In assessing potential health risks from being in such a space environment the toxicologist needs to consider: (1) microgravity does not permit the settling of airborne particles as experienced on Earth, and (2) physiological adaptations that occur from being in a weightless environment, can make a significant difference in deposition, retention and removal of inhaled particles which can influence the respiratory system response to inhaled chemicals.

See also: Absorption; Ames Test; Animal Models; Biomarkers, Human Health; Carcinogenesis; Clean Air Act; Combustion Toxicology; Donora: Air Pollution Episode; Dose–Response Relationship; Emergency Response and

Preparedness; International Agency for Research on Cancer; Mouse Lymphoma Assay; Occupational Toxicology; Pharmacokinetics/Toxicokinetics; Photochemical Oxidants; Pollution, Air; Pollution, Air Indoor; Polycyclic Aromatic Hydrocarbons (PAHs); Radiation Toxicology, Ionizing and Nonionizing; Risk Assessment, Human Health; Risk Characterization; Sick Building Syndrome; Silent Spring; Tissue Repair; Toxicity Testing, Inhalation.

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Retino *See* Vitamin A.

Rhodium

Shayne C Gad

- Representative Chemicals: Rhodium chloride ($RhCl_3$); Rhodium carbonyl chloride ($C_4Cl_2O_4Rh_2$)
- Chemical Abstracts Service Registry Number: CAS 7440-16-6
- Chemical/Pharmaceutical/Other Class: Platinum group metals; Precious metals; Transition metals
- Chemical Formula: Rh^{3+}

Uses

Rhodium is used as an alloy with platinum, and as a catalyst. It is also used as a corrosion-resistant electroplate for protecting silverware from tarnishing, for making high-reflectivity mirrors for cinema projectors, and searchlights. It can be used as a catalyst for chemical reactions, and in jewelry. In fact, rhodium is a very common plating for inexpensive jewelry because it is extremely shiny and tarnish resistant. It is actually a very expensive metal; however, only need a microscopically thin layer is needed.

Background Information

Rhodium is one of the platinum group elements, and is found at very low concentrations in the Earth's crust. Rhodium was discovered by William Hyde Wollaston (England) in 1804. The origin of the name comes from the Greek word *rhodon* meaning rose. The plated solid is very corrosion resistant and exceptionally hard. It is inert in air and acids. However, it can produce a violent reaction to chlorine, bromine pentafluoride, bromine trifluoride, and fluorine monoxide.

Exposure Routes and Pathways

The common routes of exposure are by inhalation and ingestion.

Acute and Short-Term Toxicity (or Exposure)

Animal

Oral toxicity studies in rats showed that rhodium trichloride, sodium chlororhodite, and chloropentamine rhodium chloride(III) are of low systemic toxicity.

Human

No toxic effects of rhodium have been reported from observations of human beings. Workers wearing rings coated with rhodium showed negative rhodium patch tests while all other metals in the rings gave positive results. It has been reported that inhalation of excessive amounts of fine rhodium metal powder or dust may cause irritation of the respiratory system, and that eye contact with fine powder or dust may cause irritation (mechanical irritation).

Chronic Toxicity (or Exposure)

Animal

Rhodium's carcinogenic potential has not been fully established.

Human

Rhodium is listed as A4 (not classifiable as a human carcinogen) according to the American Conference of Governmental Industrial Hygienists (ACGIH). A potential symptom of overexposure to metal fumes and insoluble compounds is respiratory sensitization. A variety of rhodium compounds have been tested against various types of tumors and have been shown to have antitumor activities. However, the toxic effects of most of the compounds studied have prevented detailed examination (e.g., clinical trials), and their mechanism of action has not been studied systematically. Recent structural studies suggest that the antitumor activity of the dirhodium(II) carboxylates may be similar to that of cisplatin, that is, by binding to adjacent guanines on DNA.

In Vitro Toxicity Data

Water-soluble complex salts of rhodium have been shown to have mutagenic potential in the *Salmonella typhimurium*/microsome test system (Ames test).

Exposure Standards and Guidelines

The ACGIH threshold limit value, 8 h time-weighted average (TWA) is $1.0\,mg\,m^{-3}$ (as the metal and insoluble compounds), and the US National Institute for Occupational Safety and Health (NIOSH) recommended exposure level, averaged over a 10 h work day is $0.1\,mg\,m^{-3}$. NIOSH's immediately dangerous to life or health value is $100.0\,mg\,m^{-3}$ (as the metal fume and insoluble compounds).

See also: Platinum.

Further Reading

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Rhododendron Genus

Alexander B Baer and Christopher P Holstege

- Representative Chemicals: Azalea; *Rhododendron*
- Synonyms: *Rhododendron catabiense*, Ericaceae (heath) family; *Catawba rhododendron*; Mountain rosebay; Purple laurel; Rhodora; Rosa Laurel; Rosebay

Exposure Routes and Pathway

Exposure is through ingestion of leaves, flowers, nectar, tea brewed from the leaves, or honey produced exclusively from the nectar.

Mechanism of Toxicity

Rhododendrons contain grayanotoxins that bind to cell membrane sodium channels and increase sodium conduction. Nerve and muscle cells are subsequently kept in a state of depolarization.

Acute and Short-Term Toxicity (or Exposure)

Animal

There are several cases of animal poisonings from Rhododendrons. Goats that ingested branches of an azalea plant presented with profuse vomiting, central nervous system depression, and fasciculations. Other animals with reported poisoning include donkeys, dogs, and kangaroos.

Human

The entire plant is potentially toxic to humans. Onset of action typically occurs within 2 h of ingestion with complete resolution by 24 h. Most exposures result in no toxicity. Grayanotoxin poisoning may result from consumption of honey acquired from a hive where

the bees obtained nectar primarily from rhododendron. Commercial vendors mix honey from several different hives thus ensuring dilution of any potential grayanotoxin contaminated honey. Consumption of the plant, tea, or contaminated honey may cause burning of the mouth followed by salivation, vomiting, abdominal pain, diarrhea, ataxia, and weakness. Hypotension, bradycardia, progressive paralysis of the limbs, and seizures have also been reported.

Clinical Management

Minimal ingestions usually do not require treatment. Activated charcoal may be considered in substantial, recent ingestions. The care of the rhododendron-poisoned patient is largely supportive. Symptomatic bradycardia and hypotension should be treated with atropine and intravenous fluids, respectively. When hypotension is refractory, vasopressors should be considered. Complete heart blocks may require cardiac pacing. Seizures should be treated with benzodiazepines.

See also: Plants, Poisonous.

Further Reading

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Rhubarb

Ann P Slattery

- SYNONYMS: *Rheum rhabarbarium*; *Rheum rhapontieum*; Garden rhubarb; Pie plant; Wine plant (the herbal rhubarb is *Rheum officinale*)

Uses

Rhubarb is a perennial plant with stalks that grow 1–3 ft in length and become reddened when ripe. The leaves are large and wrinkled with wavy margins. Rhubarb is cultivated as a food source and for medicinal purposes in many parts of the world.

Exposure Routes and Pathways

The routes of exposure are ingestion and dermal contact.

Toxicokinetics

Oxalate absorption varies greatly with an oral absorption range of 1–22%. Oxalates are excreted unchanged in the urine within 24–36 h.

Mechanism of Toxicity

Soluble oxalates in the leaves are absorbed via the gastrointestinal tract. Once absorbed, oxalates bind with calcium producing secondary hypocalcemia. Once bound to calcium, oxalate salts become insoluble and may precipitate in the renal system resulting in kidney malfunction and electrolyte imbalance. Renal damage may be due to vascular stasis.

Acute and Short-Term Toxicity (or Exposure)

Animal

Oxalate-containing plants can be a source of poisoning for grazing animals. In ruminants, a large acute exposure results in hypocalcemia and death. In chronic exposures, renal damage and urolithiasis from calcium oxalate deposition result. Other animals display gastrointestinal symptoms and renal damage. Treatment includes activated charcoal and intravenous electrolyte and calcium treatment. With rest animals become ambulatory after 1 than 8 h of treatment. Notable gastrointestinal tract mucosal edema and hemorrhage, abdominal ascites, and hyperemia have occurred.

Human

Rhubarb stalks are edible. Soluble oxalates are primarily found in the leaf blades and in much lower concentrations in the stalk. Anthraquinone glycosides are present in lesser amounts in rhubarb grown in the United States; therefore, exposures do not result in the expected cathartic effects as seen with *Rheum officinale*. Acute fatal poisonings due to ingestion of leaves are rare. Large amounts of the leaf must be ingested before symptoms develop. With ingestion of large amounts, symptoms include

abdominal pain, nausea, vomiting, weakness and drowsiness, seizures, possible liver damage, and kidney damage. Because digestion of the plant is slow, effects may be delayed several days. The ingestion of stalks and small leaf exposures are unlikely to cause serious problems.

Chronic Toxicity (or Exposure)

Animal

Animals may develop subacute toxicity if enough plant material is ingested to produce hypocalcemia and kidney damage, but no so much that the animal dies. With larger or more prolonged exposures, animals may experience larger deposits of calcium oxalate crystals that result in renal fibrosis or renal failure which can ultimately lead to death.

Human

Rhubarb is used therapeutically in many parts of the world as a stimulant laxative as well as a homeopathic remedy. Chronic stimulant laxative use may result in structural changes to the colon.

Clinical Management

For patients who present soon after substantial ingestion, treatment with activated charcoal may be useful. Low calcium levels and tetany should be treated with intravenous calcium gluconate. Serum blood urea nitrogen and creatinine should be measured, and urine should be checked for the presence of oxalates. The patient should be hydrated with fluids and electrolytes as needed. Hemodialysis may be indicated if anuria develops.

See also: Plants, Poisonous; Oxalates.

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Riboflavin

Diana Ku

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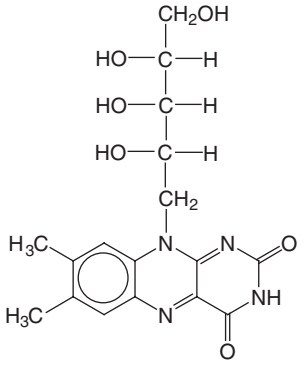
- Chemical Abstracts Service Registry Number: CAS 83-88-5
- Synonyms: Vitamin B_2; Beflavine; Flavaxin; Flaxain; Lactoflavin; Isoalloxazine; 7,8-dimethyl-10-(D-ribo-2,3,4,5-tetrahydroxyphentyl)
- Chemical/Pharmaceutical/Other Class: Water-soluble vitamin
- Chemical Formula: $C_{17}H_2ON_4O_6$
- Chemical Structure:



Uses

Riboflavin is a nutritional supplement used during periods of deficiency known as ariboflavinosis. Riboflavin deficiency usually occurs in association with malabsorption, alcoholism, or protein-calorie deficiency, and is rarely the sole vitamin deficiency. Riboflavin needs are increased during chronic debilitative stress to the body such as malabsorption diseases of the small intestine, liver disease, hyperthyroidism, alcoholism, and during pregnancy and lactation. Neonates undergoing phototherapy for hyperbilirubinema also have increased nutritional needs.

Exposure Routes and Pathways

The route of exposure is oral. Dietary sources of riboflavin include broccoli, spinach, asparagus, enriched flour, yeast, eggs, milk, cheese, mackerel, trout, poultry, liver, and kidneys.

Toxicokinetics

Riboflavin is readily absorbed from the gastrointestinal tract mainly in the duodenum. It is hepatically metabolized, moderately protein bound, and widely

distributed to tissue; however, little is stored in the liver, spleen, heart, and kidneys. Riboflavin is excreted renally almost entirely as metabolites. All riboflavin in excess of daily body needs is excreted unchanged in the urine. Riboflavin exhibits biphasic pharmacokinetics with an initial half-life of 1.4 h and a terminal half-life of 14 h.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute toxicity is not expected.

Human

Acute toxicity is unlikely following even 100 times the recommended daily allowance. There are no reports of acute toxicity from exposures to riboflavin.

Chronic Toxicity (or Exposure)

Animal

It would be unlikely for animals to be given chronic riboflavin overdoses.

Human

Chronic exposure to large doses of riboflavin may cause a bright yellow discoloration of the urine.

In Vitro Toxicity Data

There are no reports of congenital anomalies among children born to mothers who used large doses of pyridoxine during pregnancy.

Clinical Management

In cases of chronic excessive use, the patient should be instructed to discontinue the supplement.

See also: Vitamin A; Vitamin D; Vitamin E.

Further Reading

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Ricin and Other Toxalbumins

Mark A Hostetler

- Representative Chemicals: Ricin; Other toxalbumins with similar ricin-like properties: *Abrus precatorius* (jequirty pea, rosary pea), *Trichosanthes* spp. (Chinese cucumber), *Robinia pseudoacacia* (black locust), *Phoradendron* spp. (American mistletoe), *Viscum* spp. (European mistletoe), and *Wisteria* spp. (wisteria)
- Chemical Abstracts Service Registry Number: CAS 9009-86-3
- Synonym: *Ricinus communis* (castor bean)
- Chemical/Pharmaceutical/Other Class: Type 2 ribosome inactivating proteins
- Chemical Structure: Ricin is a heterodimeric ribosome inhibiting protein consisting of an A-chain (RTA), linked by a disulfide bond to the B-chain (RTB). The total molecular weight is 66 000 Da, with the A-chain contributing 32 kDa and the B-chain 34 kDa. The A-chain is a globular protein composed of 267 amino acids containing eight alpha helices and eight beta sheets, with the toxin's substrate binding site located within the cleft. The B-chain is the binding lectin composed of 262 amino acids, shaped like a barbell, and has a binding site specific for membrane sugars at each end (galactose and *N*-acetyl galactosamine)

Uses

Ricin is the most well known, and ubiquitous, of the toxalbumins. Castor beans are used in the production of castor oil, a major constituent in lubricants, brake, and hydraulic fluids. During the production process, 5–10% of the aqueous phase, also known as 'waste mash', is recoverable as ricin. In addition, castor beans and jequirty peas are also used extensively throughout Mexico and Central America for ornamental purposes in items such as necklaces, prayer or rosary beads, and the rattles in musical shakers (maracas).

Exposure Routes and Pathways

Once produced, ricin remains stable in powder, aerosol, solid pellet, or liquid form. Ricin may be

dissolved in water or weak acid, and remains stable at even the most extremes of temperatures. It is believed to be one of the most toxic naturally occurring substances in the world. The toxins are present in all parts of the plant but are most concentrated in the beans or seeds. The beans are covered by a hard, relatively impervious outer shell that must be chewed or broken in some way in order for the toxalbumin to be released.

Possible routes of exposure include cutaneous, mucosal, gastrointestinal, inhalation, and parenteral (intravenous or intramuscular). Gastrointestinal exposures are usually accidental and occur most commonly when castor (ricin) or jequirty (abrin) beans are chewed or swallowed. Cutaneous exposures are limited primarily to castor beans, which are unusually allergenic and may cause severe cutaneous hypersensitivity and systemic allergic reactions. Inhalation and parenteral exposures are generally limited to intentional, usually malicious, exposures.

Toxicokinetics

Although acute hypersensitivity and allergic reactions can be triggered by casual dermal contact, cutaneous absorption of the toxin through intact skin is negligible. Ricin powder is extremely irritating to the eyes and may result in severe inflammation and hemorrhagic conjunctivitis; however, very little is absorbed systemically. Ricin is inefficiently absorbed via the gastrointestinal tract ($LD_{50} = 30\,\mu g\,kg^{-1}$). Absorption via inhalation is much more efficient ($LD_{50} = 3\,\mu g\,kg^{-1}$). The overall lethality of an inhalational exposure is directly related to the particle size of the aerosol carrying the toxin. The smaller the particle, the higher the lethality. The highest potential lethality is seen with direct parenteral exposures ($LD_{50} = 1\,\mu g\,kg^{-1}$). Ricin does not undergo any significant hepatic or renal metabolism.

Mechanism of Toxicity

Ricin contains the two basic components necessary for it to enter cells and inhibit protein synthesis. The ligand portions of the B-chain act to bind to galactose moieties of the cell membrane and facilitate endocytosis of the entire ricin molecule into the cell where it is transported via endosomes to the Golgi apparatus and endoplasmic reticulum. Once there, the A-chain is translocated into the cytosol where *N*-glycosidase modifies a base (A_{4324}) in an exposed loop of the 28S rRNA fragment of the 60S RNA chain. Requiring no energy or cofactors, it catalytically and irreversibly inactivates the 60S ribosomal subunit halting all further protein synthesis, thereby causing severe cytotoxic effects on multiple organ systems.

Acute and Short-Term Toxicity (or Exposure)

Animal

Ricin has been used with a variety of different lectins and different specificities to map cellular patterns of glycosylation and intracellular transport in animals. Studies have confirmed the importance of *N*-acetyl galactosamine residues on the cell surface. In studies mapping the effects of ricin, ultrastructural analysis reveals that ricin binds to galactose on the cell membrane, is endocytosed, and then sequentially causes the dispersion of polyribosomes as the rough endoplasmic reticulum disorganizes into smooth vesicles. Finally, the cell bodies (perikaryon) swell, the nuclei degenerate, and the entire cell disintegrates.

Human

Although fatalities have been reported following ingestion of chewed castor beans, severe toxicity secondary to accidental exposure is rare. According to the American Association of Poison Control Centers, of the reported toxalbumin cases with known outcomes, 65% have no symptoms, and 31% have only minor symptoms. Although chewing and swallowing one bean may produce symptoms in an adult, swallowing an intact bean without chewing is unlikely to cause any serious sequelae.

Toxic effects of ricin have latent periods ranging from 2 to 24 h. Symptoms include delayed gastroenteritis, which may be severe and hemorrhagic, followed by delirium, seizures, coma, and death. Toxic effects include intestinal hemorrhage, diffuse nephritis, hepatic necrosis, and on a cellular level pyknosis of the nuclei and karyorrhexis. Patients may experience hypoglycemia, severe dehydration, and shock. Severe exposures may also result in the development of pulmonary edema, respiratory failure, and death within 36–72 h. If death has not occurred in 3–5 days, the victim usually recovers.

Clinical Management

Clinical management begins with an assessment of the most important features associated with toxic environmental exposures: identification of the substance; time, type, and duration of exposure; symptoms; treatment thus far; associated injuries; and preexisting conditions. Chief among these is to determine if any of the beans have been chewed or

swallowed. All exposures should be reported to the regional poison control center. Decontamination is essential to minimize the risk of further harm to the patient, and to reduce the risk of secondary contamination to others. Decontamination begins by removing all clothing and washing the patient's entire dermal surface with copious amounts of soap and water. An alternative is to use a dilute bleach mixture with a contact time of 15 min (0.5% sodium hypochlorite solution – approximately one part bleach to nine parts water).

Treatment options are largely supportive. An assessment should first be made for airway patency and adequacy of breathing. Circulation may become affected as shock develops secondary to severe gastroenteritis. The following laboratory studies are recommended for all symptomatic patients: computerized blood count, electrolytes, and coagulation studies (prothrobin time, activated partial thromboplastin time). In cases of uncertain or unknown exposure, there is an enzyme-linked immunosorption assay test available for the detection and verification of the presence of ricin

Patients may develop severe cutaneous hypersensivity or systemic allergic reactions. Signs may include the development of an urticarial hive-type reaction, facial or tongue swelling, bronchospasm, and acute upper airway obstruction. Treatment includes antihistamines, corticosteroids, and, if necessary, epinephrine.

Any further definitive treatment options are limited. Neither induced vomiting with syrup of ipecac nor gastric lavage is believed to be beneficial, or is recommended. Administration of activated charcoal has been suggested as a possible treatment to absorb toxin; however, the potential benefit (if any) remains unproven. Whole bowel irrigation has also been suggested as a possible treatment option to ensure rapid and complete decontamination of the gastrointestinal tract; however, the potential benefit (if any) remains unproven. There are no antidotes, and the toxins are not dialyzable. The mainstay of treatment remains, therefore, largely supportive with attention to fluid, glucose, and electrolyte replacement. Symptomatic patients should be admitted to the hospital; asymptomatic patients may be discharged safely after observation for at least 4–6 h.

Other Hazards – Ricin as a Biological Weapon

As one of the most toxic and easily produced toxins available, ricin was initially investigated as a biological weapon by the US military in World War I. Although it has never been used in battle, it has been used successfully in several small-scale killings, the most notorious of which includes the very well known assassination of a Bulgarian defector in 1978 (Georgi Markov). Although it is easy and inexpensive to produce, highly toxic, and stable in a variety of conditions, the amount required to cause a high level of lethality on an entire population is probably too massive so as to make it truly practical as a weapon of mass destruction. Its potential as a major cause for morbidity and mortality as an aerosolized agent, however, especially in enclosed environments, should not be underestimated. It could also easily be used as a food or water contaminant on a relatively large scale such that it could easily incapacitate or overwhelm an area's healthcare resources by nature of the amount of illness it would produce.

See also: Castor Bean; Plants, Poisonous; Wisteria.

Further Reading

Bradberry SM, Dickers KJ, Rice P, Griffiths GD, and Vale JA (2003) Ricin poisoning. *Toxicology Reviews* 22: 65–70.

Fernando C (2001) Poisoning due to *Abrus precatorius* (jequirity bean). *Anaesthesia* 56: 1178–1180.

Kortepeter MG and Parker GW (1999) Potential biological weapons threats. *Emerging Infectious Diseases* 5: 523–527.

Lord MJ, Jolliffe JA, Marsden CJ, *et al.* (2003) Ricin: mechanisms of cytotoxicity. *Toxicology Reviews* 22: 53–64.

Roy CJ, Hale M, Hartings JM, Pitt L, and Duniho S (2003) Impact of inhalation exposure modality and particle size on the respiratory deposition of ricin in BALB/c mice. *Inhalation Toxicology* 15: 619–638.

Rifampin

Christopher P Holstege

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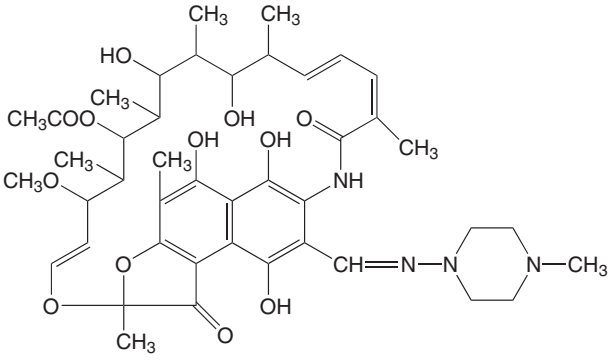
- Chemical Abstracts Service Registry Number: CAS 13292-46-1
- Synonym: Rifampicin
- Chemical/Pharmaceutical/Other Class: Antibiotic
- Chemical Structure:



Uses

Rifampin is used as an antibiotic. It is a semisynthetic derivative of rifamycin B, a macrocyclic antibiotic produced by the mold *Streptomyces mediterranei*.

Exposure Routes and Pathways

Ingestion is the most common route of exposure. Rifampin is available in oral and parenteral forms.

Toxicokinetics

Rifampin is rapidly and nearly completely absorbed from the gastrointestinal tract. Peak serum levels are seen within 2–4 h. Food, antacids, ketoconazole, and aminosalicylic acid interfere with absorption and delay peak levels. If these agents are used concurrently, they should be administered separately at an interval of at least 8 h. Massive ingestions in the overdose setting may delay absorption. Protein binding is 75–90%. The volume of distribution is $\sim 1\,l\,kg^{-1}$. Rifampin undergoes hepatic deacetylation to an active metabolite. Both rifampin and its deacetylated metabolite are excreted into the bile. Rifampin, and to a lesser extent its deacetylated metabolite, undergo enterohepatic recirculation. The half-life of therapeutic doses of rifampin is 1.5–5 h. The half-life is shortened after regular use due to induction of hepatic enzymes. Chronic liver disease increases the half-life. The kinetics is not well described in the overdose setting. In one case, the half-life was 4.4 h.

Mechanism of Toxicity

In the acute overdose setting, the mechanism of toxicity is not defined. A number of the toxic reactions occurring with intermittent dosing schedules or on reexposure are postulated to be due to the presence of antirifampin antibodies.

Acute and Short-Term Toxicity (or Exposure)

Human

Intentional overdoses of rifampin rarely lead to significant morbidity and fatalities are exceedingly uncommon. The few deaths that have been associated with rifampin have all been in individuals with a history of alcoholism or concomitant ethanol ingestion. Acute overdose with rifampin may cause a red to orange discoloration of the skin seen within 2 h of exposure, 'the red man syndrome'. Body fluids are also discolored and urine, feces, sweat, tears, and saliva may exhibit a red to orange discoloration. Symptoms associated with rifampin overdose include headache, abdominal pain, nausea, vomiting, and flushing. Pruritus, which may be limited to the scalp, may be seen, and a cutaneous burning sensation maybe noted. Lethargy and obtundation have been reported. Facial or periorbital edema may be seen. Minor and transient elevations of hepatic transaminases, bilirubin, and amylase have been reported. Rifampin may inhibit bilirubin excretion and may interfere with the bilirubin assay. An acute ingestion of 60 g was fatal in an alcoholic. Overdoses of 12 g in otherwise healthy individuals have been tolerated, as has 2 g in an 18-month-old. Because of the small number of cases, correlation of dose with severity is not possible, and serum levels are not useful.

Chronic Toxicity (or Exposure)

Animal

Offspring of rodents dosed at 150–250 $mg\,kg^{-1}\,day^{-1}$ during pregnancy have had greater than expected findings of cleft palate and spina bifida.

Human

Rifampin used daily at therapeutic doses is associated with facial flushing and itching in less than 5% of patients. More rarely, hepatotoxicity is seen and may lead to complete hepatic failure requiring liver transplantation. The risk of hepatotoxicity is increased with chronic liver disease, alcoholism, and old age. Acute renal failure, interstitial nephritis, nephrogenic diabetes insipidus, and thrombocytopenic purpura are rare complications of continuous daily use. The use of rifampin on an intermittent dosing schedule, two or three times weekly or less, is associated with a higher incidence of toxic side effects. These include a flu-like syndrome with that lasts up to 8 h following each dose of rifampin. More serious toxic effects associated with an intermittent dosing schedule include hemolytic anemia, thrombocytopenia, hepatitis, nephritis, acute renal failure, and shock. These reactions are believed to be hypersensitivity reactions and related to antirifampin antibodies. Rifampin is also a potent inducer of hepatic microsomal enzymes. Its administration may result in decreasing the half-life of numerous compounds.

In Vitro Toxicity Data

Mutagenicity studies using *Drosophila* have been inconclusive.

Clinical Management

Acute overdoses of rifampin are rarely serious. Supportive care, gastric decontamination with activated charcoal for substantial recent ingestions are all that is usually necessary. Given the extensive enterohepatic circulation of rifampin, repeated doses of activated charcoal may enhance elimination; however, the clinical utility of the procedure is questionable. Systemic toxicity associated with the chronic administration of rifampin is an indication to discontinue the drug.

See also: Liver.

Further Reading

Meisel S and Brower R (1980) Rifampin: A suicidal dose. *Annals of Internal Medicine* 92: 262–263.

Riot Control Agents

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General Considerations

Synonyms

Tear Gas, less than lethal, nonlethal, immobilizers, irritants, lacrimators, harassing agents, RCAs, crowd control agents (**Table 1**).

Table 1 Agents considered in this article

Military designation	*Chemical name*	*CAS number*
CN	1-Chloroacetophenone	532-27-4
CS	2-Chlorobenzylidene malononitrile	2698-41-1
CR	Dibenz(*b,f*)1:4-oxazepine	257-07-8
DM (Adamsite)	Diphenylaminochloroarsine	579-94-9
OC	Oleoresin capsicum	
Capsaicin		404-86-4
PAVA		
Fentanyl	*N*-Phenyl-*N*-[1-(2-phenylethyl)-4-piperidinyl]propamide	437-38-7

Pharmacological Actions

Riot control agents (RCAs) cause disabling physiological effects when they come into contact with the eyes and/or skin, or when inhaled by unprotected individuals, by interacting with sensory nerve receptors in the skin and mucosal surfaces at the site of contamination, resulting in local pain and discomfort with associated reflexes. The Kratschmer reflex causes apnea, bradycardia, and a biphasic fall and rise in aortic blood pressure, which is mediated via the olfactory (I), trigeminal (V), and glossopharyngeal (IX) cranial nerves. This reflex has been demonstrated in both animals and humans following exposure to inhaled RCAs.

Pharmacological Classes

Irritants (peripheral sensory irritants), lacrimators, sternutators, emetics, sedatives, hypnotics, serotonin antagonists, hypotensives, thermoregulator disruptors, nauseants, vision disruptors, neuromuscular blockers, malodorous substances, centrally acting anesthetics, immobilizers, tranquillizers.

*The views of the authors do not purport to reflect the position of the US Department of Defense. The use of trade names does not constitute official endorsement or approval of the use of such commercial products.

The US Military has developed a basic military classification of Class Indices for chemical and biological warfare agents. The Class Indices divides RCAs into indices: incapacitating agents (fentanyl) tear agents – halogenated (CN and CS); tear agents – nonhalogenated (CR); tear agents in solvents (OC, Capsaicin, and PAVA); and vomiting agents (DM). While the acute physiological impacts from the various agents within each of these classes are essentially the same, there are variations in the physical/chemical properties and decomposition products.

Uses

RCAs are used as nonlethal or less-than-lethal materials in riot peacekeeping operations to temporarily distract, deter, incapacitate, disorient, or disable disorderly people, to clear facilities, areas, deny areas, or for hostage rescue. They are also used in military training as a confidence builder for the protective mask. Some of these agents can be used as agents in their respective pharmacologic class, that is, fentanyl is used as a short acting central nervous system (CNS) general anesthetic and capsaicin is used as a topical analgesic. Capsaicin spray is also used in the pharmaceutical industry to induce cough for testing antitussive candidates.

Physiochemical Characteristics

RCAs are solids with low vapor pressures. They can be dispersed as fine powders; foams, as coherent jets or streams of solutions from small or large spray cans, large spray tanks, or larger weapons; and as aerosols or smokes by pyrotechnic generation (**Table 2**).

Mechanisms of Action

RCAs are considered less than lethal and nonlethal because they have a very large safety ratio. That is, their effective concentration (EC_{50}) is very low compared to their lethal dose or concentration (LCt_{50}).

CS and CN are SN2-alkylating agents with activated halogen groups that react readily at nucleophilic sites. The prime targets include sulfhydryl-containing enzymes such as lactic dehydrogenase. In particular, CS reacts rapidly with the disulfhydryl form of lipoic acid, a coenzyme in the pyruvate decarboxylase system. It has been suggested that tissue injury may be related to inactivation of certain of these enzyme systems. CS causes the release of bradykinin that can produce pain without tissue injury. The initial response to the inhalation of CS or other sensory irritants is consistent with the Kratschmer reflex and the Sherrington pseudoaffective response. It is theorized that CR aerosols also stimulate the pulmonary irritant receptors to produce bronchoconstriction and increased pulmonary blood volume by augmenting sympathetic tone. The chlorine atoms released from CS on contact with skin and mucus membranes are reduced to hydrochloride acid, which can cause local irritation and burns.

Capsaicin, like the other irritant RCAs, also causes bronchoconstriction, but the mechanism is uncertain. Capsaicin releases substance P that can cause bronchoconstriction directly by activation of specific receptors or by release of histamine or other mediators. It may also cause reflex bronchoconstriction by stimulating C fibers in both pulmonary and bronchial circulation. Therefore, bronchoconstriction may be secondary to substance P release, or to a vagal reflex. The altered neurophysiology of sensory neurons in the airway mucosa induces the release of tachykinins and neurokinin A, which causes neuromediated inflammation of the epithelium, airway, blood vessels, glands, and smooth muscles. This leads to bronchoconstriction, mucus secretion, enhanced vascular permeability, and neutrophil chemotaxis.

DM is among the group of compounds including diphenylchloroarsine (DA), diphenylcyanoarsine (DC), and chloropicrin, which are classified militarily as vomiting agents. DM has been characterized as both a vomiting agent as well as a sneezing agent (sternutator), and was used in World War I. The estimated human LCt_{50} was reported to be 11 000 mg min m^{-3}. DM effects, unlike those of CN, CS and CR, have a slightly delayed onset and have a relatively long recovery period. DM effects occur in $\sim$3 min after inhalation exposure and may last for several hours.

Table 2 Physical properties of some selected RCAs

	CS	*CR*	*CN*	*DM*	*Capsaicin*
Molecular weight	188.5	195.3	154.5	277.5	305
Melting point (°C)	93	72	54	195	64
Vapor pressure (mmHg at 20°C)	0.00034	0.00059	0.0054	2×10^{-13}	0.011
Volatility (mg m^{-3} °C^{-1})	0.71/25°	0.63/25°	1.06/52°		
Solubility[a]	loc	loc	loc	lo	loc

[a] Solubility: l = limited in water; o = soluble in organics; c = soluble in chlorinated organics.

Also unlike the tear agents, DM is more likely to cause prolonged systemic effects. Signs and symptoms of DM exposure include eye irritation, upper respiratory tract irritation, uncontrolled sneezing and coughing, choking, headache, acute pain, tightness in the chest, nausea, and vomiting as well as unsteady gait, weakness in the limbs, and trembling. Mental depression might result after exposure to DM. Inhaled high concentrations can result in serious illness and death as a result of pulmonary damage.

Fentanyls

General Pharmacology

During the Cold War (1945–91), a great deal of research was directed to chemicals that were not necessarily lethal, but would merely temporarily incapacitate enemy personnel. In particular, the United States and the former Soviet Union investigated a wide number of pharmacological agents such as depressants, hallucinogens, belladonna drugs, and opiate derivatives for their potentials as incapacitants.

A major breakthrough in opiate drugs for use in medicine was the synthesis of fentanyl in Belgium in the late 1950s and was first patented by Janssen in France in 1963. Its primary use in medicine was for anesthesia. However, its major complication is respiratory depression, which can be monitored and reversed in an operating room, but can be a problem if used operationally in the field. Since 1996, a number of different analogs of fentanyl have been introduced for use in anesthesia such as carfentanil, sufentanil, alfentanil, and remifentanil. Their pharmacological activity is characteristic of opiates and they produce all of the effects of heroin, including analgesia, euphoria, miosis, and respiratory depression. Due to their high lipid solubility, regardless of the route of administration, fentanyls reach the brain very quickly, thus providing a very fast onset of action. Some of the analogs have been synthesized specifically for sale as Persian white, China white, Mexican brown, and synthetic heroin in the illicit drug market and to circumvent regulations on controlled substances. These illicit drugs are also called designer fentanyls and are used by abusers via intravenous injection, or smoked or snorted.

Fentanyls are synthetic opiates recognized for their short acting and highly potent narcotic analgesic, anesthetic, and immobilizing properties in both animals and humans. Fentanyl is also used as an adjunct to general anesthesia, and as an anesthetic for induction and maintenance. It is primarily a mu-opioid agonist. Abuse of this drug leads to habituation or addiction. The Chemical Abstracts Service Registry numbers of some of the analogs of fentanyl are: sufentanil, CAS 56030-54-7; carfentanil, CAS 59708-52-0; and remifentanil, CAS 132875-61-7.

The feasibility of dissociating the respiratory depressant effect from the opiate-induced sedative activity of alfentanil and fentanyl with naloxone was studied. Naloxone was more effective as an antagonist to alfentanil than to fentanyl. Later studies also suggested that in the rat and ferret, dissociation of the opiate-induced sedation and respiratory depression was feasible. This was accomplished by co-administration of the opiate agonist with antagonists. The opiate-induced effects were akinesia, catalepsy, loss of righting reflex, light anesthesia, and apnea. The pharmacodynamic mechanism of the co-administration may involve competitive displacement of the opiate agonist by the antagonist at their common receptor sites within the CNS. A pharmacokinetic mechanism may also be involved such that the opiate uptake, distribution, and clearance are affected, either directly or indirectly, by the antagonist. Changes in respiratory frequency, oxygen consumption, and apnea were monitored in ferrets following the intravenous co-administration of the opiate agonist sufentanil and the antagonist nalmefene. These studies demonstrated a dissociation of the sufentanil-induced sedation/anesthesia and severe respiratory depression. Nalmefine co-administration shortened the duration, but did not significantly delay the onset of the opiate-induced sedative/anesthetic effect. Narcotic antagonists such as nalmefene, naltrexone, and naloxone have clinical application in the diagnosis of addiction, prophylactic treatment of narcotic abuse, and emergency treatment of narcotic over dosage. These antagonists displace either previously assimilated opiates from their receptor sites, or if administered prior to the narcotic, will preclude the narcotic agonist from acting at these sites.

It has been reported that the serious adverse effects of opiate analgesia such as depression of breathing are caused by direct inhibition of rhythm-generating respiratory neurons in the Pre-Boetzinger complex (PBC) of the brainstem. Serotonin 4(a) or 5-HT4(a) receptors are strongly expressed in these neurons and their selective activation protects spontaneous respiratory activity. Rats treated with a 5-HT4 receptor specific agonist overcame the fentanyl-induced respiratory depression, and reestablished stable respiratory rhythm without loss of fentanyl's analgesic effect.

Opiate effects are mediated via multiple opioid receptors such as the mu, kappa, delta, and sigma. The mu receptors mediate analgesia, euphoria, physical dependence, and depression of ventilation,

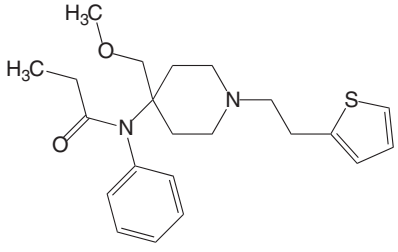
whereas kappa receptors mediate sedation and dieresis. Drugs may act at more than one opiate receptor with varying effect.

Human Toxicity

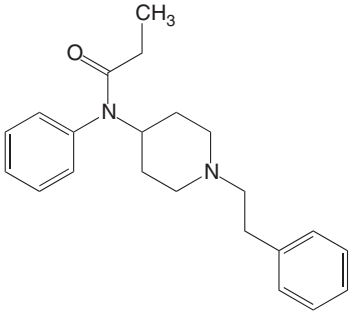
Since fentanyl is not listed in any schedules of the Chemical Warfare Convention (CWC), and is traditionally characterized by the rapid onset and short duration of action of 15–30 min of analgesia, it can be legally considered an RCA according to the definition set forth in the CWC. On October 23, 2002, at least 129 of the ~800 hostages died in the Moscow Dubrovka Theatre Center when Russian authorities subdued the hostage-takers there by pumping what many believe was fentanyl into the building; some believe that a mixture of fentanyl and halothane was used.

The Chechnian terrorists, who held the hostages, were from a particularly extreme and violent group who were on a martyrdom mission. It was also considered that the Russians might have used remifentanil since it is rather unique and extremely potent with relative fast action and short duration. The chemical structure of remifentanil also allows the body to quickly metabolize the substance into nontoxic and water-soluble forms, thus minimizing risks for both hostages and hostage-takers. Although the Russian authorities insisted that emergency personnel were prepared with 1000 antidotes in anticipation of the raid, there is still controversy whether local hospitals and physicians were adequately informed about the gas used during the operation. It has also been suggested that the Russian government revealed that a mixture of fentanyl and halothane was used to incapacitate the Chechnian terrorists in the attempt to liberate the hostages in Moscow. They further suggested that it was likely that massive doses of carfentanil were used to saturate the theatre so that maximal effect by inhalation could be achieved. Carfentanil is a potent opioid use to rapidly immobilize large, wild animals, horses, and goats. It produces rapid catatonic immobilization, characterized by limb and neck hyperextension. Adverse effects include muscle rigidity, bradypnea, and oxygen desaturation. Recycling and renarcotization have been reported as possible causes of death when low doses of antagonists are used. Although there were naloxone syringes found in the theatre, it is possible that the doses were insufficient to reverse the respiratory depression.

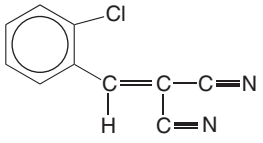
Chemical Structures



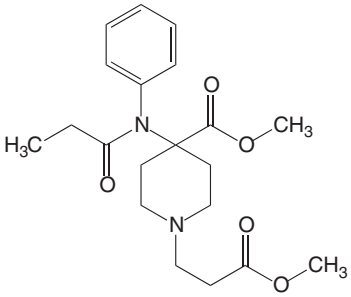
Sufentanil (SAN:BAN:INN) (RN: 56030-54-7)



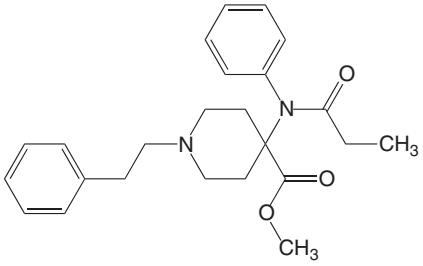
Fentanyl (RN: 437-38-7)



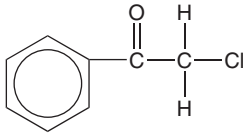
2-Chlorobenzylidene malononitrile (CS)



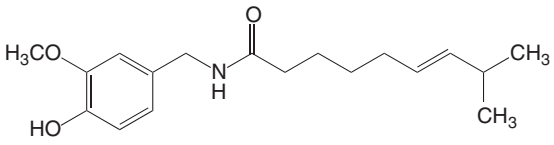
Remifentanil (RN: 132875-61-7)



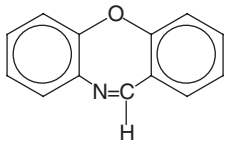
Carfentanil (RN: 59708-52-0)



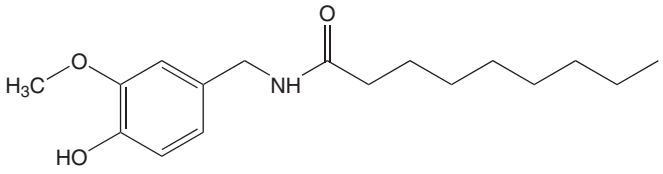
1-Chloroacetophenone (CN)



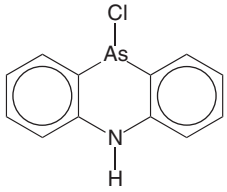
Capsaicin



Dibenz (b,f)–1:4-oxazepine (CR)



PAVA or Nonivamide



10-Chloro-5,10-diphenylarsazine (DM)

1-Chloroacetophenone

Animal Toxicology

Toxicological studies were conducted on 1-Chloroacetophenone (CN) dispersed from commercially available thermal grenades or from acetone solutions. Acute and sublethal effects following aerosol exposure in experimental animals were lacrimation, conjunctivitis, copious nasal secretions, salivation, hyperactivity, lethargy, and dyspnea, which occurred in all animals. Effects on the skin of exposed animals were primarily erythema. The estimated LCt_{50} values calculated for CN in the various animal species were 8878 mg min m^{-3} in the rat, 7984 mg min m^{-3} in the guinea pig, and 7033 mg min m^{-3} in the dog. The pathological findings in the animals that died from inhalation of CN consisted of congestion of the alveolar capillaries, alveolar hemorrhages, and excessive secretions in the bronchi and bronchioles, as well as areas of acute inflammatory cell infiltration of the trachea, bronchi, and bronchioles. It was also reported that CN was from three- to tenfold more toxic than CS in mice, rats, rabbits, and guinea pigs. The early deaths exhibited lesions of the upper respiratory tract, with marked pseudomembrane formation, excessive salivation, and nasal secretion. The animals that died later exhibited edema and hemorrhage of the lungs. In repeated exposures to lower individual concentrations than in the acute exposures for 10 consecutive days in guinea pigs, dogs, and monkeys, the toxicity of CN was found to be considerably less when administered in divided doses. Overall, studies demonstrated a lack of cumulative toxicity. Changes in biochemical endpoints measured following multiple exposures of CN and CR in mice were a decrease in hepatic glutathione and increased lipid peroxidation. Hepatic acid phosphatase increased after a 5 day exposure to CN, and the glutathione levels decreased after a 10 day CN exposure. CN-induced elevation in acid phosphatase levels reflected the release of lysosomal enzymes from the liver, indicative of tissue injury. CR exposure did not produce significant alterations in hepatic biochemical parameters. Additionally, hyperglycemia was observed after exposure to CN. Stress-mediated release of epinephrine is known to elevate glucose levels and thus may be responsible for the hyperglycemia. Significant decreases in body weight gain were also noted on exposure to these compounds with CN having a more prominent effect on body weight. These findings were consistent with results on the repeated dose effects of orally administered CR in various animal species. Histopathologic changes following CN exposures included hemorrhage, perivascular edema, congestion of the alveolar capillaries, occluded bronchioles, and alveolitis. Renal histopathology demonstrated congestion and coagulative necrosis in the cortical tubules in CN-exposed mice. Hepatic histopathology consisted of cloudy swelling and lobular and centrolobular necrosis of hepatocytes following CN exposures.

CN, particularly in solutions, is more likely to cause more serious eye effects than CS. At high concentrations, CN may result in chemical injury to the eye with corneal and conjunctival edema, erosion or ulceration, chemosis, and focal hemorrhages. CN-induced ocular effects on the rabbit eye following treatment with various formulations included lacrimation, chemosis, iritis, blepharitis, and keratitis, with severity dependent on the formulation.

CN is also a potent skin irritant, more likely to cause more serious injury to the skin than CS. These effects include diffuse and intense erythema, severe

edema, and vesication. CN is considered a more potent skin irritant and sensitizer than CS.

In 2 year carcinogenicity inhalation bioassays in rats and mice, there was no indication of carcinogenicity in male rats, while equivocal evidence was found in female rats. These findings were evidenced by increased fibroadenomas of the mammary gland. In these 2 year studies in mice, there was no evidence of carcinogenic activity in males and females.

In the body, CN is converted to an electrophilic metabolite. It is an SN2 alkylating agent that reacts with SH groups and other nucleophilic sites of biomolecules. Alkylation of SH-containing enzymes leads to enzyme inhibition with disruption of cellular processes. CN was found to inhibit human plasma cholinesterase via a non-SH interaction, and some of the toxic effects may be due to alkylation of SH-containing enzymes.

Human Toxicology

The incapacitating effects of CN in human volunteers during exposure include lacrimation, some blurring of vision, and conjunctivitis. On the nose and throat, CN causes a tingling sensation, irritation, pain, and some increases in secretions, while on the respiratory tract it causes irritation, burning, and pain. CN on the skin causes burning in the periorbital area, and other areas of tender skin, especially where sweating is present. Occasionally, nausea and gagging occur during and soon after exposure. Most of these effects disappear within 20 min after exposure, but conjunctivitis and blepharospasm usually disappear after a few days leaving no after effect. Incapacitating concentrations (IC_{50}) of CN have ranged from 20 to 50 mg min m^{-3}. The IC_{50} for CN is comparable to that for adamsite (DM), which was an early RCA that was replaced by CN. The IC_{50} values for CN and DM are greater than for CS. The estimates of human LCt_{50} values, extrapolated from animals exposed to CN dispersed from a solvent, is 7000 mg min m^{-3}, and 14 000 mg min m^{-3} when dispersed from commercially available grenades. Other estimates range from 8500 to 25 000 mg min m^{-3}. The maximum safe inhalation dosage of CN for humans has been estimated to be 500 mg min m^{-3}.

Human volunteers were tested in a wind tunnel at an air speed of five miles per hour (mph) to establish the length of time a subject could remain in the CN-containing air stream. The tolerance time varied with the subject. CN aerosols were generated from acetone solutions at a Ct of 350 mg min m^{-3} with a mass median diameter of about 0.6 μm. The immediate effects of exposure were tingling of the nose and rhinorrhea, burning of the eyes and throat, lacrimation, and blurred vision. Some subjects suffered dyspnea. These effects disappeared rapidly when the subjects left the wind tunnel. Acute injuries to the eyes, primarily from the effects of blast and missiles, may occur from tear gas weapons, such as pen guns. The immediate effects of these injuries include swelling and edema of the lids with penetration of skin, conjunctivitis, cornea, sclera, or globe by gunpowder and CN. Conjunctival ischemia and chemosis, corneal edema, erosion, inflammation or ulceration, and focal hemorrhage have also been reported just as injuries resulting from accidental discharges of tear gas guns at close range have been. Surgery was required in these cases to relieve pain and to remove foreign material. They all suffered continuing pain and some loss of sensation, apparently from the toxic action of CN on nerves.

2-Chlorobenzylidene Malononitrile

General Considerations

In addition to the nonpersistent form of 2-chlorobenzylidene malononitrile (CS), two hydrophobic variations were created, CS1 and CS2. CS1 is a micronized powder formulation containing 5% hydrophobic silica aerogel, which can persist for up to 2 weeks in normal weather conditions and CS2 is a siliconized microencapsulated form of CS1 with a long shelf life, persistence, resistant to degradation, and ability to float on water which could restrict or deny the use of water for military operations. CS is commonly used as an RCA and a simulant for training. Members of military organizations and law enforcement agencies are routinely exposed to heated CS during training. The heat vaporizes the CS for dispersion, which thus condenses to form an aerosol.

Repeated exposures of thermally dispersed CS were conduced in rats and dogs. They were exposed from 4 to 5 min day^{-1}, 5 days $week^{-1}$ for 5 weeks. The 25 day cumulative dosage (Ct) which the rats were exposed to was 91 000 mg min m^{-3} (3640 mg min m^{-3} per day), while the dogs were exposed to a cumulative dosage of 17 000 mg min m^{-3} (680 mg min m^{-3} per day). No lethality occurred in the dogs, while the rats became hyperactive and aggressive, biting noses and tails of other rats, and scratching their own noses. No changes were found in blood values for sodium, potassium, protein, albumin, or creatinine throughout the tests. Five of the 30 rats exposed died, two following the cumulative dosage of 25 000 mg min m^{-3}, and three died after 68 000 mg min m^{-3}. Gross pathological

examination of the rats that died was negative, as were those of six other rats that were sacrificed after 5 weeks of exposure. The exposed rats lost ~1% of body weight, while unexposed rats gained ~20% during the 5 weeks. There were no significant differences in organ to body weight ratios for heart, kidneys, lungs, liver, or spleen following the 5 week exposures. It was concluded that repeated exposures did not make the animals more sensitive to the lethal effects of CS. The animals that died after exposure to CS showed increased numbers of goblet cells in the respiratory and gastrointestinal tracts and conjunctiva, as well as necrosis in the respiratory and gastrointestinal tracts, pulmonary edema, and occasionally hemorrhage in the adrenals. Death appeared to result from poor transfer of oxygen from the lungs to the blood stream, probably because of edema, and hemorrhage in the lungs, and obstruction of the airways. The effects of repeated exposures to CS were studied in mice, rats, and guinea pigs to neat CS aerosols for 1 h day^{-1}, 5 days week^{-1} for 120 days. High concentrations of CS were fatal to the animals after only a few exposures, while mortality in the low and medium concentrations did not differ significantly from the controls. It was concluded that CS concentrations below 30 mg m^{-3} were without deleterious effects. Acute inhalation toxicity of CS which was generated in smoke and as an aerosol was studied in several animal species. The LCt_{50} data are presented in **Table 3**.

Other lethality estimates for CS are given below. From acute exposures to CS dispersed from a 10% CS in methylene dichloride the LCt_{50}s were as follows: mice, 627 000 mg min m^{-3}; rats, 1 004 000 mg min m^{-3}; and guinea pigs, 46 000 mg min m^{-3}. No deaths occurred in rabbits exposed to up to 47 000 mg min m^{-3}. CS at dosages up to 30 000 mg min m^{-3} did not cause any deaths in any of the monkeys with pulmonary tularemia. The combined LCt_{50} for mice, rats, guinea pigs, and rabbits was calculated to be 1 230 000 mg min m^{-3} for CS dispersed from methylene dichloride. Goats, pigs, and sheep did not exhibit hyperactivity on exposure to CS, and they were also resistant to its lethal effect. Therefore, no LCt_{50} values could be calculated for goats, pigs, or sheep. However, a combined LCt_{50} was calculated for all of the species tested, mice, rats, guinea pigs, rabbits, dogs, monkey, goats, pigs, and sheep, and was estimated to be 300 000 mg min m^{-3}. LCt_{50}s were also calculated for CS dispersed from M18 and M7A3 thermal grenades. These were 164 000 mg min m^{-3} for rats and 36 000 mg min m^{-3} for guinea pigs exposed to the M18 thermal grenade dissemination, and for the M7A3 thermal grenade the values were as follows: rats, 94 000 mg min m^{-3}; guinea pigs, 66 000 mg min m^{-3}; rabbit, 38 000 mg min m^{-3}; goat, 48 000 mg min m^{-3}; pigs, 17 000 mg min m^{-3}; dog, 30 000 mg min m^{-3}; monkey, 120 000 mg min m^{-3}. All of the acute exposure results were combined and LCt_{50} values were calculated for all rodents to be 79 000 mg min m^{-3}, and for all nonrodent species tested the value was calculated to be 36 000 mg min m^{-3}, and for all the species it was 61 000 mg min m^{-3}. The LCt_{50} values for CS2 was also calculated. CS2 is 95% CS, 5% Cal-o-Sil R, and 1% hexamethyldisilazane, and the LCt_{50} values are: rats, 68 000 mg min m^{-3}; guinea pigs, 49 000 mg min m^{-3}; dogs, 70 000 mg min m^{-3}; and monkeys, 74 000 mg min m^{-3}. The lethal effects in animals following inhalation exposures is caused by lung damage leading to asphyxia and circulatory failure, or bronchopneumonia secondary to respiratory tract injury. Pathology involving the liver and kidneys following inhalation of high dosages of CS is also secondary to respiratory and circulatory failure.

Table 3 Acute inhalation toxicity LCt_{50} (mg min m^{-3}) values for CS smoke and aerosols to various species

	CS smoke	*CS aerosol*
Guinea pig	35 800	67 000
Rabbit	63 600	54 090
Rat	69 800	88 480
Mouse	70 000	50 110

Various experimental animal species were exposed to aerosols of CS generated by various methods of exposure from 5 to 90 min. The toxic signs observed in mice, rats, guinea pigs, rabbits, dogs, and monkeys were immediate, and included hyperactivity, followed by copious lacrimation, and salivation within 30 s of exposure in all species except the rabbit. The initial level of heightened activity subsided, and within 5–15 min following initiation of the exposure, exhibited lethargy and pulmonary stress, which continued for about 1 h following cessation of the exposure. All other signs had disappeared within 5 min following removal from the exposure. When toxic signs were observed, they occurred following exposure by all of the dispersion methods.

The effects of CS inhalation were studied on embryonic development in rats and rabbits at concentrations consistent with those expected in riot control situations (~10 mg m^{-3}). Although the concentrations were low and the duration of exposure (5 min) may not have been adequate to assess the fetotoxic and teratogenic potential of CS, no significant increase in the numbers of abnormal fetuses or resorptions were noted. The mutagenic potential of CS and CS2 were studied in microbial and mammalian

bioassays. CS was positive in the Ames assay, while others reported questionable genotoxicity for *Salmonella typhimurium*, and negative when tested in *S. typhimurium* strains TA 98, TA 1535, and TA 1537 with and without metabolic activation. The mutagenic potential for CS and CS2 in mammalian assays such as the Chinese hamster ovary (CHO) test for the induction of sister chromatid exchange (SCE) and chromosomal aberration (CA), and the mouse lymphoma L5178Y assay for induction of trifluorothymidine (Tfi) resistance indicated that CS2 induced sister chromatid exchange, chromosomal aberrations, and Tfi resistance. The Committee on Toxicology of the National Research Council reported that taken in their totality, the test of CS for gene mutation and chromosomal damage provide no clear evidence of mutagenicity. Although most of the evidence is consistent with a lack of mutagenic potential, in the committee's judgment it is unlikely that CS poses a genotoxic hazard to humans. CS2 was evaluated for carcinogenicity in the NTP 2 year rodent bioassay. Compound related nonneoplastic lesions of the respiratory tract were observed. The pathologic changes observed in the exposed rats included squamous metaplasia of the olfactory epithelium, hyperplasia, and metaplasia of the respiratory epithelium. In mice, hyperplasia and squamous metaplasia of the respiratory epithelium was observed. Neoplastic effects were not observed in either rats or mice, and it was concluded that the findings suggests that CS2 is not carcinogenic to rats and mice CS in methylene chloride was also tested in mice and rats for carcinogenicity in a 2 year study, and no tumorigenic effects were observed in the CS exposed animals.

CS is absorbed very rapidly from the respiratory tract, and the half-lives of CS and its principal metabolic products are extremely short. The disappearance of CS follows first-order kinetics and spontaneously hydrolyses to malononitrile, which is transformed to cyanide in animal tissues. Metabolically, CS undergoes conversion to 2-chlorobenzyl malononitrile (CSH2), 2-chlorobenzaldehyde (oCB), 2-chlorohippuric acid, and thiocyanate. CS and its metabolites can be detected in the blood after inhalation exposure, but only after large doses. Following inhalation exposure of CS in rodent and nonrodent species, CS and two of its metabolites, 2-chlorobenzaldehyde and 2-chlorobenzyl malononitrile, were detected in the blood. In another study, human uptake by the respiratory tract, only 2-chlorobenzyl malononitrile was detected in trace amounts in the blood. CS and 2-chlorobenzaldehyde were not detected, even after high doses of CS of up to $90\,mg\,min\,m^{-3}$. This finding is consistent with the CS uptake studies in animals, and with the maximum tolerable concentration in humans, which is below $10\,mg\,m^{-3}$, it is unlikely that significant amounts of CS would be absorbed by the inhalation route at or near the tolerable concentrations. Experiments were conducted to determine the CS metabolite thiocyanate in humans exposed to amounts of CS that are intolerable. In dogs, exposure to $48\,000\,mg\,min\,m^{-3}$ of CS aerosol showed an unimpressive increase in plasma and urine thiocyanate concentration 24 h after exposure. These were lower than those observed in human subjects who smoked cigarettes. Smoking and nonsmoking human volunteers were exposed to doses up to $1.1\,mg\,min\,m^{-3}$ of CS (intolerable). Plasma and urine levels were significantly higher in smokers than in nonsmokers, and exposure to CS did not cause any significant increases in plasma and urine thiocyanate levels. Plasma and urine thiocyanate levels were measured in human volunteers following exposure to intolerable airborne concentrations of CS. Since cigarette smoking also increases thiocyanate in body fluids, levels in nonsmokers, light smokers, and heavy smokers, before and after CS exposure, were compared. There was no statistical difference in plasma or urine thiocyanate concentration between nonexposed and CS exposed volunteers. However, both light and heavy smokers' concentrations were significantly higher than nonsmokers. Thus, it was concluded that plasma and urine levels of thiocyanate CS metabolite are not high enough to detect following human exposure to intolerable levels of CS.

Human Toxicology

Exposure to CS is highly irritating to the mucous membranes that line the tissues of the eyes, nose, throat, and respiratory and gastrointestinal tracts. Irritation of the eyes may cause pain, excessive tearing, conjunctivitis, and blepharospasm (uncontrolled blinking). The nose and mouth may perceive a stinging or burning sensation with excessive rhino rhea or discharge of nasal mucous. Irritation of the respiratory tract may cause tightness of the chest, sneezing, and coughing, as well as increased respiratory secretions. Severe lung injury and subsequent respiratory and circulatory failure characterize death in experimental animals following inhalation of very high dosages of CS. Irritation of the gastrointestinal tract may cause vomiting and/or diarrhea. Following exposure of the skin, a burning sensation may be experienced, with subsequent inflammation and redness. Six minutes following exposure to CS, the irritation during exposure is so intense that the individual exposed seeks to escape.

When exposed to CS aerosols generated from solutions in acetone or methylene chloride or from thermal grenades at 3.0, 1.0, 0.5 μm MMAD, many untrained subjects were unable to don and retain their masks at low concentrations of CS, but at high concentrations were able to mask well enough to remain in the contaminated atmosphere. When properly fitted these masks will fully protect against CS. In those who were unable to mask rapidly, panic was evident. Concentrations of 9–10 $mg\,m^{-3}$ forced 50% of the subjects to leave the chamber within 30 s, 99% left at ~17 $mg\,m^{-3}$, and 100% left and were considered incapacitated at 40 $mg\,m^{-3}$ or greater. Persons who had been exposed previously to a high concentration developed a fear of the agent, and even though subsequently exposed to a lower concentration, the time to incapacitation for trained men was shorter than expected. There were no significant differences noted in the time to incapacitation in subjects exposed to CS at 0–95°F, although it appeared that the subjects appeared unable to tolerate the agent as well as those exposed at ambient temperature. At 95°F and relative humidity of 35% and 97% the skin-burning effects were much more prominent, possibly because of the excessive diaphoresis. Hypertensive subjects reacted similarly to and tolerated CS as well as normotensive individuals. However, their blood pressure elevation was greater and lasted longer than in normotensives, possibly because of the stress of exposure. The hypertensive subjects recovered as rapidly as the normotensives. Subjects with a history of peptic ulcer, jaundice, or hepatitis, and those between the ages of 50 and 60 reacted similarly to normal subjects. Persons with a history of drug allergy, hay fever, asthma, or drug sensitivity were able to tolerate CS exposure as well as the normal subjects; however, a higher percentage of this group had more severe chest symptoms than the normals. Although many of them lay prostrate on the ground for several minutes, no wheezing or ronchi were heard on ausculation, and recovery time was as rapid as for any other group tested. Hyperventilating subjects were incapacitated at much lower concentrations than normally breathing subjects, and recovery time was slightly prolonged, but only by 1–2 min. Although not significantly different, subjects exposed to CS disseminated from methylene dichloride appeared to tolerate the agent for a slightly longer period that those subjected to CS in acetone solution, nor was there any difference in CS disseminated from the miniature M18 CS smoke grenade. There was also a group exposed to a combination of CS and DM. The effects of DM were negligible when CS was effective within 30 s.

In experiments where only the eyes and respiratory tract of human volunteers were exposed to small (0.9 μm) and large (60 μm) particles of CS, the small particle size was more effective in producing eye irritation. Only two of the five men exposed to the 0.9 μm aerosol were able to tolerate the CS for 60 s, while all six men exposed to the large sized aerosol remained in the cloud for at least 60 s. Following exposure, all subjects had difficulty in seeing. Recovery times were based on the subjects' ability to sort and arrange cards. Recovery following exposure of the eyes to small particles averaged 90 s, while it took ~280 s following exposure to the large particle. The respiratory effects of exposure of the small particles were more dramatic. None of the six men could tolerate the small particles for longer than 30 s while four of the six men tolerated the larger particle exposure for at least 60 s.

A group of seven volunteers given 10 exposures of CS from 1 to 13 $mg\,m^{-3}$ in a period of 15 days revealed no clinical abnormalities. The dominant effect of the first exposure remained the dominant effect on subsequent exposures. None of the volunteers developed a tolerance to CS during the 10 exposures.

The immediate effects upon exposure to aerosols of CS were on the eyes, and were demonstrated by severe conjunctivitis accompanied by a burning sensation and pain that persisted from 2 to 5 min and usually disappeared abruptly rather than gradually. The conjunctivitis remained intense for up to 25–30 min. Erythema of the eyelids was generally present, persisted for 1 h, and was occasionally accompanied by blepharospasm. Lacrimation was invariably present, tended to be profuse and lingering for up to 12–15 min. The occasional 'tired feeling' in their eyes lasted for about 24 h. Photophobia, which was quite marked in 5–10% of the volunteers, remained for up to 1 h. On repeated exposures, the eye effects were reproduced. Rhinorrhea and salivation were profuse and persisted for up to 12 h.

The effects on the respiratory system appeared to be dependent on the duration of exposure and the depth of respiration. The first symptom was usually a burning sensation beginning in the nares and throat and then progressing down the respiratory tract, sometimes associated with coughing. As the exposure continued, the burning became painful and was rapidly followed by a 'constricting sensation' throughout the chest, which caused incapacitation for several minutes. Panic usually accompanied and accentuated this symptom, and these volunteers appeared unable to inhale or exhale. Fresh air and encouragement abated these effects. Auscultation of the chest immediately after exposure did not reveal wheezing, rales, or ronchi. Airway resistance

measured by an Asthmometer showed no significant changes, and a portable breath recording apparatus measured breathing patterns of exposed individuals. The patterns indicated that when the aerosol was inhaled, the subjects involuntarily gasped, and then held their breath or breathed slowly and shallowly. This was followed by short paroxysms of coughing that forced the individual to exit the exposure. An irregular respiratory rhythm was noted for several minutes after exposure was terminated. Many of the exposed individuals were aphonic for 1–2 min post-exposure, and several were hoarse for 24 h. It was concluded that the incapacitation cause by CS was due to the effects on the eyes, respiratory tract, or both, but regarded the effects on the respiratory system as potentially the most capable of causing incapacitation. A group of volunteers in a wind tunnel, wearing a self-contained remotely controlled breath-recording system, was exposed to 5–150 μg CS per liter for 110–120 s. Although the breathing patterns were disrupted by the CS exposure, adequate ventilation of the lungs was maintained, so they concluded that incapacitation is attributed to the unpleasant sensations rather than to any degree of respiratory failure. The apnea and cardiovascular changes observed following inhalation exposure to CS is not inconsistent with the Kratschmer reflex. These investigators also reported that sneezing was common among the observers exposed to small concentrations of CS at some distance from the exposure chamber.

Inhalation toxicity studies by aerosol dispersions of melted agents sprayed in the molten form, dry powder dispersion, sprayed from solutions of acetone or methylene dichloride, or dispersed from grenades by liberation of hot gases have been performed since World War I. Prior to the research on CS in 1958 and 1959, no toxicity studies were performed using munitions. In 1965, munitions studies were conducted with CN and DM. All of these studies demonstrated that munitions dispersed agents were less toxic than dispersion by other methods. The human LD_{50} value, based on the combined animal species toxicity data, is 52 000 mg min m^{-3} for CS by molten dispersion, and 61 000 mg min m^{-3} dispersed by the M7A3 grenade.

Although no fatalities have been validated following exposure to CS, there have been several cases of serious consequences. A documented case of pneumonia is reported in a normal 4-month-old white male infant exposed to CS gas for 2–3 h. Immediately when taken to the emergency room he was observed to have copious nasal and oral secretions, sneezed and coughed frequently, and required suction to relieve upper airway obstruction. The pneumonitis was treated aggressively and the patient was discharged from hospital on the 12th day. However, within 24 h the infant was returned to the emergency room and was rehospitalized. A repeat chest roentgenogram demonstrated a progression of the pulmonary infiltrates. Following treatment with antibiotics the chest roentgenogram was clear on the 17th day, and improvement continued and the patient was discharged after 28 days of hospitalization.

Another reported case of serious intoxication with CS tear gas was 11 days following a thorough internal medical examination that revealed no clinical or pathological findings, when a 43-year-old male was in a room in a cloud of fumes from a CS canister that a friend had ignited as a joke. Immediately he suffered from burning pains in the eyes and in his upper respiratory tract, lacrimation, and pains in his chest with dyspnea and coughing. This unusual exposure led to serious long-term complications such as toxic pulmonary edema, gastrointestinal difficulties, and indications of liver damage and passing right heart insufficiency. After 3 months of hospitalization, all tests were negative, and the patient was discharged to his home in a condition capable of work.

A reported case of major hepatitis attributable to CS inhalation exposure was described where a 30-year-old incarcerated male was sprayed with CS and was hospitalized 8 days later with erythroderma, wheezing, pneumonitis with hypoxemia, hepatitis with jaundice, and hypereosinophilia. For months he continued to suffer from generalized dermatitis, recurrent cough and wheezing consistent with reactive airway dysfunction syndrome, and eosinophilia. Systemic corticosteroids were successful, but abnormalities recurred off treatment. Although the dermatitis resolved gradually over 6–7 months, the asthma-like symptoms persisted a year after exposure. Patch testing confirmed sensitization to CS. The mechanism of the prolonged reaction is unknown, but may involve cell-mediated hypersensitivity, perhaps to adducts of CS, or a metabolite, and tissue proteins. The investigators reported this as the first documented case in which CS apparently caused a severe, multisystem illness by hypersensitivity rather than direct tissue toxicity.

Other human exposures were reported on the alleged use of tear gas in almost every major city in South Korea in June of 1987, where over 350 000 uses of CS tear gas was carried out by the government against civilians who exhibited cough and shortness of breath for several weeks. Hospitalized patients with asthma and chronic bronchitis, exposed to CS wafting through hospital wards through open windows, experienced deterioration in lung function. Persons close to the exploding tear gas

canisters and grenades sustained penetrating trauma from plastic fragments that was exacerbated by the tear gas. Lack of information and objective as well as epidemiological studies was due to fear of serious government reprisals.

There were also allegations that exposures to tear gas in Gaza and the West Bank of Israel have been associated with increases in miscarriages and stillbirths. Inquiries, by groups such as Amnesty International and Physicians for Human Rights, prompted a Government Accounting Office (GAO) investigation requested by Congressman Ronald Dellums. The GAO Report (1989) concluded that the Physicians for Human Rights fact-finding trip could not confirm any deaths linked to tear gas inhalation, nor could they substantiate the rumors of increased miscarriages. There was also no verifiable evidence available to conclude linking tear gas exposure to fetal deaths. In addition, the US State Department reported that they did not have any medical evidence to support a direct causation between tear gas inhalation and the number of deaths and miscarriages alleged. The exaggerated number of almost 400 deaths attributed to the use of tear gas by the Israeli Defense Forces (IDF) has also been repudiated by the State Department. They have concluded that at least four deaths had resulted from tear gas use in enclosed areas, and that the IDF was using primarily CN at the time.

The use of CS by the US forces in Vietnam in the years 1964–72 was to flush the enemy from bunkers and tunnels, reduce the ability of the enemy to deliver aimed fire while attacking, and to deny fighting positions and infiltration routes for extended periods of time.

Interest and possible concern developed about the adverse effects of chemicals employed in peacekeeping operations in the United Kingdom following the use of CS by the Ulster Constabulary in Londonderry, Northern Ireland, on 13 and 14 August 1969. As a result of this first use of CS for crowd control, a Committee of Inquiry was established to determine the medical effects, if any, in persons exposed to CS. Their report known as the Himsworth Reports, described that on exposure to various concentrations of CS, the effects vary from a slight prickly or peppery sensation in the eyes and nasal passages up to the maximum symptoms of profuse lacrimation, and salivation from the eyes and nose, spasm of the eyelids, retching and sometimes vomiting, burning of the mouth and throat, cough, and gripping pain in the chest. Even at low concentrations, the onset of symptoms is immediate, and they disappear when removed from the exposure. Of the many tens of thousands of military personnel in the United Kingdom who were exposed to CS in the course of their training, as well as those of the US military who undergo similar training, the signs and symptoms were similar to those described above, and there were no significant after effects. All the US military personnel exposed to CS in training and under field conditions reported similar effects. They also reported that a cluster of nine US Marine Corps Amphibious Reconnaissance students required hospitalization with pulmonary edema after strenuous exercise following exposure to CS. These patients did not become symptomatic until 36–40 h after the CS exposure and did not demonstrate evidence of airway dysfunction. It was proposed that these cases attributed to the acute pulmonary effects of CS more likely represented a cluster of incidents of either water aspiration or swimming induced pulmonary edema. Water aspiration is a well-described cause of pulmonary edema. No details were provided in the report as to whether the symptomatic marines aspirated pool or sea water or whether they were breath-hold-diving, but all became symptomatic immediately after pool or open ocean 1000–1500 m swims. Even when patients do not recall specific aspiration incidents while in the water, pulmonary edema has been described in divers and swimmers who have been immersed in cold water and strenuous swimming alone has been reported as a cause of pulmonary edema. Similar cases of pulmonary edema associated with immersion occurred at the US Basic Underwater Demolition/Seal School as well as at the Israel Naval Medical Institute. The case definition of pulmonary edema associated with immersion includes hypoxemia and radiograph air space filling that occurs during or immediately after swimming, followed by resolution of symptoms or radiographic improvement by greater than 50% within 48 h. On exposure, the eyes are red, but this disappears on leaving the contaminated atmosphere. On the skin CS causes a burning sensation on the exposed parts that can be followed by redness or the appearance of small blisters or vesicles at the points of friction. These effects are more prevalent in fair skinned persons especially if the skin is hot and moist. The Himsworth Committee reported that infants asleep in rooms where CS entered via broken windows were sufficiently distressed to awaken them crying from sleep. On snatching them out of the contaminated atmosphere, they quieted rapidly and required no hospitalization. They also found no special susceptibility to CS associated with old age. Human volunteers and members of the Himsworth committee over 50 years of age were exposed to $35\,\mathrm{mg\,m^{-3}}$ and the symptoms experienced and the time to recover from these were no

different from those in young adults. Exposure to CS was determined not to have had any effect on pregnancy since comparison of the 9 months following exposure compared to the 9 months of the previous year demonstrated no difference in abortions, stillbirths, or congenital abnormalities. Middle aged and elderly people who had chronic bronchitis and had been significantly exposed to CS did not show exacerbation different than that caused by natural causes. Following the riots of 1969, there was no increase in the death rate from chronic bronchitis and asthma. Asthmatics, especially children who were exposed to CS, did not show any difference in the number of attacks from their experience prior to the exposure. The committee reported that there is ample evidence that if CS causes unconsciousness in humans, it can do so only rarely and that many, if not all of the cases reported are more probably the result of other conditions that occur in riot situations. In animals, unconsciousness does not occur after inhaling CS. The Himsworth reports, considered to be the most extensive study of the use of CS agent on humans, by United Kingdom forces in Northern Ireland in the late 1960s, found that no deaths and no long-term injuries resulted from the widespread use of CS agent there.

Contamination of the skin with solutions of CS causes transient rises in both systolic and diastolic blood pressure. Contamination of the eye with solutions of CS also causes increases in blood pressure, together with transient rises in intraocular pressure.

Dibenz(*b*,*f*)-1:4-Oxazepine

Animal Toxicology

The mammalian toxicology in various animal species indicates that the acute toxicity (LD_{50} and LCt_{50}) of dibenz(*b*,*f*)-1:4-Oxazepine (CR) is less than that of CS and CN by all routes of exposure. Animals exposed to CR exhibited ataxia or incoordination, spasms, convulsions, and tachypnea or rapid breathing. In the animals that survived, these effects gradually subsided over a period of 15–60 min. Increasing respiratory distress preceded death. The animals that died following intravenous and oral administration demonstrated congestion of liver sinusoids and alveolar capillaries. At necropsy, the surviving animals did not show any gross or histological abnormalities. The toxic signs following intraperitoneal administration included muscle weakness and heightened sensitivity to handling. These effects persisted throughout the first day of exposure. Some animals also exhibited central nervous system effects. Surviving animals did not exhibit any gross or histological abnormalities at necropsy. Several animal species were exposed to the acute inhalation of CR aerosols and smokes for various time periods. Rats exposed to aerosol concentrations from 13 050 to 428 400 mg min m^{-3} manifested nasal secretions and blepharospasm or uncontrollable closure of the eyelids, which subsided within 1 h after termination of the exposure. There were no deaths during or following these exposures. There were also no deaths in rabbits, guinea pigs, or mice exposed to CR aerosols of up to 68 000 mg min m^{-3}. Animals exposed to CR smoke generated pyrotechnically, had alveolar capillary congestion, and intra-alveolar hemorrhage, as well as kidney and liver congestions.

The potential of CR aerosols to produce physiological and ultrastructural changes in the lungs was evaluated by electron microscopy. Rats exposed to CR aerosols of 115 000 mg min m^{-3} did not reveal any effects on organelles such as lamellated osmiophilic bodies. Lungs of animals exposed to aerosols of CR at dosages of 78 200, 140 900, and 161 300 mg min m^{-3} were found to appear normal on gross examination. On microscopic examination, however, the lungs revealed mild congestion, hemorrhage, and emphysema. Electron microscopy showed isolated swelling and thickening of the epithelium, as well as early capillary damage, as evidenced by ballooning of the endothelium. It was concluded that these very high dosages of CR aerosols produced only minimal pulmonary damage.

Repeated inhalation exposures in mice and hamsters to concentrations of 204, 236, and 267 mg m^{-3} CR for 5 days $week^{-1}$ for 18 weeks produced death in both species at the high concentrations, but no single cause of death could be ascertained, although pneumonitis was present in many cases. Chronic inflammation of the larynx was observed in mice. Although alveologenic carcinoma was found in a single low dose and a single high dose group of mice, the findings and conclusions were questioned because the spontaneous occurrence of alveologenic carcinoma is high in many mouse strains. Further, this tumor type differs in many respects from human lung tumors. No lung tumors and no lesions were found in hamsters exposed to CR aerosols. Histopathology revealed hepatic lesions in mice, but these were of infectious origin, and not CR related. CR exposures at high concentrations reduced survivability and produced minimal organ specific toxicity at many times the intolerable human dose, which has been reported as 0.7 mg m^{-3} (IC_{50}) within 1 min and 0.15 mg m^{-3} (IC_{50}) within a minute. The effects in rats of CR and CN aerosols on plasma glutamic oxaloacetic transaminase (GOT), plasma glutamic pyruvate transaminase (GPT), acid phosphatase, and alkaline

Table 4 Comparative acute animal toxicity LCt_{50} (mg min m^{-3}) values for CR, CS, and CN to various species

	Pyrotechnically generated			*Aerosol*		
	CR	*CS*	*CN*	*CR*	*CS*	*CN*
Mouse	203 600	76 000	No data	169 500	67 200	18 200–73 500
Rat	139 000	68 000	23 000	428 400	88 460	3 700–18 800
Rabbit	160 000	63 000	15 800	169 000	54 100	5 840–11 480
Guinea pig	ND[a]	ND	ND	169 500	50 010	3 500–13 140

[a] ND = no data.

phosphatase exhibited no change in any of these parameters for CR, while there were significant increases in all of these parameters in rats exposed to CN, suggesting that CN could cause tissue damage.

Comparative data for acute inhalation toxicity to various animal species for CR, CS, and CN are presented in **Table 4**.

The cardiovascular effects of CR administered intravenously demonstrated a dose-dependent increase in blood pressure of short duration and an increased heart rate and arterial catecholamines. The cardiovascular effects of CR were postulated to be related to sympathetic nervous system effects as evidenced by the abolition of CR induced presser effects by phentolamine and 6-hydroxydopamine.

Repeated cutaneous application of CR was conducted in experimental animals 5 days week^{-1} for 12 weeks with little effect on the skin. In view of the absence of any specific organ effects, it was postulated that absorption of even substantial amounts of CR would have little effect.

Mild and transitory eye effects such as mild redness and mild chemosis were observed in rabbits and monkeys after a single dose of 1% CR solution. Multiple doses over a 5 day period, of 1% CR solution to the eye produced only minimal effects. No signs of eye irritation in animals following single or multiple dose applications of 1% CR solutions was reported while moderate conjunctivitis following the application of 5% CR solution to the eyes of rabbits was reported. Although histological examination revealed normal corneal and eyelid tissues, aerosol exposures of 10 800 and 17 130 mg min m^{-3} resulted in mild lacrimation and conjunctival injection, which cleared in 1 h, while in solution, produced reversible dose-related increases in corneal thickness. It was concluded that CR produced considerably less damage to the eye than CN, and that there was a much greater degree of safety for CR than for CN. On skin it was reported to produce only transient erythema, but did not induce vesication or sensitization, and did not delay the healing of skin injuries.

The reproductive and developmental effects of CR were studied on rabbits and rats exposed to inhalation of aerosolized CR at concentrations of 2, 20, and 200 mg m^{-3} for 5 and 7 min. Groups of animals were also dosed intragastrically on days 6, 8, 10, 12, 14, 16, and 18 of pregnancy. No dose-related effects of CS were observed in any of the parameters measured and the number and types of malformations observed. No externally visible malformations were seen in any group and no dose-related effects of CR were noted in any of the fetuses in any group. Based on the overall observations, the author concluded that CR was neither teratogenic nor embryotoxic to rabbits and rats.

The mutagenic potential of technical grade CR and its precursor (2-aminodiphenyl ether) in the various strains of *S. typhimurium* as well as in mammalian assay systems were negative in all the assays, suggesting that CR is not mutagenic. Further testing is required to exclude the genetic threat to humans, as well as to determine the carcinogenic potential and its ability to cause other chronic health effects.

CR aerosols are very quickly absorbed from the respiratory tract following inhalation. The plasma half-life ($t_{1/2}$) is about 5 min, which is about the same following intravenous administration. CR metabolism *in vitro* and *in vivo* supported the conclusions that the major metabolic fate of CR in the rat is the oxidation to the lactam, subsequent ring hydroxylation, sulfate conjugation, and urinary excretion.

Human Toxicology

Studies at the Edgewood Arsenal and other research centers have been conducted to assess the effects of CR on humans following aerosol exposures, drenches, and local application. The human aerosol and cutaneous studies conducted at Edgewood Arsenal have been summarized by the National Academy of Sciences. The respiratory effects following aerosol exposures included respiratory irritation with choking and difficulty in breathing or dyspnea, while the ocular effects consisted of lacrimation, irritation, and conjunctivitis. The effects of dilute CR solutions on humans following splash contamination of the face, or facial drench, were an immediate increase in blood pressure, concomitant with decreased heart

rate. Humans exposed to whole body drenches also faced the same effects of immediate hypertension and bradycardia. Although it was theorized that insufficient amount was absorbed to cause systemic effects, it was suggested that the cardiovascular effects resulted via the sympathetic nervous system. Additionally, solutions of CR contaminating the eye caused a transient increase in intraocular pressure. Following aerosol exposure to a mean concentration of $0.25\,mg\,m^{-3}$ CR with a particle size of $1–2\,\mu m\,h^{-1}$, the expiratory flow rate decreased ~ 20 min after the onset of exposure. The investigators postulated that CR stimulated the pulmonary irritant receptors to produce bronchoconstriction and increased pulmonary blood volume by augmenting sympathetic tone. The human LCt_{50} was estimated to be $100\,000\,mg\,min\,m^{-3}$, while the incapacitant IC_{50} was estimated to be $\sim 1\,mg\,m^{-3}$.

Oleoresin Capsicum

General Considerations

Oleoresin capsicum (OC), pelargonic acid vallinylamide (PAVA), and capsaicin are derived from the pepper plant. The ingredients in hot peppers that are responsible for 'the heat' are called capsaicinoids. Capsaicinoids are a family of chemicals and they come with various heat qualities. The mixture used contains the active ingredient capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) as well as other compounds. PAVA is a pepper derivative that is extremely hot. PAVA (capsaicin II) is the hottest of the capsaicin family.

OC is a reddish-brown oily liquid obtained by extracting dried, ripe fruit of chili peppers, usually *Capsicum annuum* or *Capsicum fruitescenes*. The oleoresin is a mixture of many compounds. Its composition is variable and depends on factors such as maturity of the fruit and the environment in which the plants are grown, as well as the conditions of the extraction. More than 100 compounds have been identified in oleoresin capsicum. Among the branched-and straight-chain alkyl vanillylamides isolated from oleoresin capsicum, capsaicin is the major pungent component in many peppers, and it is particularly noted for its irritant properties. Depending on the variety of chili pepper, the oleoresin contains from 0.01% to 1.0% capsaicinoids on a dry mass basis. Other components of the oleoresin such as phenolic compounds, acids, and esters may also possess irritant properties.

OC is considered a highly effective irritant that has received much attention as a less-than-lethal agent in civilian, governmental, and military sectors. OC spray or pepper spray has gained popularity as a law enforcement weapon in recent years. Since OC is a natural product, it is considered safe – a viewpoint not necessarily accurate.

Animal Toxicology

Not much is known about the toxicology of OC, but because it is a natural product and much utilized food component, it is considered to be relatively safe, with a low order of toxicity. The pharmacology and toxicology of capsaicin, on the other hand, have been well characterized in both animal and human studies. The acute toxicity of capsaicin in several species found capsaicin to be highly toxic by all routes of administration except gastric, rectal, and dermal. The intravenous doses of capsaicin caused convulsions within 5 s and times to death were from 2 to 5 min. The toxic signs observed included excitement, convulsions with limbs extended, dyspnea, and death due to respiratory failure. A comparison of intravenous LD_{50}s in mice with other well-known chemicals is presented in **Table 5**. This was done since there was no known comparative inhalation LD_{50}s in mice, as all of these chemicals and the intravenous route has been considered very close to the inhalation route of exposure. This demonstrates that capsaicin's acute toxicity in mice is between that of nicotine and strychnine, two well-known potent poisons. Additionally, the intraperitoneal acute toxicity of capsaicin to the oleoresin capsicum in female mice indicated the extract to be four times more toxic than the capsaicin with LD_{50}s of 1.51 and $6.50\,mg\,kg^{-1}$, respectively. Guinea pigs appeared to be more susceptible than mice and rats, while hamsters and rabbits were less vulnerable to the toxic actions of capsaicin.

The pulmonary pharmacology and toxicology of capsaicin has been studied in some detail. Inhalation of capsaicin is consistent with the induction of the Kratschmer reflex, which is apnea, bradycardia, and a biphasic fall and rise in aortic blood pressure.

Table 5 Mouse intravenous LD_{50} values ($mg\,kg^{-1}$) for various RCAs

Botulinum toxin	0.00001	Fentanyl	11.2
Ricin	0.005	Parathion	13
VX	0.012	DM	35
GB	0.10	CR	37
Nicotine	0.30	CS	48
Capsaicin	0.40	Caffeine	62
Strychnine	0.41	CN	81
Potassium cyanide	2.60	Cocaine	161
Mustard gas	3.30	Isopropyl alcohol	1509
Methamphetamine	10	Ethyl alcohol	1973

Exposure to capsaicin causes bronchoconstriction in animals and humans, the release of substance P, a neuropeptide, from sensory nerve terminals, as well as mucosal edema. The pulmonary effects of capsaicin appear to be species related. In guinea pigs, intravenous and intraarterial administration causes bronchoconstriction. The bronchoconstriction in the dog and cat following intravenous capsaicin is dependent on a vagal cholinergic reflex, as is the bronchoconstriction in the cat following aerosol exposure. In guinea pigs, the bronchoconstriction following aerosol exposure suggests both a vagal-cholinergic and noncholinergic local axon reflex. The cardio respiratory effects following intravenous administration resulted in a triphasic effect on blood pressure and altered cardiac parameters. The complex effects on the cardiovascular system consist of tachypnea, hypotension (Bezhold–Jarish reflex), bradycardia, and apnea.

If capsaicin or pepper spray is the preferred agent of choice for self-defense or riot control, it has been suggested that much research is required, and preferably compared in parallel to CS and CR. Alternatively, capsaicin or an analog could be synthesized and evaluated as a single agent, rather than a mixture with undetermined interactions of the multiple components. These components vary qualitatively and quantitatively dependent on their natural origin (variety, species, and maturity of fruit, hydrology, geology, and meteorology). Such a synthetic equivalent pelargonyl vanillamide (PAVA or Nonivamide), a potent sensory stimulant, has become available and is being used by police forces in the United Kingdom and other countries. The available data are very limited. PAVA is also used as a food flavor in quantities up to 10 ppm, and in human medicine as a topically applied rubifacient, as is capsaicin. In the United States, PAVA, as a food flavor, has been given GRAS (generally recognized as safe) status by the Food and Drug Administration.

Human Toxicology

OC has been incorporated into a variety of formulations and marketed as pepper gas, pepper mace, and pepper spray for self-defense, criminal incapacitation, law enforcement, and riot control purposes. It has also been formulated in combination with CS and CN for the same purposes. OC exposure induces involuntary closing of the eyes and lacrimation. It also causes respiratory related effects such as severe coughing and sneezing, nasal irritation, bronchoconstriction, and shortness of breath. It causes burning sensations of the skin and loss of motor control. As a result, many exposed individuals can be easily subdued. Acute effects of capsaicin and capsaicinoids cause edema, hypertensive crisis, and hypothermia. Since 1990, there have been over 100 deaths reported following the use of OC spray. Although a causal relationship has not been established, most of the reported deaths had occurred within 1 h following exposure. The causes of these in-custody deaths remain controversial, but the most common explanation is death from positional asphyxia. Other suggested causes that should be considered include excited delirium, heat prostration, drug interactions, cardiopulmonary sensitization, and the compromised Kratschmer reflex.

Capsaicin was prepared and being evaluated by the military as early as the 1920s in the United States. Interest in its development waned when CS was synthesized, and research efforts were redirected to the development of CS as an RCA. Unlike the other RCAs such as CS, CR and CN, which have definite chemical compositions, OC is a mixture of compounds containing capsaicinoids, various acids and esters, alcohols, aldehydes, ketones, and carotenoid pigments. Capsaicin as the major component is considered to be the active ingredient without consideration as to the activity or interactions of the other capsaicinoids or components. Although the activity of the other capsaicinoids is similar, they differ in potency.

PAVA or nonivamide, a synthetic equivalent of capsaicin, which is pelargonyl vanillamide, is a potent sensory stimulant and has become available and is being used by police forces in the United Kingdom and other countries. Although it is being used as a food flavor in quantities up to 10 ppm and in human medicine as a topically applied rubifacient, as is capsaicin, there are limited data available for its use as a self-defense spray or RCA. Following a pilot exercise by the Sussex police force in the United Kingdom, they and the Northampton police force, as well as some police forces in other European countries and in North American, are now using PAVA spray as an alternative chemical incapacitant to CS spray. The spray used is a 0.3% solution of PAVA in 50% aqueous ethanol and is dispersed from handheld canisters by a nitrogen propellant. The coarse liquid stream spray pattern is considered to be directional and precise. The maximum effective range is 8–15 ft, aimed at the subject's face, especially the eyes. Users are cautioned not to use it at a distance of less than 3 f in order to avoid pressure injury to the eyes. The particle size of the spray indicates that the bulk of the droplets are over 100 μm, but a small proportion is in the range of 2–10 μm, with trace amounts below 2 μm. Thus it is unlikely that large amounts of PAVA will reach the respiratory system.

Volunteers, including mild asthmatics, were exposed to aerosols of PAVA generated using a nebulizer that provides respiratory particles to study the effects on the respiratory and cardiovascular systems. The normal volunteers experienced transient cough on exposure, and minimal effects on FEV_1 (forced expiratory volume in one second (1% reduction)), heart rate (15% increase), and blood pressure (8% increase). Similar results were noted in mild asthmatics also exposed to 0.1% PAVA. These were 3% reduction in FEV_1, 5% increase in heart rate, and 5% increase in blood pressure. It was noted that, in actual use, subjects might experience a high level of stress that could lead to clinically significant bronchospasm. Experience did not indicate any significant adverse effects or any persistent harm to skin or eyes of those exposed. However, based on the animal experiments, it is an eye irritant, and thus might cause marked effects in subjects wearing contact lenses. In view of the limited data available, a complete assessment of its adverse health effects is not possible.

Diphenylaminochlorarsine

Animal Toxicology

Various animal species including monkeys have been exposed to diphenylaminochlorarsine (DM). Following acute exposures the animals exhibited ocular and nasal irritation, hyperactivity, salivation, labored breathing, ataxia, and convulsions. Histopathology did not reveal any abnormalities at exposure dosages of below $500\,mg\,min\,m^{-3}$. At higher dosages, animals that died or were killed demonstrated hyperemia of the trachea, pulmonary congestion and edema, and pneumonia. These effects were consistent to exposure to pulmonary irritants. DM toxicity values are presented in **Table 6**.

Monkeys were exposed to varying concentrations and durations. At a Ct dosage of $2565\,mg\,min\,m^{-3}$, only one animal responded, and that was with oral and nasal discharge, and diminished response to stimuli. A Ct of $8540\,mg\,min\,m^{-3}$ resulted in ocular and nasal conjunctival congestion, facial erythema, and decreased responses, all of which were resolved within 24 h. Exposure to the high dosage of $28\,765\,mg\,min\,m^{-3}$ resulted in hyperactivity, copious nasal discharge, conjunctival congestion, marked respiratory distress, as well as gasping and gagging in all of the exposed monkeys. Eight of these exposed monkeys died within 24 h of exposure. Necropsy of these animals revealed congestion and extremely edematous lungs. Microscopic examination revealed ulceration of the tracheobronchial tree and pulmonary edema. Studies were also conducted in which monkeys were exposed to low target concentrations of 100 and $300\,mg\,m^{-3}$ DM for 2–60 and 2–40 min, respectively. As the exposure duration increased toxic signs increased, characteristic of exposure to irritants. At the maximum dosage of $13\,200\,mg\,min\,m^{-3}$, the animals exhibited nausea and vomiting, oral and nasal discharge, and conjunctival congestion. Below $1296\,mg\,min\,m^{-3}$, the only signs were blinking.

Table 6 Acute toxicity of DM

Species	*LCt_{50} (mg min m^{-3})*	*LD (mg kg^{-1})*[a]
Mice	22 400	17.9
Rats	3 700	14.1
Guinea pigs	7 900	2.4

[a] Theoretical dose calculated from respiratory volume, LCt_{50}, and estimated percent retention.

The effects of DM on the gastrointestinal tract were suggested as a possible cause of death. Dogs were dosed both intravenously and orally with lethal doses of DM, while the following parameters were monitored: central venous pressure, right ventricular pressure, cortical electric activity, alveolar CO_2, respiratory rate, heart rate, electrocardiogram, and gastric activity. DM caused a marked elevation of both amplitude and rate of gastric activity for 15–20 min and then returned to normal. Pretreatment with trimethobenzamide, an effective antiemetic for peripheral and centrally acting emetics did not prevent DM gastric activity, but chlorpromazine was effective. The authors concluded that DM affects the stomach directly, and that the primary cause of death following exposure to DM is its effects on the lungs.

The effects of DM on the eyes and skin of rabbits were studied with DM suspended in corn oil instilled into the eyes of rabbits in doses of 0.1, 0.2, 0.5, 1.0, and 5.0 mg. No effect was observed at 0.1 mg, but at 0.2 mg, mild conjunctivitis was observed. At 0.5 mg, mild blepharitis was also seen. Corneal opacity persisted over the 14 day observation period in rabbit eyes that were dosed with 1.0 and 5.0 mg. Corn oil suspensions of DM ($100\,mg\,ml^{-1}$) were placed on the clipped backs of rabbits at doses of 1, 10, 50, 75, and 100 mg. At 10 mg and higher, necrosis of the skin was observed. The skin sensitization potential of DM in guinea pigs was negative.

Human Toxicology

The earliest human studies describing the effects of DM inhalation exposures date back to 1922. The effects begin with acute pain in the nose and sinuses followed by pain in the throat and chest, with

sneezing and violent coughing. Then there is eye pain, lacrimation, blepharospasm, rhinorrhea, salivation, nausea, and vomiting. Recovery is usually complete in 1–2 h after exposure. The onset of signs and symptoms is delayed for several minutes, unlike the onset for CS and CN, which is almost immediate. The slow onset for DM allows for the absorption of much more DM before a warning is perceived. Threshold concentrations were estimated for irritation of the throat, lower respiratory tract, and initiation of the cough reflex to be 0.38, 0.5, and 0.75 mg m^{-3}, respectively. Varying concentrations were tested on human subjects, and it was agreed that men could tolerate concentrations of 22–92 mg m^{-3} for 1 min or more, and with concentrations in a range of 22–220 mg m^{-3}, it appears to be intolerable to 50% of a population for 1 min. Dosages from 49 to 370 mg min m^{-3} have been estimated to cause nausea and vomiting. Inhalation of high concentrations has resulted in severe pulmonary damage and death. DM is considered less effective as a riot control or incapacitating agent than CS and CN, and it was conjectured that there is greater differences in susceptibility among people to DM than to the other agents. DM, like CS, is considered to be a cholinesterase inhibitor, which may be responsible for its lacrimatory effect. DM also has a direct effect on gastric activity, but evidence suggests that the lethal effect is respiratory.

Essential Summary

The toxicological effects, which are actually the pharmacological effects of RCAs, but are perceived as adverse or toxicological effects, can be local or topical as well as systemic following absorption. In addition, the effects can be acute or long term. Also, the exposure can be acute, long, or repeated. The disposition of the agent in the exposed individual also needs to be considered. That is, absorption, distribution, metabolism (biotransformation), and excretion (ADME). RCAs have been described as nonlethal or less-than-lethal agents. Exposure to these compounds involve ocular, dermal, and inhalation effects, and indirectly oral or gastrointestinal. Their primary action is the local or topical effect on the eye, which appears to be the most sensitive target organ. They also act on the skin and respiratory tract. The immediate effects on exposure to these irritants include intense irritation of the eyes, marked irritation of the nose, throat, and lungs, as well as irritation of the skin. The margin of safety or the safety ratio between the dose eliciting the intolerable effect and that dose which causes serious adverse effects is large. Examples of these are presented as human estimates for incapacitation concentrations (IC_{50}) and lethal dosages (LCt_{50}) in **Table 7**.

Table 7 Estimates of IC_{50} values (mg m^{-3}) and LCt_{50} values (mg min m^{-3}) for various RCAs

Agent	*LCt_{50}*	*IC_{50}*	*Safety ratio (LCt_{50}/IC_{50})*
CN	8 500–25 000	20–50	425–500
CR	>100 000	−1	100 000
CS	25 000–150 000	5	5 000–30 000
DM	11 000–35 000	20–150	550–233

Relevant Ocular and Cutaneous Effects

Exposure to RCAs causes an immediate stinging sensation in the eyes and tearing, resulting in a temporary disabling effect. These effects are reversible and noninjurious at low concentrations. At high concentrations, however, some irritants can cause ocular damage. Moderate injury to the eye following exposure results in corneal edema, which is reversible. Most serious injury may include corneal opacification, vascularization, scarring of the cornea, and corneal ulceration. Ocular injuries are more prevalent following use of explosive- or thermal-type tear gas devices as contrasted with solvent spray-type devices (**Table 8**).

RCAs at low concentrations also produce a tingling or burning sensation and transient erythema of the skin. At higher concentrations, agents such as CN, CS, and DM can cause edema and blistering. They can also induce an allergic contact dermatitis after an initial exposure. These effects are successfully treated

Table 8 Estimates for human ocular sensory irritancy

Compound	*Onset/action*	*Threshold concentration (mg m^{-3})*	*Intolerable concentration (mg m^{-3})*	*10 min exposure lethal concentration (mg m^{-3})*
CN	Immediate	0.3	5–30	850
CR	Immediate	0.002	1	10 000
CS	Immediate	0.004	3	2 500
DM	Rapid	1	5	650
Acrolein	Rapid	2–7	50	350
OC	Rapid			

with topical steroid preparations and oral administration of antihistamines for itching. Appropriate antibiotics can be administered to treat secondary infections.

RCAs do not usually cause long-term or permanent toxic effects, although the risk for serious toxic effects, long-term sequelae, or even death increases with higher exposure concentrations and greater exposure durations, in enclosed spaces or in susceptible individuals. Overall, however, the toxicity of acute and short-term repeated exposures to RCAs is well characterized.

See also: Bio Warfare and Terrorism: Toxins and Other Mid-Spectrum Agents; Blister Agents/Vesicants; Chemical Warfare During WW1; G-Series Nerve Agents; Nerve Agents; V-Series Nerve Agents: Other than VX.

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Risk Assessment, Ecological

Steven Bartell

Introduction

Managers and decision makers face daunting challenges in solving complex environmental issues associated with the ever increasing pressures that humans place on valued natural resources and their life-sustaining ecosystems. These challenges are made difficult by the large number and diversity of human disturbances and perhaps even more difficult by the complexity and dynamics of imperfectly understood natural ecological systems. The process of ecological risk assessment (ERA) was designed to address ecological complexity and incorporate uncertainty in assessing the impacts of disturbances on ecological resources.

ERA applies methods of systems analysis to integrate ecology, environmental chemistry, environmental toxicology, geochemistry, hydrology, and other fundamental sciences in estimating the probabilities of undesired ecological impacts. In theory, ERA applies to both human-induced and natural disturbances. ERA can be viewed as a subset of basic disturbance ecology. However, in practice, most of the ERAs derive from specific needs to assess human-induced impacts on the environment. Many ERAs conducted in the United States are motivated by legislation, including the National Environmental Policy Act (NEPA), the Toxic Substances Control Act (TSCA), and the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA or 'Superfund'). Other ERAs are undertaken by private industry to determine future ecological risks and potential liabilities associated with the development, use, and disposal (i.e., life-cycle) of new or existing products (e.g., herbicides, pesticides, industrial chemicals).

Several different approaches for performing an ERA have been proposed both nationally and internationally. While no single methodology has been officially sanctioned, the approach outlined by the US Environmental Protection Agency is being increasingly used to guide ERAs in the United States. The following discussion focuses, therefore, on the US EPA methodology, which consists of four basic components: problem formulation, exposure analysis, effects assessment, and risk characterization. This presentation will attempt to minimize the use of jargon that has proliferated during the evolution of the ERA methodology. Thus, the discussion might

not correspond in exact detail to the ERA methodology, although the major aspects of this approach will be described.

Problem Formulation

This initial and perhaps most important part of the assessment defines the nature and scope of the ERA, describes the sources of potential risk ('stressors'), identifies the ecological resources at risk ('endpoints'), considers the nature of the ecological impacts in relation to the stressors, and produces a conceptual model of the overall assessment. Thus, problem formulation essentially encapsulates the entire ERA process. Execution of this step requires collaboration among risk managers and risk assessors to define the assessment objectives and develop the corresponding conceptual model. This model, like most, should be viewed as dynamic and subject to change throughout the ERA in relation to modifications to the objectives and the development of new data and information.

Much of the original emphasis on ERA concerned the ecological effects of toxic chemicals. Importantly, the continuing evolution of ERA frameworks reflects the recognition that physical, geological, hydrologic, and biological stressors can produce undesired ecological effects. Examples of these kinds of stressors include physical habitat degradation, erosion of soils or sediments, drought/floods, and introductions of exotic species. Therefore, the set of stressors addressed by ERA has expanded to include other kinds of disturbance, some of which are influenced by human activities (e.g., introduction of exotic species, hydrology, climate change).

In assessing risks posed by toxic chemicals, 'exposure' (see exposure analysis below) refers to mechanisms of direct contact, ingestion, inhalation, or indirect accumulation through the consumption of contaminated food. The concept of exposure has been expanded to include analogous pathways and mechanisms that define the intersection in space and time of other stressors with individual organisms or their ecological support systems.

The large number and different kinds of ecological effects that are of potential concern distinguish, in part, ERA from more traditional human health risk assessment. An ERA might address alterations in basic physiological processes (e.g., photosynthesis, respiration), lethal or sublethal (i.e., growth) effects on individual organisms, changes in population dynamics (e.g., growth rate, fluctuations, local extinction), alterations in community structure, species diversity, ecosystem function (e.g., primary production, total system respiration, decomposition, nutrient cycling), and landscape-level impacts (e.g., habitat distribution). ERAs commonly identify more than one kind of ecological effect of concern in problem formulation. The ecological effects of concern identified during problem formulation should be ecologically important, sensitive to the stressor(s), and relevant to risk management.

Following construction of the conceptual model, problem formulation continues by developing a plan to implement the conceptual model of the ERA. The resulting analysis plan further characterizes the stressors, identifies specific ecological effects of concern, and identifies applicable data, as well as measures or models that can be used to quantitatively relate the stressors to the expected ecological effects.

The initial interactions between risk managers and risk assessors might also involve other organizations and concerned members of the public ('stakeholders'). Initial discussions can help ensure that all important aspects of the assessment are identified, included as part of the problem formulation, and represented in the conceptual model. Such interactions can also ensure that the kinds of results produced by the ERA can be used effectively in the process of risk management and decision-making. The US EPA approach argues for the separation of risk managers and risk assessors during the course of risk estimation. Upon completion of risk estimation, risk managers, risk assessors, and stakeholders may reconvene to discuss the nature and interpretation (e.g., conclusions, assumptions, caveats) of the results in the context of the overall assessment objectives. Possible outcomes of these interactions include revisions to the conceptual model, collection of new data, and subsequent iterations of risk estimation until the ERA needs of risk managers and decision-makers are fulfilled.

Exposure Analysis

Exposure is analyzed through characterizing the processes and mechanisms that bring organisms into contact with the stressor(s) of concern and quantifying the frequency, magnitude, and duration of such contact. Clearly, the nature of the stressor(s) and the kinds of ecological effects of concern will strongly influence the exposure analysis. Each identified stressor will suggest a relevant spatial and temporal scale for analysis. The scales might be local and relatively short-term, as for accidental spills of toxic, yet readily degraded or volatilized chemicals that result from hazardous waste management. Conversely, some stressors (e.g., fire, climate change) can exert ecological impacts over large expanses and for durations that greatly exceed the generation time of most

organisms. Stressors are also evident at intermediate scales, for example, major oil spills (e.g., Exxon Valdez) and certain exotic species (e.g., gypsy moth, zebra mussel, Asian long-horned beetle).

The nature of specific stressor(s) can provide information concerning the processes or mechanisms of exposure that will have to be evaluated in an ERA. Chemical contaminants introduced into the environment are naturally transported by the movements of wind and water. Certain chemicals can accumulate in organisms and be transmitted throughout complex food webs. Some organic chemicals are rapidly sorbed to soils and sediments, while others effectively remain in solution. Natural movements of biological stressors might be augmented by private and commercial transportation (e.g., cars, trucks, ships, airplanes) systems.

The kinds of ecological effects included in the conceptual model can also provide insights into exposure analysis for an ERA. Organisms occupy certain dimensions in space and time. Habitats have measurable spatial extent; ecological processes exhibit characteristic rates. Such observations can guide the analysis of exposure. For example, knowledge of the timing and duration of a sensitive life stage (e.g., eggs, larvae) can focus the corresponding measurement of stressors of concern and provide more meaningful quantification of exposure than longer term averages or monitoring that might completely miss the necessary time period for measurement. Seasonal changes in light, temperature, precipitation, and other physical factors can result in spatial–temporal variability in exposure. The important point is that variability in both the processes that influence the stressor and the characteristics of the ecological entities should be addressed in developing a meaningful analysis of exposure.

Alternative approaches can be used in a sequential manner to assess exposure. Worse-case scenarios can be developed that assume maximum values of the stressor. For example, 'end-of-pipe' concentrations of toxic chemicals can be used without accounting for physical dilution, chemical alterations, or biological degradation that would otherwise reduce the concentrations experienced by the organisms of concern. This approach can overestimate risk. If acceptable risks were estimated using these extreme exposures, the assessment process might reasonably be terminated. As an alternative to worse-case scenarios, exposures might be measured. Actual measures of exposure are undoubtedly the most easily defended scientifically (presuming competent sampling and analysis) and the most realistic inputs to an ERA. Finally, exposures might be estimated using physical (e.g., microcosms, mesocosms) or mathematical models. For example, several models have been developed and used to simulate the transport, fate, and distribution of toxic chemicals in the environment.

The product of exposure analysis is an exposure profile. For chemicals, the profile should include the nature of the source; pathways of exposure; environmental media of concern (e.g., soils, water, sediments, contaminated biota); exposure concentrations (magnitude, timing, duration, recurrence); and uncertainties associated with these exposures. Analogous exposure profiles would be developed for nonchemical stressors included in an ERA.

Effects Assessment

This component of the overall ERA methodology develops the functional relationships between the stressors and the selected ecological responses. The stressor–response functions are central to ERA. In short, ERA can be described as the development and application of uncertain stressor–response functions in assessing ecological impacts. The functions should estimate the severity of the ecological response in relation to the magnitude, frequency, and duration of the exposure. The derivation of stressor–response functions depends on the quantity and quality of available data.

Sources of data that might be used in the construction of stressor–response functions include: the results of toxicity tests (lethal, chronic) performed under controlled laboratory conditions, direct measures of exposure and response in controlled field experiments, and the application of statistical relationships that estimate the biological effects of chemicals based on physical or chemical properties of specific toxicants. The order of preference among these sources of data lists field observations as the most valuable, followed by laboratory toxicity tests, and finally by the use of empirical relationships. In the absence of directly relevant data, the development of stressor–response functions may require the use of extrapolations among similar stressors or ecological effects for which data are available. For example, effects might have to be extrapolated from the available test species to an untested species of concern in an ERA. Similarly, toxicity data might be available only for a chemical similar to the specific chemical stressor of concern in an ERA, and thereby require an extrapolation from one chemical to another to perform the assessment.

Risk Characterization

Risk characterization combines the exposure profiles with the stressor–response relationships to estimate

ecological risks in ERA. A variety of methods and tools are available for risk estimation. For assessing risks posed by toxic chemicals, one simple method simply divides the exposure concentrations by the toxicity reference values. Quotients equal to or greater than 1.0 imply risk; quotients less than 1.0 suggest minimal or no risk. Such quotients can prove useful in initial screening-level assessments to reduce the number of stressors that should be analyzed in greater detail. The screening assessments may be particularly effective if exposure estimates used in risk characterization are biased toward overestimating risk. This approach is limited in the context of using single-value estimates of exposure and toxicity to estimate risk.

Depending on the availability of data, distributions of exposure and toxicity can be constructed and compared. Risk can be estimated by comparing the degree of overlap between these distributions: the greater the overlap, the higher the risk. Using comparisons of distributions in screening-level assessments can extend the single-value quotient approach by including more information, including uncertainty, in the estimation of risk.

Experiments under field conditions or more controlled conditions in the laboratory (e.g., microcosms, mesocosms) can be used to characterize ecological risks. Experimental systems provide opportunities to physically impose the stressors of interest on the ecological resources of concern. Such experiments may be the only practical method for assessing risks posed by stressors not intended to be introduced into the environment. This approach may also prove essential in assessing risks posed by stressors that are virtually unknown or whose attributes are proprietary.

Mathematical and computer simulation models can be used to estimate ecological risks. There was a comprehensive review and evaluation of the existing ecological models for potential application in assessing ecological risks. Following decades of model construction in support of basic research and development, it stands to reason that some of these models might prove useful in estimating ecological risks posed by various stressors on individual organisms, populations, communities, and ecosystems. The critical aspect in adapting these models for assessing risk is the ability to derive a stressor–response relationship for the stressor(s) and ecological impacts of interest.

Uncertainty

Risk implies uncertainty. ERA was designed expressly to include uncertainty as an integral component of the assessment process. Sources of uncertainty include natural variability in ecological and environmental phenomena, as well as bias and imprecision associated with the stressor–response functions. This latter source of uncertainty can be exacerbated if extrapolations were involved in the derivation of the functions (e.g., laboratory to field, across species).

Uncertainties inherent to the risk assessment process can be quantitatively described using, for example, statistical distributions, fuzzy numbers, or intervals. Corresponding methods are available for propagating these kinds of uncertainties through the process of risk estimation, including Monte Carlo simulation, fuzzy arithmetic, and interval analysis. Computationally intensive methods (e.g., the bootstrap) that work directly from the data to characterize and propagate uncertainties can also be applied in ERA. Implementation of these methods for incorporating uncertainty can lead to risk estimates that are consistent with a probabilistic definition of risk.

Methods of numerical sensitivity and uncertainty analysis can be used to examine uncertainty and identify the key sources of bias and imprecision in quantitative estimates of risk. Once identified, limited resources (e.g., time, funding) can be efficiently allocated to obtain new information and data for those major sources of uncertainty and reduce it. These analyses can be repeated until uncertainties associated with the risk estimates are of an acceptable degree or until uncertainties cannot be further reduced.

Risk Communication and Management

If carefully crafted during problem formulation, the risk estimates derived from the previous step will provide information compatible with the process of risk management. The nature of the risk estimates should also facilitate their description and interpretation to stakeholders. Successful risk communication and management will likely require the risk assessors to again collaborate with managers and decision-makers to ensure proper interpretation of the risk estimates. Such collaboration can importantly help in developing and evaluating alternative management actions directed at the original goals and objectives of the ERA. Finally, discussions among risk managers and risk assessors can also lead to revision of goals and objectives, modifications of the conceptual model, and subsequent iteration of the risk assessment process.

See also: Cumulative Risk Assessment; Ecotoxicology.

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Risk Assessment, Human Health

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Risk assessment is a process that can be used to qualitatively and/or quantitatively evaluate the potential for an event or events to occur. The process can be used to gain a better understanding of potential risks associated with a broad range conditions, including the following:

- risk of adverse health effects occurring after chemical or radiation exposure;
- risk of injury and/or death during air, highway, or rail travel; and
- risk of certain catastrophic events occurring, such as nuclear accident, industrial accident, or earthquake.

The following discussion focuses on the use of the process to better understand the potential for chemicals and agents to adversely impact human health. Typically, this process integrates science, science policy, and specific methodologies as defined under policy or regulatory mandate to identify and characterize risks. Used as a predictive tool, the risk assessment process generates information that can support risk management and decision-making. Typically, risk management is regarded as distinct from the risk assessment process, and decision-making is

based on an integration of the results of the risk assessment and other considerations. Other considerations may include engineering data; potential social, economic, and political impact; general feasibility; and cost–benefit analysis.

Evaluating the potential for exposure to a chemical or chemicals to pose an unacceptable risk of causing harm to people can be complex. The environment is composed of chemicals (e.g., soil, water, air) and living things are composed of chemicals and sustained by chemicals (e.g., food, drink, air). The bottom line is balance. An old toxicological adage states, the dose makes the poison. Almost any chemical can cause harm if the dose is high enough. For example, chromium at very low concentrations is a micronutrient and necessary for good health. At high enough concentrations it can cause a broad range of adverse effects and has been associated with an increase in the risk of cancer. The likelihood that chemical exposure will cause harm and the severity of effects depends on the inherent toxicity of the chemical, the individual' sensitivity to the effects of the chemical, the level of exposure (concentration in contact medium and/or amount absorbed into the body), and how long the individual is exposed (duration of exposure). Generally, the shorter the duration of exposure, the higher the concentration needs to be to cause harm. 'Poisons' are generally chemicals that can cause harm at very low doses in a relatively short time frame.

Generally, human health risk assessment is used to evaluate circumstances where the 'normal condition' has been changed or may change and there is a need to understand the potential health consequences of the change. Risk assessments may be used by regulators as well as the regulated community to more effectively manage benefits and risks and can answer questions like the following:

- Is it likely that the use a of particular pesticide at levels needed to control pests on particular crops will cause harm to end users (e.g., use on food crops and use on plants as tobacco and cotton).
- Could exposure to a specific chemical over a short term (e.g., accidental exposure, large-scale incidents) or over longer periods (such as residual impacts to environmental media like soil, air, and groundwater) cause significant health problems?
- How much of the chemical would someone have to be exposed to before it is likely to cause harm?
- What additional lifetime risk of cancer is associated with exposure to a specific chemical or chemical mixture?
- What levels of particular chemicals in drinking water are acceptable within a particular regulatory context?

Risk assessments may need to be completed by a team of specialists, including toxicologists, epidemiologists, physicians, biologists, chemists, fate and transport specialists, and engineers. Typically, a regulatory mandate will dictate in what circumstances and which chemicals need to be evaluated and controlled. For example, at the federal level US Environmental Protection Agency (US EPA) administers the Safe Drinking Water Act (SDWA), which provides for limits on certain chemical contaminants in drinking water, the Clean Air Act, which regulates chemical emissions into the ambient air, and the Resource Conservation and Recovery Act, which regulates hazardous waste handling, storage, and disposal. The Occupational Safety and Health Administration (OSHA) administers the Occupational Safety and Health Act (OSHAct) and regulates human exposure to chemicals in the work environment. The regulatory framework generally defines what is acceptable and what is not.

There is an ongoing effort to improve the risk assessment process. Changes often provide for integration of more of the underlying science to reduce uncertainty as well as exploring new approaches to evaluating risk. The following are examples of areas of current interest and activity.

- The use of physiologically based pharmacokinetic models (PBPK) to translate applied dose in animal studies to predict dose and risk in humans is increasing. Many regulatory programs recommend use of PBPK modeling when data and information are adequate.
- Consideration of effects of chemical exposure in sensitive populations with a focus on children's health is currently being evaluated by a number of regulatory agencies. There is new guidance and recommendations for addressing these issues in the risk assessment process.
- The standard approaches for using dose–response relationship information in the risk assessment process is the focus of ongoing efforts. Guidance provides for use of a point of departure when data are appropriate. The benchmark dose approach is commonly used.
- The consideration of mode of action in carcinogen risk assessment is becoming standard practice. When data are adequate to demonstrate use of the standard default low dose extrapolation models such as the 'linearized multistage model is not appropriate, alternate approaches, including threshold approaches are now being used.
- New guidance is available for addressing cumulative risk and looking at the potential consequences of exposure to mixtures.

National Academy of Science's Paradigm

The risk assessment process, as used to evaluate the impact of chemicals on human health, was formally defined in the National Academy of Science (NAS) 1983 report, *Risk Assessment in the Federal Government: Managing the Process* (the 'Red Book'). The study on which the report was based was carried out by a committee of the National Research Council (NRC) Commission on Life Sciences with support from the Food and Drug Administration. The NRC is a principal operating agency of the NAS. The report was developed to provide the federal government with a systematic approach for evaluating risks to human health associated with chemical exposure. The NAS framework was designed to strengthen the reliability and objectivity of the scientific basis of risk assessment as well as ensure that the best scientific data were integrated into the process and to ensure that there was consistency in the approach used by federal agencies. It was intended to minimize controversy and allow for more consistent and rational decision-making with regard to human health. The process was defined in broad and general terms and has also been applied to evaluation of risks associated with other organisms (e.g., wildlife) and the environment. The NAS divided the process into four major steps: hazard identification, dose–response assessment, exposure assessment, and risk characterization.

Hazard Identification

Hazard identification is the step in the risk assessment that qualitatively characterizes the inherent toxicity of a chemical. Scientific data are evaluated to establish a possible causal relationship between the occurrence of adverse health effects and chemical exposure. This step includes characterization of acute, subchronic, and chronic effects; the potential for local versus systemic effects; the influence of the route of exposure; the relevance, to humans, of effects seen in animals; an evaluation of the biological importance of the observed effects; the likelihood of the effects occurring under certain conditions; and the potential implications for public health. This step should be based on a thorough review of all the data that may provide information that is relevant to evaluating the potential chemical hazard. This may include data describing the effects on a variety of test animals, *in vitro* studies that characterize mechanisms of toxicity, metabolism, physiologically based pharmacokinetic studies, structure–activity relationships, short-term human studies, and epidemiological studies. Animal studies may focus on particular types of effects and may include reproductive toxicity studies, immunotoxicity studies, neurotoxicity studies, genotoxicity studies, and cancer bioassays. Each study must be evaluated with respect to quality, design, interpretation of the data, and statistical considerations to ensure that conclusions are valid before they can be integrated into the assessment.

Dose–Response Assessment

Dose–response assessment characterizes the quantitative relationship between exposure (usually determined in toxicity studies) and the occurrence of adverse health effects. Typically applied or administered dose, rather than effective tissue dose, is used to develop the dose–response relationship. As a rule, the higher the dose, the greater the frequency or intensity of the adverse reaction to a chemical. Often, different effects are observed at high and low doses. The approaches used to extrapolate the dose–response relationship from high experimental doses administered to relatively few animals used in laboratory animal studies to relatively low-dose human exposure anticipated to occur in the environment (e.g., via ambient media) vary and are critical to assessing potential risks. For noncarcinogenic effects and certain nongenotoxic carcinogens the lowest dose or doses at which no adverse effects are identified (or, if not available, the lowest dose at which adverse effects are observed) in the most sensitive species are commonly used as the basis for setting what are anticipated to be reasonably safe exposure levels (doses associated with acceptable risk) under certain conditions. For carcinogens regulated based on excess lifetime cancer risk, extrapolations are made from the incidence of cancer found at the high experimental doses to the doses expected to be associated with human environmental exposures.

In general, under US regulatory programs the results of the dose–response assessment usually differ depending on whether carcinogenic or noncarcinogenic effects are being assessed. For carcinogens, the outcome is an estimate of the potency of the chemical; that is, the probability of a certain incidence of cancer associated with a given dose. In contrast, for noncarcinogens, the assessment leads to an acceptable daily intake value. In the United States, this is a 'reference dose' (RfD) or 'reference concentration' (RfC); in other countries, this may be a 'tolerable daily intake' or 'acceptable daily intake'. In many regulatory programs around the world both carcinogens and noncarcinogens are evaluated using a 'threshold' approach.

Practices are constantly changing to provide for the use of as much scientific information in the risk assessments as possible. Current practice provides for

using a 'threshold' approach to developing exposure criteria if there is an adequate understanding of the mode of action. If the chemical is not mutagenic and does not cause cancer by heritable genotoxic lesions, a nonthreshold approach is deemed more appropriate. More detail is provided in US EPA's updated guidance for carcinogen risk assessment.

Toxicity values developed in the above described process are used to develop exposure criteria. Exposure criteria in a particular media represent levels of exposure at which the risk of adverse effects occurring are determined to be acceptable under a particular regulatory framework or as defined by a particular body (e.g., World Health Organization). Exposure criteria may be expressed as media concentrations (e.g., milligrams of chemical per kilogram of soil, liters of drinking water, or cubic meters of breathing zone air) or in terms of dose (e.g., milligrams of chemical per kilogram of body weight of the animal per day).

Exposure Assessment

Exposure assessment qualitatively and quantitatively characterizes the potential for exposure to occur in particular circumstances and includes an estimate of dose when possible. The assessment includes an estimation or measurement of chemical concentration in the contact media (e.g., soil, water, air, a particular food crop, a consumer product), an estimation of the length of time over which contact will occur, characterization of potential routes of exposure (inhalation, ingestion, and skin contact), and the likelihood for a chemical to be absorbed through those routes. In certain circumstances, direct measurements or fate and transport modeling may be used to estimate chemical concentrations in ambient media. For certain assessments, a quantitative estimate of the total dose of a chemical over a particular time frame and in the given circumstances is made.

Risk Characterization

Risk characterization provides for both qualitative and quantitative descriptions of risk. The step involves integrating the results of the hazard identification, dose–response assessment, and exposure assessment to characterize risk. Often, a direct comparison between exposure criteria developed in the first two steps and the results of the exposure assessment (concentration in the environmental media or the estimated dose, as appropriate) provide a basis for determining whether risks are acceptable. Typically, if criteria are exceeded, the risk is not acceptable. What is defined as acceptable, as well as the way risk is expressed, is often a function of the agency, law, and/or regulation that drives the analysis. Risk may be expressed as excess cancer risk, hazard index, or in terms of a margin of safety. The risk characterization must incorporate considerations of the uncertainties in the assessment. Each step of the risk assessment process contributes uncertainty and uncertainties must be clearly defined and integrated into the conclusions of the assessment.

Risk Management

Risk management was defined in the NAS report as the process of weighing policy alternatives and selecting the most appropriate regulatory actions. It is considered to be separate from the risk assessment process. Risk management decisions are based on the results of the risk assessment and other concerns that are relevant to the situation.

Human chemical exposure is regulated under a number of different laws in the United States, including SDWA, OSHAct, CERCLA, Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Toxic Substance Control Act, Hazardous Substance Act (HSA) (TSCA), Clean Air Act, and many state laws. These laws are administered by different agencies (e.g., US FDA, US EPA, OSHA, Consumer Product Safety Commission, and US Department of Agriculture). The approaches and problems associated with estimating exposure and risk, defining acceptable risk, and developing exposure criteria differ both under different regulatory mandates and the circumstances in which exposure is likely to occur.

The risk assessment process as described in the NAS paradigm identifies the critical information needed to evaluate risk in the broad sense; however, the resources expended to obtain the information defined in each step may vary in different circumstances. For example, for a new drug, a dose that provides therapeutic benefits and has an acceptably low risk of significant side effects must be identified prior to acceptance for use on the general public. The hazard identification and dose–response assessment steps are likely to be resource intensive. Drugs must be tested in a series of animal tests and clinical trials. The risks of side effects occurring (severity and likelihood of occurrence) have to be well characterized and balanced against the benefit derived from use of the drug. The exposure assessment portion of a risk assessment conducted to evaluate risks associated with environmental contamination regulated under certain state or federal laws (e.g., CERCLA) may be much more resource intensive than the hazard identification and dose–response assessments. The nature and extent of contamination in each environmental

media (e.g., soil, groundwater, and water) and the likelihood of human exposure has to be evaluated. Characterization of chemical concentrations in environmental media may require extensive environmental sampling and complex fate and transport modeling.

Exposure criteria are used in many regulatory programs and may be used as legally binding standards or provide guidance. A number of government agencies and other organizations develop criteria that are useful in different circumstances. These include OSHA (permissible exposure levels), American Congress of Governmental Industrial Hygienists (ACGIIH) (threshold limit values), Agency for Toxic Substances and Disease Registry (ATSDR) (minimal risk levels), US EPA (maximum contaminant levels), Health Canada, and the World Health Organization's International Programme on Chemical Safety.

Risk Assessment at Sites of Environmental Concern

US EPA's Approach to Assessing Human Health Risks Associated with Environmental Contamination under Superfund

Chemicals may be introduced into the environment (e.g., air, soil, surface water, and groundwater) during ongoing industrial and commercial activities, improper chemical handling, accidental spills and releases, as well as the routine disposal of chemical wastes by communities (e.g., household cleaners and solvents).

The risk assessment process has been used to identify situations in which contamination of the environment results in significant risks. CERCLA (Public L. No. 96-510, 40 CFR 300) was enacted on December 11, 1980, and gave the federal government the authority to act when hazardous substances were released (or could be released) into the environment in quantities that had the potential to endanger the public health. It provided for a system to clean up hazardous waste (e.g., spills, leaks, and abandoned dumpsites), for immediate reporting of chemical releases over specific amounts (reportable quantities), for fines and penalties, for a trust fund to pay for remediation at contaminated sites, and for recovering costs of remediation when the responsible party or parties can be identified. The Superfund Amendments and Reauthorization Act (SARA) to CERCLA was passed in 1986 and provided for more stringent and permanent remedies at sites regulated under the law.

Under CERCLA (sometimes referred to as the 'Superfund Law'), actions taken to remediate environmental contamination must protect both human health and the environment. US EPA administers CERCLA and has used the risk assessment process to characterize potential risks at sites of environmental concern. US EPA has developed guidance for conducting quantitative human health risk assessments at Superfund sites. Guidance documents include: *Risk Assessment Guidance for Superfund* (RAGS) originally published in 1989, *Human Health Evaluation Manual and Supplemental Guidance*, and *Exposure Factors Handbook*. This guidance was intended for use during the remedial investigation/feasibility study process at Superfund sites but has been widely used to address sites regulated under other environmental laws. The guidance continues to be updated and expanded to reflect changes in policy and approaches to evaluating risk.

US EPA's approach, as defined in RAGS, incorporates the principles defined in 1983 by NAS. The US EPA RAGS identifies four steps in an environmental risk assessment: data collection and evaluation, exposure assessment, toxicity assessment, and risk characterization. Tasks involved in characterizing the environmental media have greater emphasis because they often require tremendous resources and time.

Data collection and evaluation involves characterization of the concentration of contaminants in the media (e.g., soil, groundwater, and air) at the site in question. It includes the collection of samples to characterize soil and groundwater at contaminated property. This phase of a risk assessment may be complex and require significant resources but it is critical to providing the data needed to support the exposure assessment.

Exposure assessment includes both qualitative and quantitative evaluations of the potential for exposure to site-related chemicals to occur. Assessments commonly address both current and likely future uses of the property (e.g., residential, commercial, industrial, and agricultural). Typically, a conceptual model is developed that summarizes how site-related chemicals may contact receptors (e.g., humans, wildlife, and ecological). The model includes identification of chemical sources, impacted media, potential movement through the environment, identification of the appropriate exposure scenarios, and identification of the points at which contact between receptors and site-related chemicals are likely to occur. Chemical concentrations in environmental media may be estimated based on site data and using statistical analyses and/or fate and transport modeling. An estimate of the dose (intake) attributable to contact with environmental media through significant and completed pathways is made for chemicals of concern at

the site. This estimate is based on an estimation of the amount of time over which contact will occur, characterization of potential routes of exposure (ingestion, inhalation, and skin contact), and the likelihood for the chemical to be absorbed from the contaminated media through those routes.

Toxicity assessment includes characterization of the toxicity of a chemical, development of a dose–response relationship, and ultimately the development of exposure criteria. Toxicity values express a dose that is associated with either a given risk of cancer occurring over a lifetime of exposure (e.g., slope factors and unit risks) or a dose that is not expected to cause harm (e.g., RfDs). Some toxicity values are used as the basis for developing exposure criteria (RfDs) and some can be used as exposure criteria (e.g., RfCs). US EPA has developed toxicity values for many chemicals commonly associated with environmental contamination. Verified US EPA criteria are available in the Integrated Risk Information System (IRIS).

The toxicity values and criteria developed in the toxicity assessment combine the approaches and procedures described in the hazard identification and response steps described in the NAS paradigms. Because criteria are typically provided by regulatory agencies, for chemicals commonly identified as a concern the effort associated with this step for a given site may be limited. However, if conditions on the site appear to be unacceptable, these may be refined as part of the process.

Risk characterization includes a comparison between toxicity values and/or exposure criteria and exposure (dose or media concentration) to determine whether the exposure is acceptable. US EPA developed a formalized system that is commonly used to determine whether chemicals are likely to present an unacceptable risk based on current and likely future use of the property. The estimated dose is used to calculate an additional lifetime cancer risk for each chemical regulated as a carcinogen. Typically, a total site risk (sum of the risk associated with all carcinogens identified at the site) is presented. Acceptable risk is defined by the agency, in the appropriate laws, or by regulations that govern the site. Acceptable risk is a function of policy or law but is supposed to be rooted in science.

The contamination levels at which noncarcinogenic effects are likely to occur are also evaluated. Total dose is compared to a dose that is considered likely to be safe (exposure criteria; e.g., RfD). In a quantitative assessment the site-related dose is divided by the criteria and the resulting fraction is defined as the hazard quotient. This simply indicates whether the hypothetical dose exceeds the threshold criteria identified as likely to be safe. The hazard quotients for all chemicals at the site are summed and presented as a hazard index for the site. If the total does not exceed 1, it is assumed that the dose attributed to the site will not exceed the criteria and the potential exposure is deemed acceptable. If the hazard index for a site exceeds 1 (hypothetical dose exceeds criteria), a more refined analysis can be completed. Chemicals can be grouped according to target organ and a refined set of indexes can be developed for the site. The sum of the fractions for each target should not exceed 1. Uncertainties associated with each step in the process must be clearly defined and integrated into the conclusions of the assessment.

US EPA's approach is essentially an application of NAS', tailored to provide guidance for assessing risk associated with contamination in environmental media. Adverse health impacts associated with exposure to the chemical of concern are identified through the hazard identification, dose response, and toxicity assessment. Exposure is evaluated during the exposure assessment and data collection and evaluation steps.

Risk management is the decision-making process that follows the completion of a risk assessment. The risk assessment provides important information that supports decision-making and is integrated with other factors, including economic, feasibility, and cost–benefit analysis, in the risk management process.

See also: Carcinogen Classification Schemes; Dose-Response Relationship; Exposure Assessment; Exposure Criteria; Hazard Identification; Risk Assessment, Ecological; Risk Based Corrective Action (RBCA); Risk Characterization; Risk Communication; Risk Management; Uncertainty Analysis.

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Risk Based Corrective Action (RBCA)

Shawn L Sager

Introduction

Risk-based decision-making can be utilized to focus both remedial measures and limited funds while being protective of human health and the environment (i.e., prioritize) and to facilitate timely closure of impacted sites. The approach combines the information gathered during a site investigation together with data on the health effects of the site-related constituents to evaluate whether a particular site requires remedial action. A risk assessment demonstrating protection of human health and the environment can be helpful in determining if, and to what degree, active remediation is warranted at a site, and whether active remediation may be discontinued prior to removing all constituents from a medium at a site. Therefore, considerable savings can be realized while protecting human health and the environment.

A risk-based decision-making approach toward remediation is increasingly becoming an integral component in most regulatory programs under supervision by both federal and state agency personnel. American Society for Testing and Materials (ASTM) International committees have prepared Risk Based Corrective Action (RBCA) standards for evaluating petroleum sites (E 1739-95), chemical release sites (E 2081-00), and ecological resources (E 2205-02). RBCA is a process that quantifies: (1) the potential risks to identified receptors associated with exposure to site-related constituents, or (2) site-specific remediation goals for impacted media that are protective of human health if exposure to the identified receptors occurs. The process integrates components of the site assessment, risk assessment, risk management, and corrective action into a holistic site-specific approach that is consistent and technically defensible while still being practical and cost effective.

Sites with limited impacts do not require an extreme level of analysis. Utilizing RBCA to identify the project termination point minimally involves conducting an exposure assessment to identify complete exposure pathways by which receptors (people and/or the environment) could potentially be exposed to site-related constituents. Sites with a minimum amount of contamination can be handled through a Tier 1 Lookup Table, representing a health-protective generic screening approach. A majority of service station sites probably will be addressed through a Tier 2 quantitative approach involving assumptions of realistic current and future site use, simplistic fate and transport analysis, and health-protective site-specific exposure parameters. For those sites where multiple sources exist in a complex setting, a more detailed and comprehensive risk assessment may be warranted (Tier 3).

Background

RBCA was developed initially for underground storage tank (UST) programs. State programs developed

or adopted regulatory cleanup standards based on the needs of other programs. In some states, a standard for total petroleum hydrocarbons (TPH) was adopted based on intuition rather than on protection of human health and the environment explicitly. Finally, state agencies were finding that the procedures adopted for other programs did not necessarily fit UST programs.

There is a federal statutory requirement requiring evidence of ability to pay the costs of remediation at sites with UST releases. The costs of corrective action are borne by many state financial assurance funds or through the purchase of insurance policies by owners and operators. Many of these funds have experienced revenue shortfalls as the money paid out for corrective action is not covered by the money paid in. When funds start experiencing these shortfalls, corrective action slows down.

Each of these issues contributed to the establishment of a task group within ASTM to develop a standard for UST release sites. The goal of the ASTM RBCA process was to develop a procedure that would protect human health and the environment while at the same time being cost-effective. This meant that cleanup goals would be targeted toward actual exposures, not necessarily toward protection of unrealistic uses of the property. The vision of the ASTM RBCA standard was to provide a framework that state agencies could adopt to be consistent with state regulations and practices already in place.

ASTM RBCA Process

The key to the ASTM RBCA process is a step-by-step process from site investigation through decision-making to take a site to closure. The RBCA process is not very different from how site investigations have been conducted previously. The only additional information required focuses on the information required to make risk-based decisions. Briefly, the steps involved are described below.

Site Characterization

A site assessment is the process designed to collect data necessary to evaluate whether contamination is present at the site and, if necessary, direct future work at the site. Under the ASTM RBCA process, limited data are collected to characterize the site with respect to the source area, location of actual or potential receptors, and any other information necessary to perform the Tier 1 screening analysis. By only collecting limited data, the site assessment focuses only on the information required to make an initial decision on the site. Additional data can be collected during the Tier 2 or Tier 3 assessments to fill data gaps, if necessary.

The site characterization provides site-specific background information essential to visualizing site conditions and potential receptors and pathways. Characteristics of the site, such as history, climate, topography, local land use and populations, soil type, depth to groundwater, groundwater flow direction and rate, and distance to groundwater discharge, are considered. While all of these data are not required for the Tier 1 assessment, this information commonly is collected during site investigations and activities designed to delineate the extent of the hydrocarbon impacts. Further, this information will be readily available if a Tier 2 assessment becomes necessary. Site characterization data provide the basis for a realistic assessment of exposure pathways and are used as input in any models utilized.

A key component in the initial site assessment is the identification of human and environmental receptors potentially impacted by the site. An exposure pathway analysis relies on transport information to identify receptor or exposure points. For example, potentially significant transport and exposure pathways may include groundwater transport, vapor migration into buildings or utilities, etc. Current and potential future land use is identified as well as the potential for future installation of groundwater drinking water wells. If surface water has been impacted by the release, then appropriate surface-water exposure pathways will be identified.

Site Classification

Using a risk-based decision-making approach to determine if residual constituent concentrations are protective of human health and the environment can be cost-effectively conducted using a tiered approach. Under RBCA, site-specific data may be used to prioritize sites (in the case of limited remedial funds and resources). The ASTM classification scheme utilizes four classes. Class 1 sites are those posing an immediate threat to human health or the environment. For these sites, an interim response can be required to reduce this threat. Class 2 sites are those that may pose a short-term threat within 2 years or less. A Class 2 site may be one in which there are less than 2 years until impacted groundwater reaches a downgradient receptor. Class 3 sites are those that pose a longer term threat. For example, an aquifer may have been impacted, but the groundwater flow is such that the travel time to the nearest receptor is greater than 2 years. Some states have incorporated a classification or prioritization scheme into their RBCA process. States with an existing

classification have integrated it into their RBCA program, rather than starting with a new system. A number of states did not incorporate a classification system into their new RBCA program.

Tier 1

Tier 1 uses conservative, health-protective levels to compare to site-specific data to eliminate those sites not requiring remediation beyond monitoring (the obvious monitoring-only sites). The Tier 1 risk-based screening levels (RBSLs) typically incorporate very conservative or worst-case exposure assumptions. However, many sites with concentrations exceeding the RBSLs do not necessarily pose a threat to human health. Therefore, exceeding values of RBSLs by themselves do not indicate that remedial action should be actively undertaken to protect human health and the environment.

To pose a risk, a constituent must be present in the environment at a concentration high enough to cause a toxic effect if exposure occurs. Complete pathways must exist, and exposure to a constituent at that concentration must occur. Without the potential for exposure to a constituent, there is no risk; therefore, the driving force behind any remediation would not be the protection of human health. If no complete exposure pathways exist now or are not anticipated to exist in the foreseeable future at a site, the RBCA process technically can be terminated at this point as there is no risk. One example of this concept is the lack of exposure to impacted subsurface soil beneath a building that houses an operating business. However, it is often difficult to determine the future use of a site, and it may be necessary to evaluate potential risks associated with hypothetical future exposure. A key to the Tier 1 evaluation is the development of a conceptual site model that evaluates the possible exposure pathways for the site under current and hypothetical future conditions.

The data evaluation step in Tier 1 identifies the constituents of potential concern and the concentrations at which they occur in impacted media as determined by investigations conducted at the site. Most states do not require evaluation of historical data as part of the Tier 1 evaluation, although they may require submission of these data in the RBCA report as a basis for comparison (e.g., to demonstrate the decrease in constituent concentrations over time and/or demonstrate plume stability). However, the data that best represent the current environmental conditions at the site should be used in the risk-based decision-making approach.

A decision based on the Tier 1 evaluation may be to go to a monitoring program to verify that the conditions at the site, in fact, do not pose a threat to human health and the environment, to move to Tier 2, or to remediate to the Tier 1 levels. The costs required to collect additional data, along with the cost to perform the Tier 2 assessment and the benefits of higher cleanup goals, will be weighed against the remediation costs to meet Tier 1 levels to determine the best approach for sites with concentrations exceeding the Tier 1 RBSLs. UST sites with minimal impacts most likely will be addressed by this level of effort and sophistication. Estimates are that $\sim$10–15% of the sites will be closed under Tier 1.

Tier 2

Tier 2 produces site-specific target levels (SSTLs) that are protective of human health and the environment, but utilizes more site-specific data than Tier 1. This level of sophistication should address $\sim$70–80% of the sites. This more site-specific assessment involves the assumption of reasonable use exposure assumptions, considers actual beneficial uses of resources, and provides a tool for determining points of compliance.

The Tier 2 evaluation tends to focus on more realistic exposure pathways and may include fate and transport modeling to project whether site-specific concentrations will reach receptors where exposure could occur. Tier 2 will allow the exclusion of those pathways not expected to occur at the site. Groundwater flow models can be used in conjunction with contaminant transport models to describe the flow paths and rate of hydrocarbon movement and, thus, estimate exposure points concentrations. For example, if monitoring data indicate that a groundwater plume at a site has not migrated to a well or other exposure points where contact could occur, groundwater transport modeling may be used to predict if the plume will, in time, reach the exposure point at concentrations greater than regulatory standards or health-based concentrations. In Tier 2, generic assumptions used in Tier 1 modeling are replaced with site-specific data to ensure that the results accurately represent actual site conditions. In many cases, verification of the modeling results through monitoring is required by the state.

Tier 3

Tier 3 is more complex and detailed. It relies on more site-specific data. While Tier 2 may have relied on simple and relatively uncomplicated fate and transport models, Tier 3 will utilize more sophisticated models and will include additional site-specific data. Tier 3 also may rely on site-specific exposure assumptions, if appropriate. Some states allow the

incorporation of Monte Carlo techniques into the Tier 3 process. As the site evaluation and risk assessment process increases in tier level and complexity, the costs, data requirements, and level of sophistication required to complete the process also increase. It is anticipated that only ~5–10% of the UST sites will be closed under Tier 3.

Corrective Action

Corrective action can be a combination of passive and aggressive actions designed to reduce constituent concentrations to levels considered protective of human health and the environment. These can include natural attenuation, soil vapor extraction, source removal, etc. In each case, the type of corrective action is selected to meet the remediation goals (i.e., RBSLs or SSTLs) developed under the appropriate tier for the site. This allows the project to focus only on those areas or media posing a threat to human health or the environment.

No Further Action

The final step in the RBCA process is to take the site to closure. This may involve a monitoring program for a period of 1–2 years to verify that the assumptions included in the RBCA analysis were appropriate for the site. It is anticipated that RBCA will allow for closure of sites in a consistent and cost-effective manner. This will allow resources to be focused on those sites posing the greatest threat to human health and the environment. It will also allow sites posing little risk to potentially be placed in a monitoring program with the goal being to justify closure using natural attenuation and no active remediation.

Summary

RBCA and the use of risk-based decision-making to establish health-protective remedial measures and controls at a site is a process that is producing health-protective and cost-effective corrective action sites. RBCA is used to decide the level of corrective action necessary at a site to protect human health and the environment, site-specific remediation goals (RBSLs or SSTLs), and the concentrations of constituents that can remain at the site because they will not impact human health and the environment. For sites already undergoing remediation, the RBSLs or SSTLs can be used to determine when the site no longer poses a threat to human health and the environment. Therefore, RBCA is useful both for newly discovered releases as well as for old releases that may have treatment systems with constituent concentrations reaching asymptotic levels.

See also: Pollution, Air; Pollution, Soil; Pollution, Water; Risk Assessment, Ecological.

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Risk Characterization

Michael A Kamrin

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Risk characterization is the final step of the risk assessment process as laid out in the classic National Research Council report: *Risk Assessment in the Federal Government: Managing the Process*. In this step, the risk from a specific agent (chemical or physical) or group of agents in a particular setting is evaluated. This evaluation is based on a comparison of the results of the dose–response assessment for these agents with the outcome of the exposure assessment for these same agents in the situation of interest. For example, a risk characterization may address the risk from chemicals at a hazardous waste site to those living near the site. While a risk characterization can provide either qualitative or quantitative evaluations of risk, quantitative outcomes are generally most useful and so will be the focus of this article.

Because the dose–response assessment step is performed differently for agents considered to be carcinogens compared to those classified as non-carcinogens, the risk characterization process for these two kinds of agents differs as well. The result of the dose–response assessment for carcinogens is some measure of the potency of the agent; for example, the

daily dose that produces a specific number of additional cancers in a given population. In the case of noncarcinogens, the toxicity is expressed as an acceptable daily intake; that is, the maximum daily dose unlikely to be associated with adverse health effects. This acceptable daily intake may be described in a variety of ways. In the United States, the Environmental Protection Agency (EPA) calls this value a reference dose (RfD) or reference concentration (RfC).

Since the cancer potency and/or acceptable daily intake values are characteristics of the agent, they do not vary from situation to situation. Exposure, however, does. Exposure assessments provide an estimate of the dose to which individuals may be exposed via all possible routes in a specific circumstance. The result of the exposure assessment is usually expressed as a single number; for example, the average daily dose. However, since no two individuals are likely to have the same exposure it may also be expressed as a distribution. This distribution provides estimates of the exposures of particular segments of the population; for example, the top 95% of exposed individuals.

Quantitative risk characterization thus results in either an estimate of the additional cancers expected (for carcinogens) or an estimate of whether or not individuals will be exposed to doses that exceed the acceptable daily intake – and perhaps the magnitude of this exceedence (for noncarcinogens). However, as can be seen from the US EPA definitions of cancer potency: "Cancer potency is estimated as the 95% upper confidence limits of the slope of the dose response in the low dose region. This method provides an upper estimate of the risk; the actual risk may be significantly lower and may actually be zero." and the RfD: "An estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime," there are considerable uncertainties associated with the dose–response estimates. In addition, because of difficulties in estimating exposure due to lack of information about environmental levels of agents and about human behaviors, uncertainties in exposure assessments may also be large.

Thus, while the results of a risk characterization may be expressed as a single value; for example, X additional cancers per million people exposed, a full description of the risk must include a discussion of the uncertainties in the risk estimate. Unfortunately, many of these uncertainties are hidden in the design of the dose–response studies and, in addition, there are many implicit value judgments that are part of both the dose–response and exposure assessment processes. Ideally, all of these uncertainties and judgments would be part of the risk characterization results but in reality many are not included. While a full description of all of the uncertainties and value judgments would lead to a cumbersome risk characterization result that would be difficult to use in making risk management decisions, including more information of this type than is presently used would aid those who manage risk.

Guidelines for performing and reporting the results of risk characterizations have been promulgated by governmental bodies. In the United States, the EPA is the agency that is responsible for risk assessment guidance for environmental contaminants and it has developed and issued such guidelines. These documents are written primarily for the use of federal risk assessors and risk managers. However, state governments also must deal with risks from environmental agents and they generally utilize these same guidelines although they are often not as well equipped as the federal government to appreciate the implicit uncertainties and value judgments in the risk characterization. Thus, their ability to comprehensively characterize risk and make sound risk management decisions may be compromised.

Perhaps more importantly, each citizen is a risk assessor and risk manager with regard to his or her own health and the health of loved ones. Members of the public make daily decisions about risk and may influence government decisions on these same risks but they are generally poorly equipped to appreciate the uncertainties and value judgments in risk characterizations. They are also most likely to be presented with single value risk characterization results tempered little by attendant discussion of uncertainties. This not only limits their ability to make the best management choices but also makes them vulnerable to unfounded and misleading claims made by advocacy groups.

In sum, risk characterization is the last and critical step in risk assessment. While it is often portrayed as providing objective, scientific best estimates of risk, the value judgments that are integral parts of the characterization process clearly reflect that this is not the case. Greater appreciation of this aspect of the risk characterization process would provide agency and citizen risk managers with a better understanding of the meaning of risk values and thus how to improve risk management decisions.

See also: Dose–Response Relationship; Exposure Assessment; Risk Assessment, Human Health; Risk Management.

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Risk Communication

Michael A Kamrin

Although communication about risk has a long history, risk communication has been formalized as an area of study for only ~15 years. It is an area that draws from a number of disciplines in the natural and social sciences. Research in this field includes studies of human perception and decision-making as well as investigations of a socioeconomic nature. Risk communication practitioners include government officials, business and industry representatives, public interest group members, academics, media professionals, and the general public.

Because of its short history, the concept of risk communication is still evolving. In its landmark report in 1989, *Improving Risk Communication*, a National Academy of Sciences panel defined risk communication in part as "An interactive process of exchange of information and opinion among individuals, groups and institutions." However, this definition describes one group's consensus as to what risk communication ought to be and does not reflect either the diversity of opinion on this topic or the reality of risk communication as it is currently practised.

Reduced to its essentials, risk communication is just one example of communication in general, a process by which information is transferred by one party to another through a variety of channels. The components of the process are the risk communicator or source, the message, the message channel, and the recipient of the message. While this description suggests a linear series of events, communication is often, but not always, an interactive process which includes feedback among the components. For example, the responses of the recipient may lead to alterations in the source, message, and/or channel and may result in a change in outcome.

Based on this conceptual framework, and using a variety of experimental approaches and case studies, risk communication professionals have been able to identify and, in some cases, roughly quantify many of the factors that determine the effectiveness or success of risk communication. One fundamental problem is, however, that effectiveness can be defined in a number of ways.

From one point of view, success can be measured as increased understanding of risk on the part of the message recipient, whether or not this results in changes in behavior. However, from another perspective, effectiveness is determined by the degree of behavioral change brought about by the risk message being transmitted. There are some who have argued that increased understanding is directly linked to changes in behavior but research suggests that this is not necessarily the case; for example, smokers who become aware of the risk do not always quit.

Many recent communication efforts about public health risks reflect the latter view. For instance, the success of risk communication programs about radon have been judged by the number of people who test for radon or take remedial action to reduce radon levels. Similarly, campaigns on smoking are assessed by the number of individuals who stop smoking. When behavior change is the criterion of success, the accuracy of the risk communication is not as important as its impact.

However, other risk communication efforts reflect an educational focus rather than a persuasive one. Understanding is considered the most important goal and a lack of change in behavior is not judged as a failure in communication; rather it is the result of decisions by the information recipient who may take into consideration a variety of other factors, such as offsetting benefits. Accuracy is considered critical so that the recipient can make an informed decision based on the best information.

The factors that affect the success of risk communication efforts, irrespective of the criteria for judgment, can be considered in the context of the components of the communication process. Studies of the risk messenger (source) have focused on the issue of trust and, not surprisingly, reveal that the effectiveness of the communication increases as trust in the communicator increases. This trust may be related to the degree of expertise of the communicator but more often is determined by other factors such as the messenger's perceived objectivity or social class and the accuracy of previous communications by this messenger or other messengers representing the same organization. For example, state agencies may have difficulties in effective risk communication for decades after taking actions or making statements seen as untrustworthy.

The content and form of the message are also critical and it has been demonstrated that the same risk expressed or framed in different ways can have different impacts. For example, describing the risk from a disease or surgery as 60% chance of survival will lead some people to different decisions than if it is stated as 40% chance of death. Similarly, a risk presented in terms of the total number of fatalities may be perceived differently than if it is presented as a probability.

Furthermore, there are a number of factors that come into play if the risk is presented in comparison to other risks – a communication strategy that is often employed. Research suggests that audiences are most receptive to comparisons of the same risk at one time or place to another time or place. For example, an effective message may describe the risk as high 2 years ago but having decreased continuously and now less than one-tenth of what it was. Risk comparisons that are not as successful involve comparisons between hazards that are thought of as having incomparable qualities (e.g., smoking and water contaminated with bacteria). Smoking is controllable and the effects delayed, while bacterial contamination of water is involuntary and the effects immediate.

The channel of communication is also an important variable in the communication process. Survey researchers have examined the relative credibility of various channels, including print media, radio and television, magazines, and advertising, and have found differences in the degree of trust people have in each. These differences depend not only on the class of channel but also on the specific representative of that class. For example, coverage of a risk issue in a local newspaper may be viewed differently from stories on the same issue in the national press.

Last, the perception of the recipient is critical to the process and is an aspect of risk communication that has been studied very intensively. This research has clearly shown that this perception depends both on the way that humans tend to internalize knowledge and on the background of the information recipient. For example, a scientist may perceive risk as a specific quantity and compare risks based on a quantitative approach (e.g., a risk of one in a million is much lower than a risk of one in a thousand).

However, potentially affected citizens may look at these quantitative descriptors as only partially describing the risk. They may consider a number of other factors such as the voluntariness, catastrophic potential, familiarity, and controllability of the hazard. Thus, contrary to the scientist, they may consider the one in a million risk to be more serious than the one in a thousand risk. Government officials are often reminded of this at public meetings, when people who smoke express great concern about very low levels of environmental contaminants which pose much lower health risks than smoking.

The results of research on the factors that influence successful risk communication are the bases for sets of risk communication principles that have been developed. One well-known set of principles is titled *Seven Cardinal Rules of Risk Communication*. This guide includes steps to increase trust such as to 'coordinate and collaborate with other credible sources', steps to increase the recipients' control such as to 'accept and involve the public as a legitimate partner', and steps to increase the interactive nature of the process such as to 'listen to the public's specific concerns'.

In the main, research to date has focused on understanding individual differences in risk perception and on the interactions between sources and recipients. However, recently, increasing attention has been paid to the social context in which risk communication is performed. Research in this area has led to a greater awareness of the sociocultural factors that affect the transmission of risk information.

These factors include the ethnic and socioeconomic characteristics of populations as well as the structure and history of communities that must deal with risk concerns. For example, a 'company' town may react quite differently than a rural agricultural village or an inner-city neighborhood to information about risk from an environmental chemical.

Another important social issue that influences risk communication is environmental justice and the perceived fairness of the risk to a community or subculture. For example, the perceived risk of adverse health effects from a landfill may depend on whether the waste deposited there is generated locally or transported from somewhere else. Similarly, the perception of risks associated with locating new

facilities in an area may be colored by environmental equity concerns.

These sociocultural factors can affect all aspects of the communication process. The credibility of a particular source may vary greatly depending on the cultural experience of the recipient with the organization the source represents, for example, industry or state government. In addition, the way that the message is framed may have quite different impacts in different cultural settings; an effective comparison in one setting may be an ineffective one in another. Communication channels may be seen as more or less reliable by different socioeconomic classes, for example, certain media may be seen as more trustworthy by different social groups. Furthermore, the relative importance of the factors that influence perception, for instance, controllability and immediacy, may be different in various cultural settings.

Research in this area, while in the formative stage, has been increasing. One aspect of this expanded effort involves studies aimed at understanding why certain communities have responded to risk, for example, from Superfund sites, quite differently than other communities, or why media focus on some risks has led to increased awareness and concern while attention to other risks has been met with little reaction. It is hoped that research into the reasons behind these differences will help in identifying the roles of various sociocultural factors in risk communication.

It is clear that there are many gaps in our current understanding of the risk communication process. For example, how exactly do age and gender affect the way risks are perceived? Other questions that are yet to be answered include exactly how the framing of the message affects its impact; how uncertainty in the risk message affects the perception of the risk and risk communicator; and why some risks that appear to share the same characteristics are perceived differently by individuals and/or communities.

Future research will address these questions and our understanding of the influence of various factors on the risk communication process will undoubtedly increase. In addition, innovative ways to present risk messages will certainly be developed. However, some fundamental issues with respect to the goals of risk communication will likely remain.

See also: Risk Assessment, Ecological; Risk Assessment, Human Health; Risk Characterization; Risk Management.

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Risk Management

Xuannga Mahini

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Risk management, in simplified terms, is the decision-making process by which risk assessment results are integrated with other information to arrive at decisions about the need for, method of, and extent of risk reduction. This definition covers many levels of decision-making. At one level, it deals with the question of what programs should be undertaken to reduce risk to the population of the country. At another level, it is the allocation of tax dollars to improve the quality of life in a city. At yet another level, it is the decision-making process for mitigating risk from a specific chemical in a specific use or at a specific contaminated site.

This latter level of risk management is discussed in some detail in the 1983 National Academy of Sciences (NAS)/National Research Council (NRC) report on risk assessment (*Risk Assessment in the Federal Government: Managing the Process* – the so-called Red Book). In that report, NAS advocated a clear conceptual distinction between risk assessment and risk management, since this distinction would help prevent, for example, the tailoring of risk

assessments to the political feasibility of regulating the substance in question. According to the Red Book, risk assessment is a four-step process: (1) Hazard assessment – Does the chemical have the intrinsic potential to cause an adverse health effect, for example, cancer? (2) Dose–response assessment – What is the relationship between low doses in humans (generally estimated from high-dose studies in laboratory rodents) and the incidence and severity of the potential adverse health effect? (3) Exposure assessment – What is the actual or hypothetical exposure of humans to the chemical in question? (4) Risk characterization – What is the estimate of probability or likelihood of specific harm to an exposed individual or population? Risk management – How do we mitigate this risk? – is typically the logical next step following the risk assessment process. This is "the process by which the results of risk assessment are integrated with other information – such as political, social, economic, and engineering considerations – to arrive at decisions about the need and methods for risk reduction" (NAS definition). In the 1994 *Science and Judgment in Risk Assessment*, NAS further recommended an iterative approach to risk assessment that would allow improvements to be made in the risk estimates (more realistic risk estimates with lesser degrees of uncertainties) and that would lead to an improved relationship between risk assessment and risk management.

Risk management decisions are based on the technical risk assessment information, but they also incorporate other information. Specifically, risk managers consider costs, benefits, risk of alternative actions, and risk of no actions. For example, some drugs have a very small safety margin between the level that is effective and the level that is toxic, but the benefits of the drug are judged to outweigh the risks; therefore, it is decided that these drugs can be used. At the other end of the spectrum, some food-coloring additives have very large safety margins between what is toxic and what is in the diet, but the benefits of food coloring are minimal and alternatives exist, so this risk would generally face a higher level of scrutiny in a risk management decision.

These examples emphasize that risk management decisions are based in part on level of risk, but other factors are also considered to most effectively protect the public. In making risk management decisions, it is also necessary to understand the level of uncertainty in the technical data. For example, there is considerable uncertainty in predictions of potential impacts of global warming, but the consequences are potentially very large. It is often the case in risk assessment that there is considerable uncertainty in the predictions of risk. These uncertainties need to be carried forward into the hands of the risk manager so that they are weighed in the final decision. The necessary level of public protection and the magnitude of margins of safety properly belong in the domain of risk management.

At a more global level, risk management refers to the activity of allocating resources (time and money) to reduce risk in general to the public. For example, the US Congress does this when they allocate money to US federal agencies. The trade-off in agency funding addresses the question of whether more resources should be allocated to improve air and water quality (US Environmental Protection Agency, EPA), ensure that our food is more strictly inspected and regulated (US Food and Drug Administration (FDA) and USDA, improve highway safety (NHSTA), improve air safety (FTA), or bolster national defense systems (armed forces). We do not have unlimited resources to expend, so Congress makes these allocations roughly proportional to where it thinks the money is most effectively spent. These allocations reflect risk management on a global level.

The level of risk management closest to what most individuals experience is risk reduction at a city management level. Cities are responsible for ensuring that their drinking water is tested and is safe relative to bacteria, disease, and chemicals. They are also responsible for disposing of garbage in either landfills or incinerators, for keeping beaches and swimming places safe, and for minimizing vehicle risk to pedestrians and vehicle occupants. A number of cities have a significant problem with keeping their drinking water and beaches clean because of storm water overflowing into sanitary water systems. Risk management at the local level involves deciding whether solving this storm water issue should be the highest priority for the next tax dollars being spent or whether road repair or expanding the capabilities of the local ambulance and hospital system should be highest priority.

The 1997 Presidential/Congressional Commission's Risk Management Framework

In the 1997 *Framework for Environmental Health Risk Management – Final Report* published by The Presidential/Congressional Commission on Risk Assessment and Risk Management (Commission), risk management was defined as "the process of identifying, evaluating, selecting, and implementing actions to reduce risk to human health and to

ecosystems." The goal of risk management is scientifically sound, cost-effective, integrated action that reduces or prevents risks while taking into account social, *cultural*, *ethical*, political, and *legal* considerations (italicized words are new considerations since the 1983 Red Book). This new definition of risk management is broader than the traditional definition, which is restricted to the process of evaluating alternative regulatory actions and decision-making only by regulatory agencies.

There are two reasons for the broadened scope of risk management beyond regulatory actions typically taken previously by federal, state, and local government agencies:

- Government risk managers now often consider both regulatory and voluntary approaches to reducing risks, as society is challenged to solve more complex risk problems with limited resources.
- Risk management is being conducted outside of government arenas, by 'stakeholders' (individual citizens, businesses, workers, industries, farmers, fishermen, etc.). So the decision-making process needs to be improved by the involvement of those affected by risk problems.

To help meet these needs, the Commission developed a systematic, comprehensive Risk Management Framework that has six stages:

1. Define the problem and put it in the appropriate public health or ecological context.
2. Analyze the risks associated with the problem in context (e.g., multisource, multimedia, multichemical, multirisk) based on identified risk management goals.
3. Examine options for addressing the risks (e.g., comparative risk analysis, expected effectiveness, feasibility, potential adverse consequences, priority to prevent risks not just controlling them).
4. Make decisions about which options to implement.
5. Take actions to implement the decisions.
6. Conduct an evaluation of the actions.

The Commission's proposed Risk Management Framework is conducted: (1) in collaboration with stakeholders and (2) using iterations if new information is developed that changes the need for or nature of risk assessment, while avoiding 'paralysis by analysis'. By emphasizing the importance of collaboration, communication, and negotiation among stakeholders (so that public values can influence risk management strategies), the new Risk Management Framework is designed to produce risk management decisions that are more likely to be successful than decisions made without adequate and early stakeholder involvement.

The most important tools to carry out the Commission's proposed Risk Management Framework are effective risk communication, comparative risk analysis, and benefit/cost analysis (BCA) and cost/effectiveness analysis (CEA). Effective risk communication is critical to successful implementation of the Risk Management Framework. Risk communication is the process of informing people about the risks or hazards. Like all communication, communicating risk is a two-way exchange to inform the stakeholders or target community about possible hazards, but also to gather information about those affected by the risk. The purpose of risk communication is to help stakeholders understand risk assessment and risk management, form scientifically valid perceptions of the likely hazards, and participate in making decisions about how the risk should be managed. Effective risk communication include knowledge and tools on how to communicate to the public/stakeholders the risks involved in a particular situation, and how to make day-to-day decisions regarding that risk.

Comparative risk analysis is a methodology that uses sound science, policy, economic analysis, and stakeholder participation to identify and address the areas of greatest environmental risks and provide a framework for prioritizing environmental problems. The results of a comparative risk analysis can be used to provide a technical basis for targeting activities, management priorities, and resource allocation. In this analysis, different risk management strategies beside 'command and control' can be explored: (1) education; (2) incentives (e.g., market-based, subsidies, alternative compliance); (3) monitoring; (4) surveillance; and (5) research.

BCA and CEA are together referred as economic analysis. The Commission recommended that where practicable, environmental equity needs to be considered in economic analysis. BCA has the advantage of helping make choices among policies and actions with quite different benefits and costs. It is guided by what stakeholders or members of society are thought to be willing to pay to reduce risks. CEA, in contrast, does not require that benefits be monetized. It defines some physical measures (e.g., tons of pollutants to be reduced or number of cancer deaths avoided) as effectiveness of risk management actions. These nonmonetized benefits cannot, however, be aggregated.

Successful examples of the Risk Management Framework cited by the Commission are: (1) stakeholders and EPA identified risk management options for the pulp and paper and the steel industries;

(2) environmental management plan to control pollution in the San Francisco Bay; (3) transportation policy that considered alternatives to highway expansion in Maine.

See also: Risk Assessment, Ecological; Risk Assessment, Human Health; Risk Characterization; Risk Communication; Sensitivity Analysis; Uncertainty Analysis.

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Risk Perception

Patricia M Nance

Risk perception can play a critical role in the daily behavior of humans, and needs to be considered for developing effective risk communication. Risk perception is the apprehension or opinion of the likelihood of risk(s) associated with performing a certain activity or living a certain lifestyle. Many factors play a role in perception of risk. Some of these factors are personal experience with the risk, perceived importance of the risk, the credibility of the communicator and their organization, and the language and presentation format. Each individual has their own way of thinking and decision-making ability, which can make risk communication a challenge. Dramatic and memorable risks are less acceptable than uninteresting and forgettable ones. The factors that make a risk dramatic and memorable, such as airline crashes, may distort risk perceptions. Events that are highly publicized in the media become well remembered and appear to have happened more frequently than normal, hence creating a larger perceived risk.

Familiarity with a risk also skews the perception. Unfamiliar risks are not as acceptable and tend to be perceived to be as higher risks than familiar ones. The public tends to overestimate the risks of seldom occurring events and underestimate the risks of common, everyday risks. For example, the perceived risk of being in an automobile crash is perceived to be low compared to the risk of being in an airplane crash. In an automobile, the individual has a feeling of control, which allows the individual to feel safer than in an airplane where someone else is in control.

Trust and accuracy are two very important factors in risk perception and risk communication. If the public does not trust the experts, the perceived level of risk may be high. To build this trust, accurate information must be given to the public. No potentially important information should be left out and the public should not perceive the experts as hiding the key facts. There are two basic situations when dealing with trust: high trust, low concern and low trust, high concern.

The awareness of the risk also plays a crucial role in risk perception. If the public lacks the knowledge to understand the risk, then the risk can be overestimated or underestimated. Researchers have shown that experts and lay people are typically overconfident about their risk estimates. The role of experience is related to the knowledge of the risk. Individuals who have previous experience with the specific risk or those having a direct economic relationship to the risk usually have a more accurate perception of the risk. Experience does not mean that the individual must have personally been involved in the risk but has awareness of the risk's affects. Experience can also be influenced by the risk frequency. If an individual is exposed to a similar risk more frequently, it can create an overestimate of the risk due to the frequency of exposure.

There has been an increasing amount of research done in the area of risk perception in a variety of fields, such as sociology, political science, psychology, anthropology, and even geology. This research is leading to a better understanding of how individuals perceive a variety of risks in different situations. One expert (Paul Slovic) has stated, "Perhaps the most important message from this research is that there is wisdom as well as error in public attitudes and perceptions. Each side, expert and public, has something valid to contribute. Each side must respect the insights and intelligence of the other."

See also: Chemical Hazard Communication and Material Safety Data Sheets; Risk Communication; Risk Management.

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Rotenone

Carey N Pope

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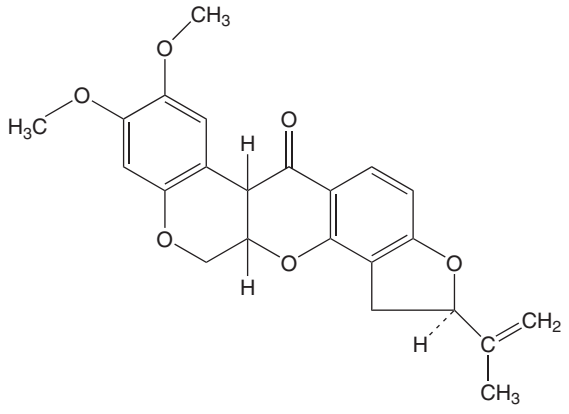
- Chemical Name: Benzopyrano(3,4-*b*)furo(2,3-*h*)(1)benzopyran-6(6*aH*)-one,1,2,12,12*a*-tetrahydro-2-α-isopropenyl-8,9-dimethoxy
- Chemical Abstracts Service Registry Number: CAS 83-79-4
- Synonyms: Barbasco; Mexide; Ro-Ko; Fish-Tox; Chem Fish; Cube'; Derrin; Derris root; Nicoulins; Nusyn Nox fish; Prentox; Noxfish; Rotenone dust; Timbo powder
- Chemical Structure:



Uses

Rotenone has been used for centuries as a fish poison. Rotenone is used as an insecticide around the garden to control chewing insects on vegetables, fruits, and forage crops. Rotenone is also used as a dust on cattle and in dog and sheep dip formulations for scabies, chiggers, fleas, ticks, lice, and mange.

Background Information

Rotenone is a naturally occurring alkaloid (rotenoid) extracted from the roots, leaves, seeds, and barks of certain tropical plants, such as the Jewel Vine or Flame tree (*Derris* spp.), Lacepod (*Lonchocarpus* spp.), or hoary pea (*Tephrosia* spp.).

Exposure Routes and Pathways

Dermal and ocular exposures are most common, but rotenone may also be ingested or inhaled.

Toxicokinetics

Parenteral exposure is more hazardous than oral exposure; gastrointestinal absorption is slow and incomplete. Fats and oils increase rotenone absorption from the gastrointestinal tract. Rotenone exhibits a significant first-pass effect following oral exposure. Biotransformation of rotenone in rats leads to hydroxylated metabolites (rotenolones). In addition, *O*-demethylation inactivates rotenone. Rotenone distributes to lipid-rich tissues, including the nervous system. Elimination of rotenone from the body is primarily through the fecal route.

Mechanism of Toxicity

Rotenone inhibits the electron transport chain by blocking transport between the flavoprotein and ubiquinone. The oxidation of pyruvate in rat mitochondria is virtually completely blocked by rotenone *in vitro* ($<1\,\mu mol\,l^{-1}$ concentration).

Acute and Short-Term Toxicity (or Exposure)

Animal

Depression of the respiratory center appears to be the primary cause of death. The acute oral LD_{50} values in laboratory rats range from about 130 to 1500 mg kg^{-1}. Death following high oral doses in animals can occur within 2 days or as long as 2 weeks after exposure. Rotenone is more toxic by inhalation and intraperitoneal exposure. Rabbits appear markedly

less sensitive than rodents to oral rotenone exposures ($LD_{50} < 1\,g\,kg^{-1}$).

Human

The estimated oral LD_{50} in humans is 300–500 mg kg^{-1}. Ocular exposure to rotenone dusts can cause severe irritation. Inhalation exposure can cause irritation of the nose and throat, and a temporary anesthetic effect may occur. Significant exposures may cause nausea, vomiting, cramps, muscle tremors, loss of coordination, dyspnea, and seizures.

Chronic Toxicity (or Exposure)

Animal

Dogs given dietary rotenone for 6 months at dosages up to 10 mg kg^{-1} day^{-1} showed reduced food consumption and reduced weight gain. Lesions of the gastrointestinal tract were noted. Reproductive and developmental effects were only noted at maternally toxic levels of exposure. Hamsters given oral dosages as high as 120 mg kg^{-1} day^{-1} for 18 months showed no increased tumor incidence.

Human

Long-term exposure to rotenone may cause fatty liver and kidney damage.

In Vitro Toxicity Data

Rotenone was negative in bacterial mutagenesis assays.

Clinical Management

Respiratory and cardiovascular function should be supported with oxygen, assisted ventilation, and parenteral fluids. If eyes or skin are contaminated, they should be washed immediately. Gastrointestinal decontamination procedures should be used appropriately depending on the patient's level of consciousness and the amount of rotenone ingested. Oils or fats should not be administered because they can promote rotenone absorption. Activated charcoal should be used to block absorption with oral exposure. In animals, 10 mg of menadione (intravenously) reversed rotenone's blocking of mitochondrial oxidative phosphorylation; however, it is not known if this has been tried in humans.

Environmental Fate

Rotenone is rapidly degraded in soil and water, with half-lives of 1 and 3 days, respectively. Rotenone does not substantially leach into groundwater. Photodegradation also occurs such that little residue is left within 2–3 days of summer sunlight. It is also sensitive to heat, with much of the rotenone quickly lost at high temperatures.

Ecotoxicology

Rotenone is only slightly toxic in birds. The LD_{50} for rotenone in mallards and pheasants are $> 1.5\,g\,kg^{-1}$. A dietary LC_{50} of 4500–7000 ppm was reported in Japanese quail. Rotenone is highly toxic to fish whereas aquatic invertebrates have a wide range of sensitivity. Rotenone does not appreciably bioaccumulate. Rotenone is practically nontoxic to honey bees.

Exposure Standards and Guidelines

The reference dose for rotenone is 0.004 mg kg^{-1} day^{-1}. The 8 h permissible exposure limit for rotenone is 5 mg m^{-3}.

See also: Plants, Poisonous.

Further Reading

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Relevant Websites

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Rotenone.

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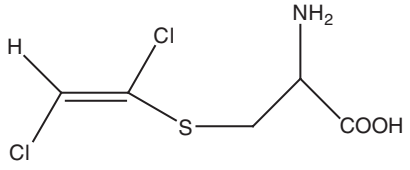
S

S-(1,2-Dichlorovinyl)-L-Cysteine

Vishal S Vaidya and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 627-72-5
- Chemical/Pharmaceutical/Other Class: Halogenated hydrocarbons
- Chemical Formula: $C_5H_7Cl_2NO_2S$
- Chemical Structure:



Uses

S-(1,2-Dichlorovinyl)-L-cysteine (DCVC) is a model nephrotoxicant and cataractogen used to induce acute renal failure and cataracts in experimental animals to study the biochemical, physiological, and molecular mechanisms underlying the disease.

Exposure Routes and Pathways

The only way humans are potentially exposed to DCVC is through trichloroethylene (TCE) because it is a known metabolite of TCE, which is a common industrial solvent used for degreasing metals. TCE is produced in the United States at ~130 000 metric tons per year and is the most commonly found chemical contaminant of groundwater at many chemical waste sites. It is in the Agency of Toxic Substances and Disease Registry's National Priority List of Hazardous Chemicals and is an established animal carcinogen. Toxic and carcinogenic effects of TCE in the kidneys are hypothesized to be due to its metabolism by glutathione (GSH) conjugation, subsequent metabolism to the cysteine conjugation, DCVC, and metabolism of DCVC by the cysteine conjugate β-lyase to form reactive compounds. This chapter will not focus on the exposure pathways and potential toxic effects of TCE because that is discussed in a separate chapter on TCE.

Toxicokinetics

TCE conjugates with GSH yielding *S*-(1,2-dichlorovinyl)-glutathione, and which upon further metabolism yields DCVC. DCVC further undergoes *N*-acetylation to yield mercapturate, which because of its polarity, is readily excreted in the urine. The *N*-acetylation reaction is catalyzed by a cysteine *S*-conjugate *N*-acetyltransferase found in the endoplasmic reticulum (**Figure 1**). The mercapturate can also be deacetylated intracellularly, thus regenerating the cysteine conjugate. When [^{35}S]- and [^{14}C]-DCVC was administered intraperitoneally or intravenously to male Fisher 344 rats, a rapid initial half-life of 2.0 and 2.8 h, respectively was observed. The major plasma metabolite identified was inorganic sulfate, followed by pyruvate and *N*-acetyl-DCVC (NAcDCVC). Metabolite formation was rapid and peak plasma concentrations reached a maximum at 30 min, remained elevated for a few hours, and then decreased. In contrast to plasma the major urinary metabolite was NAcDCVC, followed by inorganic sulfate and pyruvate. The NAcDCVC that is secreted into the tubular lumen of the kidney or which arrives there by glomerular filtration and is not transported back into the renal epithelial cell for deacetylation is excreted into the urine. NAcDCVC has been recovered from rats, mice, and humans after exposure to TCE.

Mechanism of Toxicity

Cell death is initiated by the metabolism of DCVC via renal cysteine conjugate β-lyase, to a sulfur-containing reactive thiol radical that covalently binds to macromolecules (**Figure 1**). The findings that the nephrotoxicity and cataractogenesis of DCVC can be blocked by aminooxyacetic acid (a selective inhibitor of β-lyase) and probenecid (organic anion transport inhibitor) provide evidence for the roles of cysteine conjugate β-lyase and the organic anion transport system, respectively, in

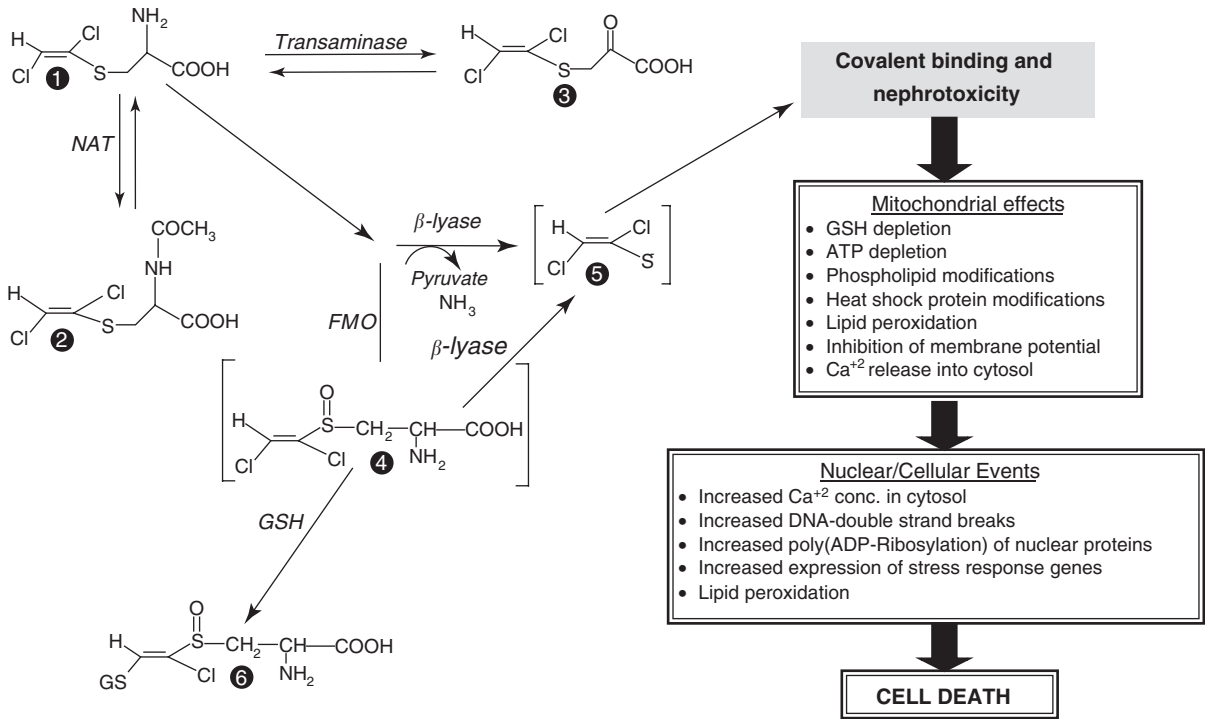


Figure 1 Metabolism of DCVC and mechanism of nephrotoxicity. ❶ *S*-(1,2-dichlorovinyl)-L-cysteine; ❷ *S*-(1,2-dichlorovinyl)-*N*-acetyl-L-cysteine; ❸ the α-keto acid metabolite of *S*-(1,2-dichlorovinyl)-L-cysteine; ❹ *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide; ❺ 1,2-dichlorovinylthiol; ❻ *S*-[1-chloro-2-(*S*-glutathionyl)vinyl]-L-cysteine sulfoxide.

DCVC-induced nephrotoxicity. Although the β-lyase enzyme is considered to be the major bioactivating enzyme for DCVC (**Figure 1**), other bioactivating enzyme activities have been described, and some of these may have relevance to risk assessment. Studies have shown that renal FMO3 can also metabolize DCVC to form DCVC sulfoxide thereby causing nephrotoxicity. Reports from several laboratories indicate that the cytotoxicity of DCVC is mediated at the mitochondrial level (**Figure 1**). Depletion of GSH, mitochondrial lipid peroxidation and GSSG formation, inhibition of mitochondrial lipoyl dehydrogenase activity, release of Ca^{2+} from mitochondria, and inhibition of mitochondrial membrane potential have been observed prior to renal cell death and correlated well with cytotoxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute toxicity of DCVC is well characterized in various animal models including young calves, dogs, cats, rabbits, guinea pigs, turkeys, rats, and mice. With the possible exception of young calves, the primary target for DCVC is proximal tubules in the kidney. It selectively damages the S3 portion of the proximal tubule and causes a focal necrosis of the tubular epithelium in the outer stripe of the outer medulla. Various factors modulating DCVC nephrotoxicity *in vivo* and *in vitro* are summarized in **Table 1**. DCVC nephrotoxicity is usually followed by a nephrogenic repair response, which is characterized by an early stage of increase in proliferation of cells at the wound site by 24 h exposure, loss of differentiated character in the regenerative epithelium, and cessation of cell growth and redifferentiation between days 5 and 13. Studies also suggest that tissue repair is a critical determinant of the ultimate outcome of nephrotoxicity.

Human

Acute toxicity data for humans in unavailable and is unlikely to occur because this compound is a metabolite of TCE.

Chronic Toxicity (or Exposure)

Animal

When male Swiss Webster mice were given $0.1\,mg\,ml^{-1}$ of DCVC in drinking water for 37 or 46 weeks, by 26 weeks all the mice developed cortical cataracts. Cytomegaly, nuclear hyperchromatism and multiple nucleoli were noted in the cells of pars recta

Table 1 Factors modulating DCVC nephrotoxicity

	Effect	*Proposed Mechanism*
Age	Similar renal histological damage in mice of age 5, 15, 25 days old as compared with adult mice in spite of having an increasing accumulation of ^{14}C-DCVC in kidney with respect to age	Unknown
		No change in kidney β-lyase activity with respect to age
Sex	Higher toxicity in female mice as compared to male mice when administered a low dose (5 mg kg^{-1}, p.o.)	Higher β-lyase activity in the female mouse as compared to male mouse. However, this hypothesis is not consistent with the observed reversal in toxicity with respect to high dose
	Higher toxicity in male mice as compared to female mice when administered a higher dose (25 mg kg^{-1}, p.o.)	Sex differences in gastrointestinal biotransformation and/or absorption of DCVC
Species	Higher sensitivity of guinea pigs to DCVC-induced nephrotoxicity as compared to rats	Higher *in vivo* β-lyase activity in guinea pigs as compared to rats
		Lower cysteine *S*-conjugate *N*-acetyltransferase activity as compared to rats
Aminooxyacetic acid	Significantly reduces toxicity of DCVC *in vivo* and *in vitro*	Inhibits β-lyase activity
α-Ketoacids	Potentiate toxicity of DCVC *in vitro* and *in vivo*	α-Ketoacids increase the β-lyase activity
Probenecid	Inhibits both kidney binding and toxicity of DCVC	Inhibitor of organic anion transport in kidney

region of the kidney by 4 weeks and these mice later developed renal tubular atrophy and early interstitial fibrosis. This suggested that chronic DCVC ingestion in drinking water results in cataract formation and severe kidney injury but no incidence of renal tumors. These studies also indicate that the mutagenic potential of DCVC may not be expressed *in vivo*, perhaps due to natural repair and defense mechanisms. To put these findings into perspective, a 2 year bioassay in rats/mice is required.

Human

Some epidemiologic studies suggested a correlation between long-term, occupational exposures to high doses of TCE and the development of kidney tumors, whereas acute or chronic exposures to high levels may induce tubular necrosis in humans. However, others have failed to find any correlation between TCE exposure and renal cancer in humans. Conjugation of TCE with GSH yields *S*-(1,2-dichlorovinyl)-glutathione, and which upon further metabolism yields DCVC. Although GSH conjugation is not the predominant metabolic pathway for TCE, it may be the most relevant to the development of nephrotoxicity. GSH conjugation of TCE has been demonstrated *in vivo* in rats and humans exposed to TCE by detection of mercapturic acid in urine although the amount excreted as the mercapturate is very low. That the mercapturic acid and/or the corresponding GSH and cysteine S-conjugates are potent nephrotoxicants *in vivo* and cytotoxic *in vitro* is evidence that GSH conjugation is important in the development of the nephrotoxicity of TCE.

In Vitro Toxicity Data

Isolated proximal tubular cells from rat kidneys are susceptible to DCVC-induced necrosis at relatively high doses (>0.2 mmol l^{-1}). Similarly, high concentrations (>0.2 mmol l^{-1}) of DCVC are also required to produce significant necrosis in suspensions of freshly isolated human proximal tubular cells. DCVC has also been shown to induce apoptosis in primary cultures of rat proximal tubular cells and in the LLC-PK1 cells.

Clinical Management, Environmental Fate

These sections are the same for TCE and DCVC because human exposure to DCVC occurs only via potential formation of DCVC *in vivo* following TCE exposure.

See also: Kidney; Trichloroethylene.

Further Reading

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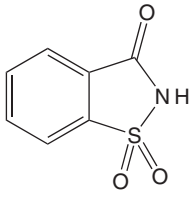
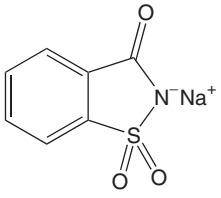
Saccharin

Robin C Guy

- Chemical Abstracts Service Registry Numbers: CAS 81-07-2; CAS 128-44-9 (sodium saccharin); CAS 6485-34-3 (calcium saccharin)
- Synonyms: 1,2-Benzisothiazol-3(2*H*)-one; 1,1-Dioxide benzosulfimide; 1,2-Benzisothiazol-3(2*H*)-one, 1,1-dioxide, sodium salt; 1,2-Benzisothiazol-3(2*H*)-one, 1,1-dioxide, calcium salt
- Chemical/Pharmaceutical/Other Class: Artificial sweetener
- Chemical Formula: $C_7H_5NO_3S$
- Chemical Structures:



Saccharin



Saccharin sodium salt

Uses

Saccharin is used as an artificial sweetener in foods, beverages, and personal care products.

Background Information

Saccharin has been produced commercially produced commercially in the United States for over 80 years. In the 8th report, 1998 National Toxicology Program (NTP) Report on Carcinogens from the (US) National Institute of Environmental Health Sciences (NIEHS), and until 2002, saccharin was classified as a 'reasonably anticipated carcinogen' in the United States. The Calorie Control Council submitted a nomination to the NTP to consider removing saccharin from the Report on Carcinogens based upon mechanistic data related to development of urinary bladder cancers in rats. Following a formal review by NTP, saccharin was delisted from the 10th Report on Carcinogens, because the rodent cancer data are not sufficient to meet the current criteria to list this chemical as 'reasonably anticipated to be a human carcinogen'. This is based on the observed bladder tumors in rats that arise by mechanisms not relevant to humans, and the lack of data in humans suggesting a carcinogenic hazard. Saccharin was finally removed from the carcinogen list in 2002.

The Human Health Assessment Group in the US Environmental Protection Agency's (EPA's) Office of Health and Environmental Assessment has evaluated saccharin for carcinogenicity. According to their analysis, the weight-of-evidence for saccharin is group C, which is based on inadequate evidence in humans and limited evidence in animals. As a group C chemical, saccharin is considered to be possibly carcinogenic to humans.

The International Agency for Research on Cancer (IARC) has judged that there is inadequate evidence in humans for the carcinogenicity of saccharin salts used as sweeteners; however, there is sufficient evidence in experimental animals for the carcinogenicity of sodium saccharin, and there is inadequate evidence in experimental animals for the carcinogenicity of saccharin (acid form) and calcium saccharin. Overall evaluation: in making the evaluation, the working group concluded that sodium saccharin produced urothelial bladder tumors in rats by a non-DNA reactive mechanism that involves the formation of a urinary calcium phosphate containing precipitate, cytotoxicity, and enhanced cell proliferation. The mechanism is not relative to humans because of critical interspecies differences in urine composition. Saccharin and its salts are not classifiable as to the carcinogenicity to humans (group 3).

The European Commission Scientific Committee for Food in 1997 established 1% sodium saccharin in the diet as a clear no-observed-effect level (NOEL) in relation to male rat bladder tumors and for other non-neoplastic effects of saccharin. In response to primarily updated experimental data and the extensive epidemiological data with no evidence of any relationship between saccharin intake and bladder cancer in humans, the Committee set a full acceptable daily intake (ADI) for sodium saccharin of 0–5 mg kg^{-1} body weight. If the ADIs were expressed in terms of the free acid, since sodium saccharin is not the only salt used, and taking into account of the molecular weight difference between sodium saccharin (molecular weight 241) and the free acid (molecular weight 183), then ADI expressed as the free acid is 0–3.8 mg kg^{-1} body weight.

Exposure Routes and Pathways

Oral: Occupational exposure to saccharin may occur through inhalation of dust particles and dermal contact with this compound at workplaces where saccharin is produced or used. The general population

may be exposed through the ingestion of food products such as soft drinks, table sweeteners, and candy that contain this product.

Toxicokinetics

Saccharin is excreted primarily unchanged in the urine.

Mechanism of Toxicity

There is evidence that saccharin is a promoter for bladder cancer, primarily in rodents. It has been delisted from the US NTP Report on Carcinogens, because the rodent cancer data are not sufficient to meet the current criteria to list this chemical as 'reasonably anticipated to be a human carcinogen'. This is based on the judgment perception that the observed bladder tumors in rats arise by mechanisms not relevant to humans, and the lack of data in humans suggesting a carcinogenic hazard. Studies indicate that the observed urinary bladder cancers in rats are related to the physiology of the rat urinary system including urinary pH (>6.5), decreased urine osmolality, increased urine volume, and the presence of urinary crystals or precipitate, and urothelial damage triggering a hyperplasia following consumption of dietary concentrations of 3% or higher with inconsistent findings at lower dietary concentrations. The factors thought to contribute to tumor induction by high doses of sodium saccharin in rats would not be expected to occur in humans.

Acute and Short-Term Toxicity (or Exposure)

Animal

The mouse oral LD_{50} is $17\,g\,kg^{-1}$.

Human

The effects found range from headaches, dizziness, severe depression, and palpitations. Tachycardia has been reported.

Chronic Toxicity (or Exposure)

Animal

In four studies of up to 30 months duration, sodium saccharin was carcinogenic in Dawley male rats as evidenced by a dose-related increased incidence of benign or malignant urinary bladder neoplasms at dietary concentrations greater than 1%. Slight increases (not statistically significant) in urinary bladder cancer have also been observed in female rats from studies showing a positive effect in males. In addition, several initiation/promotion studies in different rat strains have shown a reduced latency and/or increased incidence of similar urinary bladder cancers in male and female rats fed sodium saccharin subsequent to treatment with different urinary bladder initiators. The mouse data are inconsistent and require verification by additional studies.

Human

Results of several epidemiology studies indicate no clear association between saccharin consumption and urinary bladder cancer. Although it is impossible to absolutely conclude that it poses no threat to human health, sodium saccharin is not reasonably anticipated to be a human carcinogen under conditions of general usage as an artificial sweetener. Most of the relevant human epidemiology studies have examined associations between urinary bladder cancer and artificial sweeteners, rather than saccharin *per se*. The time trend data for bladder cancer show no clear indication that the increased use of saccharin or artificial sweeteners commencing in the 1940s is associated with a general increase in bladder cancer when controlled for confounding factors, chiefly smoking. Risks of bladder cancer in diabetics, who presumably consume greater amounts of artificial sweeteners compared to the general population, are not greater than risks in the general population.

In Vitro Toxicity Data

Studies of the genotoxicity of saccharin have shown generally negative but occasionally conflicting results. Sodium saccharin is essentially nonmutagenic in conventional bacterial systems but is weakly clastogenic or genotoxic in short-term *in vitro* and in some *in vivo* test systems. Urine from mice treated with sodium saccharin was mutagenic in the Ames test in one study. Saccharin does not covalently bind to DNA and does not induce unscheduled DNA synthesis in bladder urothelium.

Environmental Fate

Saccharin's production and use as a nonnutritive sweetener may result in its release to the environment through various waste streams. Saccharin will exist in both the vapor and particulate phases in air, and vapor-phase saccharin will be degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 3 days. Particulate-phase

saccharin will be removed from the atmosphere by wet and dry deposition. Volatilization from water surfaces is not expected to be an important fate process based upon this compound's estimated Henry's law constant. An estimated bioconcentration factor (BCF) of 3 suggests bioconcentration in aquatic organisms is low. This compound has the potential to chemically hydrolyze in aqueous environments to *o*-sulfamoylbenzoic acid and ammonium *o*-sulfobenzoic acid, but the kinetics of the potential hydrolysis is unknown. The importance of biodegradation in soil and water is unknown, but amides are usually susceptible to microbial metabolism.

Ecotoxicology

No ecotoxicity issues exist.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value, 8 h time-weighted average (TWA) is $10\,mg\,m^{-3}$. The (US) Occupational Safety and Health Administration permissible exposure limit, 8 h TWA is $15\,mg\,m^{-3}$.

See also: Carcinogenesis.

Further Reading

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International Agency for Research on Cancer (IARC) (1999) Saccharin and its salts. *Monographs on the Evaluation of the Carcinogenic Risks to Humans* 73: 517–624.

Taylor JM, Weinberger MA, and Friedman L (1980) Chronic toxicity and carcinogenicity to the urinary bladder of sodium saccharin in the *in utero*-exposed rat. *Toxicology and Applied Pharmacology* 54: 57–75.

Whysner J and Williams GM (1996) Saccharin mechanistic data and risk assessment: Urine composition, enhanced cell proliferation, and tumor promotion. *Pharmacology & Therapeutics* 71: 225–252.

Relevant Website

http://ehp.niehs.nih.gov – US National Institute of Environmental Health Sciences (NIEHS), National Toxicology Program. Report on Carcinogens from the National Toxicology Program. Agents, substances, mixtures, or exposure circumstances delisted from report on carcinogens.

Safe Drinking Water Act, US

Robert Kapp

- AGENCY: US Environmental Protection Agency (EPA)
- YEAR OF ENACTMENT: 1974; amended 1986 and 1996

Background

The first known public water system in the United States was built in Philadelphia as early as 1799. Over 400 additional water systems had been built in major cities throughout the nation by 1860, and this increased to over 3000 by the early 1900s. With these systems came several major outbreaks of disease because when the supply water was contaminated, the system was an efficient way to spread the contamination throughout the community. In 1849, there were cholera epidemics in New York City that claimed 8000 lives and another in New Orleans that claimed 5000, which were clearly linked to the water distribution system.

Federal legislation on the quality of drinking water began in 1914 when the US Public Health Service (USPHS) set standards for bacterial quality. These original standards applied to contaminants that could cause contagious disease in water systems, which provided drinking water to interstate carriers such as ships, trains, and buses. The USPHS subsequently revised and expanded these standards in 1925, 1946, and 1962. It is estimated that there were over 19 000 public water systems by 1960 growing to ~62 000 operating water systems by 1980. The 1962 standards regulated 28 different substances. By 1962 nearly all of the 50 states adopted the USPHS standards as either standards or guidelines. However, as society developed and became more sophisticated utilizing many man-made chemicals in agriculture and industry, many of these new substances began to appear in water supplies. Further, it was believed that many of these new chemicals were suspected of

causing health problems. The USPHS conducted a survey in 1969 which disclosed that only 60% of the nation's public water systems met the 1962 water standards. There were major deficiencies in over 50% of the systems. In addition, the water systems serving 500 people or less had the most deficiencies. These and other findings led to the passage of the Water Quality Improvement Act of 1970 (Public Law 91-224), which expanded the federal government's authority to set water quality standards.

Continuing concerns over water pollution led to the enactment of the Federal Water Pollution Control Act of 1972 (Public Law 92-500), which contained comprehensive provisions for restoring and maintaining all bodies of surface water. Another USPHS study conducted in 1972 found 36 chemicals, including trihalomethanes, detected in the treated water from treatment plants that were drawing water from the Mississippi River in Louisiana. Based upon the concerns over the findings in these and other studies, several legislative proposals were introduced to Congress in 1973. The Safe Drinking Water Act (Public Law 93-523) was signed into law by President Ford in 1974 to protect human health from contaminants in drinking water and to prevent contamination of existing groundwater supplies.

Overview of the Safe Drinking Water Act

The primary focus of the Safe Drinking Water Act (SDWA) was to set national contaminant-based drinking water standards. These included primary standards intended to address adverse health effects and consist of maximum contaminant level goals (MCLGs), which are nonenforceable goals, and maximum contaminant levels (MCLs) which are enforceable limits set as close as possible to the MCLGs. The MCLs represent an upper limit on the permissible concentrations of regulated contaminants in public drinking water supplies. The MCLGs are the maximum concentrations below which no negative human health effects are known to exist.

Also included in the legislation were secondary standards such as odor and appearance of the drinking water, which were not enforceable. In this law, public water systems were defined to include any water system that serves water to more than 25 people (or 15 service connections). The SDWA required EPA to promulgate interim national primary drinking water standards in order to "protect health to the extent feasible taking costs into consideration." Each contaminant was to determine an MCL or a treatment technique for its control. The interim regulations were replaced with recommendations for the MCLs based upon peer-reviewed science from the (US) National Academy of Sciences.

1986 SDWA Amendments

In 1986, the SDWA Amendments were passed to move EPA closer to enforcing the original Act. Only 23 contaminants had been established since the 1974 legislation and no treatment techniques had been established for any of the contaminants. The 1986 legislation required EPA to set standards (MCLs and MCLGs) for a total of 83 contaminants in the next 3 years. EPA was also directed to prescribe regulations for two treatment techniques for public water systems – namely filtration and disinfection. The 1986 Amendment also gave EPA the authority to fine violators as much as $25 000 per day per violation.

1996 SDWA Amendments

On August 6, 1996, the SDWA was amended again with the goals of establishing scientifically based programs there are flexible with technical and infrastructure assistance. The 1996 Amendments established water contamination prevention requirements including source water protection, capacity development, and operator certification.

The SDWA required formalization of the procedure to set enforceable health-based drinking water standards as follows:

1. Determine whether a contaminant should be regulated based upon peer-reviewed science.
2. Set an MCLG. These goals do not take into account available technology and therefore are sometimes set at levels which public water systems cannot attain. These levels are not enforceable.
3. Propose an enforceable standard in the form of an MCL or a treatment technique (TT). MCLs are set as close to the MCLGs as feasible considering available technology and cost. Required monitoring schedules are part of the enforceable standard. Upon determination of a proposed MCL or TT that is close to the MCLG as possible based upon affordable technology, EPA must perform a cost/benefit analysis to determine whether or not the benefits justify the costs.
4. EPA sets an enforceable MCT or TT. Upon review of all of the data, EPA sets an enforceable MCT or TT level including required testing and reporting schedules.
5. States are authorized to grant variances from EPA standards for water systems serving less than 3301 people if the systems cannot afford to comply with

the ruling. State variances to systems with 3301–10 000 people need EPA approval. No systems are permitted to have variances for microbial contaminants.

Also included were consumer information requirements comprising the development of consumer confidence reports and new notification requirements. The final right-to-know SDWA legislation requires specific information on the following:

1. What contaminants are found in the tap water distributed by the water system.
2. What the water source is for the water system in question.
3. Any known pollution sources responsible for detected contaminants.
4. Listing and details of any violations during the previous 12 months.

In addition, the water system is responsible for the following:

1. Sending a report to all water system customers.
2. For making a good-faith effort to get the report to tenants and others who would not receive a water bill, but who would otherwise use the water.
3. The report must not be cluttered or obscured with extraneous data, which is not directly critical to the purpose of the report.
4. Tabular data cannot be obscured with irrelevant information or presented in a way that is difficult for the recipient to interpret.

The 1996 Amendments further require EPA to establish a mechanism to identify and select new contaminants, as well as specific efforts to establish criteria for arsenic, sulfates, radon, and disinfection by-products. The SDWA required EPA to establish a list of contaminants every five years that are known or anticipated to occur in public water systems and may require further investigation and possible regulation under SDWA. The list is divided into those materials that are candidates for additional research, those that need additional occurrence data, and those that are priorities for consideration in rulemaking. The EPA then must prioritize the critical substances in each category and develop a plan of action for making regulatory decision for the most appropriate candidates.

The National Contaminant Occurrence Database, which stores data on the occurrence of both regulated and unregulated materials, was established by EPA. The monitoring data provides the basis for identifying contaminants that may be placed on future Contaminant Candidate Lists and support the Agency's decisions to regulate contaminants in the future.

See also: Clean Water Act (CWA), US; Pollution, Water.

Relevant Website

http://www.epa.gov – US Environmental Protection Agency (EPA), the Safe Drinking Water website.

Safety Pharmacology

S Satheesh Anand and Harihara M Mehendale

Definition

Safety pharmacology is defined as the field that investigates the potentially undesirable pharmacodynamic effects of a substance on physiological functions in relation to exposure in the therapeutic range and above. It is also called functional safety.

Background

Successful drug development requires precise drug safety assessment. The importance of evaluating the safety of medicinal products before they are allowed into the market was realized following unacceptable levels of unanticipated deaths after drugs have entered the market. The eventual regulatory requirements were reached at different times in different regions. In the United States, a tragic mistake in the formulation of a children's syrup in the 1930s and in Europe, the thalidomide tragedy in the 1960s, are some examples that triggered the regulations requiring product authorization. Safety pharmacology has been an emerging discipline within the pharmaceutical industry in which unanticipated effects of new drug candidates on major organ function are critically assessed in a variety of animal models. Traditionally, drug safety studies are designed to examine effects other than the primary therapeutic effect of a drug candidate. While these studies evaluate the toxic profile of the drug candidate at

maximum tolerated dose and in selected organs, the effects on normal physiological functions at therapeutic doses have long been neglected. Deaths and adverse effects of drugs have been reported in patients and clinical trial participants due to failure in physiologic functions. Pharmacoepidemiology studies in Europe and the United States show that adverse drug reactions now may account up to 10% of admissions in hospitals. In the United States from 1954 until 1980, 7 000 000 people participated in clinical trials. Safety pharmacology studies are developed to help protect clinical trial participants and patients receiving marketed products from potential adverse effects of pharmaceuticals, while avoiding unnecessary use of animals and other resources. Until recently, there have been no internationally accepted definitions, objectives, or recommendations on the design and conduct of safety pharmacology studies. The harmonization in regulation was impelled by concerns over rising costs of healthcare, escalation of the cost of R&D, and the need to meet the public expectation that there should be a minimum delay in making safe and efficacious new treatments available to patients in need.

In 1990, representatives of the regulatory agencies and industry associations of Europe, Japan, and the United States proposed International Conference on Harmonisation (ICH) to develop harmonized guidance on technical issues aimed at ensuring that good quality, safe, and effective medicines are developed and registered in the most efficient and cost-effective manner. These activities are pursued in the interest of the consumer and public health, to prevent unnecessary duplication of clinical trials in humans and to minimize the use of animal testing without compromising the regulatory obligations of safety and effectiveness. The ICH guideline on safety pharmacology (ICHS7A) was recommended for adoption by regulatory bodies in the European Union, the United States, and Japan in November 2000 and it has become effective worldwide since 2001. However, the efforts are in preliminary stage. With this guideline calling for tests on the effects of compounds on vital functions of the human body, the data providing specific information on the safety profile of a new potential therapeutic agent used by clinicians designing clinical studies and by regulatory agencies in their assessment of the safety of a new product is crucial. A brief description of the safety pharmacology studies is presented in this article.

Types of Pharmacological Studies

Pharmacology studies can be divided into three categories: primary pharmacodynamic, secondary pharmacodynamic, and safety pharmacology studies. Studies on the mode of action and/or effects of a substance in relation to its desired therapeutic target are primary pharmacodynamic studies. Studies on the mode of action and/or effects of a substance not related to its desired therapeutic target are secondary pharmacodynamic studies. These have sometimes been referred to as part of general pharmacology studies.

Safety pharmacology studies investigate adverse effects of drugs on vital functions in mammalian species at therapeutic concentrations. Primary evaluations are cardiovascular, respiratory, and central nervous system assessment. The supplemental evaluations are renal/urinary function, gastrointestinal function, and immune function assessment. In addition to these batteries of tests, knowledge of potential harmful interactions or interactions between medications that may neutralize the beneficial effects is becoming increasingly important to evaluate the coadministration of drugs.

Objective

The objectives of safety pharmacology studies are: (1) to identify undesirable pharmacodynamic properties of a substance that may have relevance to its human safety; (2) to evaluate adverse pharmacodynamic and/or pathophysiological effects of a substance observed in toxicology and/or clinical studies; and (3) to investigate the mechanism of the adverse pharmacodynamic effects observed and/or suspected.

Study Design

It is important to adopt a rational approach when selecting and conducting safety pharmacology studies. The specific studies that should be conducted and their design will vary based on the individual properties and intended uses of the pharmaceuticals. Some safety pharmacology endpoints can be incorporated in the design of toxicology, kinetic, clinical studies, etc., while in other cases these endpoints should be evaluated in specific safety pharmacology studies. Although the list is neither exhaustive, nor comprehensive, the following list serves as an example of factors considered:

1. effects related to the therapeutic class of the test substance, since the mechanism of action may suggest specific adverse effects (e.g., proarrhythmia is a common feature of antiarrhythmic agents);
2. adverse effects associated with members of the chemical or therapeutic class, but independent of

the primary pharmacodynamic effects (e.g., antipsychotics and QT prolongation);
3. ligand binding or enzyme assay data suggesting a potential for adverse effects;
4. harmful effects or neutralization of intended effect of a therapeutic substance as a result of interactions (e.g., barbiturates);
5. results from previous safety pharmacology studies, from secondary pharmacodynamic studies, from toxicology studies, or from human use that warrant further investigation to establish and characterize the relevance of these findings to potential adverse effects in humans.

During early development, sufficient information (e.g., comparative metabolism) may not always be available to rationally select or design the studies in accordance with the points stated above; in such circumstances, a more general approach in safety pharmacology investigations can be applied. The methods used must be validated, well established, and should be in common use and should give reliable, reproducible results every time.

A hierarchy of organ systems can be developed according to their importance with respect to life-supporting functions. Vital organs or systems, the functions of which are acutely critical for life, such as the cardiovascular, respiratory, and central nervous systems, are considered to be the most important ones to assess in safety pharmacology studies. Other organ systems, such as the renal or gastrointestinal system, the functions of which can be transiently disrupted by adverse pharmacodynamic effects without causing irreversible harm, are of less immediate investigative concern. Safety pharmacology evaluation of effects on these other systems may be of particular importance when considering factors such as the likely clinical trial or patient population, for example, gastrointestinal tract in Crohn's disease, renal function in primary renal hypertension, and immune system in immunocompromised patients.

Test Systems

Consideration should be given to the selection of relevant animal models or other test systems so that scientifically valid information can be derived. Selection factors can include the pharmacodynamic responsiveness of the model, pharmacokinetic profile, species, strain, gender, and age of the experimental animals, the susceptibility, sensitivity, and reproducibility of the test system and available background data on the substance. Data from humans (e.g., *in vitro* metabolism), when available, should also be considered in the test system selection. The time points for the measurements should be based on pharmacodynamic and pharmacokinetic considerations. Justification should be provided for the selection of the particular animal model or test system. Animal models as well as *ex vivo* and *in vitro* preparations can be used as test systems. *Ex vivo* and *in vitro* systems can include, but are not limited to: isolated organs and tissues, cell cultures, cellular fragments, subcellular organelles, receptors, ion channels, transporters, and enzymes. *In vitro* systems can be used in supportive studies, for example, to obtain a profile of the activity of the substance or to investigate the mechanism of effects observed *in vivo*. In conducting *in vivo* studies, the use of unanesthetized animals is preferred. In the use of unanesthetized animals, the avoidance of discomfort or pain is a foremost consideration. The use of the same species is preferred for *in vivo* tests as those used in drug metabolism, pharmacokinetics, and toxicology – generally, rats and dogs.

Appropriate negative and positive control groups are included in the experimental design. The expected clinical route of administration should be used when feasible. Regardless of the route of administration, exposure to the parent substance and its major metabolites should be similar to or greater than that achieved in humans when such information is available.

Dose Levels or Concentrations of Test Substances and Metabolites

Safety pharmacology studies are intended to define the dose–response relationship of the adverse effect observed. The time course (e.g., onset and duration of response) of the adverse effects should be investigated, when feasible. Generally, the doses eliciting the adverse effects should be compared to the doses eliciting the primary pharmacodynamic effect in the test species or the proposed therapeutic effect in humans, if feasible. Since there are species differences in pharmacodynamic sensitivity, doses should include and exceed the primary pharmacodynamic or therapeutic range. In the absence of an adverse effect on the safety pharmacology parameter(s) evaluated in the study, the highest tested dose should be a dose that produces moderate adverse effects in this or in other studies using similar route and duration.

In vitro studies should be designed to establish a concentration–effect relationship. The range of concentrations used should be selected to increase the likelihood of detecting an effect on the test system. The upper limit of this range may be influenced by

physicochemical properties of the test substance and other assay specific factors. In the absence of an effect, the range of concentrations selected should be justified.

Safety pharmacology studies are generally performed by a single dose administration. When pharmacodynamic effects occur only after a certain duration of treatment, or when it results from repeat dose nonclinical studies, or results from use in humans, give rise to concerns about safety pharmacological effects, the duration of the safety pharmacology studies to address these effects should be rationally based.

Generally, any parent compound and its major metabolite(s) that achieve, or are expected to achieve systemic exposure in humans should be evaluated in safety pharmacology studies. Evaluation of major metabolites is often accomplished through studies of the parent compound in animals. Additionally, if metabolites from humans are known to substantially contribute to the pharmacological actions of the therapeutic agent, it may be important to test such active metabolites.

Safety Pharmacology Core Battery of Tests

The purpose of the safety pharmacology core battery of tests is to investigate the effects of the test substance on vital functions. In this regard, the cardiovascular, respiratory, and central nervous systems are usually considered the vital organ systems that should be studied in the core battery of tests. In some instances, based on scientific rationale, the core battery may be supplemented by other tests, or some of the tests may become unnecessary.

Central Nervous System

Effects of the test substance on the central nervous system should be assessed appropriately. Motor activity, behavioral changes, coordination, sensory/motor reflex responses, analgesia test (hot plate), proconvulsant activity, barbiturate-induced sleeping time, and body temperature should be evaluated. For example, a functional observation battery, modified Irwin's, or other appropriate tests can be used.

Cardiovascular System

Effects of the test substance on the cardiovascular system should be assessed appropriately. Blood pressure, heart rate, and the electrocardiogram should be evaluated. *In vivo*, *in vitro*, and/or *ex vivo* evaluations, including methods for repolarization and conductance abnormalities, should also be considered.

Respiratory System

Effects of the test substance on the respiratory system should be assessed appropriately. Respiratory rate and other measures of respiratory function (e.g., tidal volume or hemoglobin oxygen saturation) should be evaluated. Clinical observation of animals is generally not adequate to assess respiratory function, and thus these parameters should be quantified by using appropriate methodologies.

Supplemental Safety Pharmacology Studies

Supplemental studies are meant to evaluate potential adverse pharmacodynamic effects on organ system functions not addressed by the core battery of tests or repeated dose toxicity studies when there is a cause for concern.

Renal/Urinary System

Effects of the test substance on renal parameters should be assessed. For example, urinary volume, specific gravity, osmolality, pH, fluid/electrolyte balance, proteins, cytology, and blood chemistry determinations such as blood urea nitrogen, creatinine, and plasma proteins can be used.

Autonomic Nervous System

Effects of the test substance on the autonomic nervous system should be assessed. For example, binding to receptors relevant for the autonomic nervous system, functional responses to agonists or antagonists *in vivo* or *in vitro*, direct stimulation of autonomic nerves and measurement of cardiovascular responses, baroreflex testing, and heart rate variability can be used.

Gastrointestinal System

Effects of the test substance on the gastrointestinal system should be assessed. For example, gastric secretion, gastrointestinal injury potential, bile secretion, transit time *in vivo*, ileal contraction *in vitro*, and gastric pH measurement can be used.

Other Organ Systems

Effects of the test substance on organ systems not investigated elsewhere should be assessed when there is a reason for concern. For example, dependency potential or skeletal muscle, immune and endocrine functions can be investigated.

Adverse effects may be suspected based on the pharmacological properties or chemical class safety pharmacology core battery, clinical trials, pharmacovigilance, or from literature reports. When such

potential adverse effects raise concern for human safety, these should be explored in follow-up or supplemental safety pharmacology studies, as appropriate. Follow-up studies are meant to provide a greater depth of understanding, or additional knowledge to, than that are provided by the core battery of tests on vital functions. The following studies may be conducted to further evaluate these organ systems for potential adverse pharmacodynamic effects. These lists are not meant to be comprehensive or prescriptive, and the studies should be selected on a case-by-case basis after considering factors such as existing nonclinical or human data.

Central Nervous System

The studies on the central nervous system include those on behavioral pharmacology, learning and memory, ligand-specific binding, neurochemistry, visual, auditory, and/or electrophysiology examinations, etc.

Cardiovascular System

These studies concern with cardiac output, ventricular contractility, vascular resistance, the effects of endogenous and/or exogenous substances on the cardiovascular responses, etc.

Respiratory System

Airway resistance, compliance, pulmonary arterial pressure, blood gases, blood pH, etc. are studied.

The core battery of tests as well as the supplementary safety pharmacology studies can be conducted at the very beginning of *in vivo* screening and, at the latest, before first studies in man. The safety pharmacology core battery of tests as well as follow-up and supplemental studies should be conducted in compliance with good laboratory practice (GLP). Any study or study component not conducted in compliance with GLP should be adequately justified, and the potential impact on evaluation of the safety pharmacology endpoints should be explained.

Conditions Under which Studies are not Necessary

Safety pharmacology studies may not be needed for locally applied agents (e.g., dermal or ocular) where the pharmacology of the test substance is well characterized, and where systemic exposure or distribution to other organs or tissues is demonstrated to be low. For biotechnology-derived products that achieve highly specific receptor targeting, it is often sufficient to evaluate safety pharmacology endpoints as a part of toxicology and/or pharmacodynamic studies, and therefore safety pharmacology studies can be reduced or eliminated for these products. In addition, testing is not required for new salts having similar pharmacokinetics and pharmacodynamics, and cytotoxic agents for treatment of end-stage cancer patients.

Conclusions

Pharmacology studies have been performed worldwide for many years as a part of the nonclinical evaluation of pharmaceuticals for human use. Safety pharmacology studies are focused on identifying adverse effects on physiological functions at therapeutic doses. These studies are necessary not only to protect the patients treated with drugs, but also the healthy volunteers participating in the clinical trials. The 1960s and 1970s saw a rapid increase in laws, regulations, and guidelines for reporting and evaluating the data on safety, quality, and efficacy of new medicinal products. Until recently, although different regulatory systems were based on the same fundamental obligations to evaluate the quality, safety, and efficacy, the detailed technical requirements were different from each other. Because the pharmaceutical industries have to deliver the safe therapeutics rapidly due to many factors, the ICH was established in 1990 as a joint regulatory/industry project to improve, through harmonization, the efficiency of the process for developing and registering new medicinal products in Europe, Japan, and the United States. The ICH guideline on safety pharmacology has become effective worldwide since 2001. The effects of a test substance on the functions listed in the safety pharmacology core battery should be investigated prior to first administration in humans. Any follow-up or supplemental studies identified as appropriate, based on a cause for concern should also be conducted. No simple formula or set of tests is ideal for safety pharmacology studies for all kind of therapeutic compounds. Knowledge of the pharmacology of the compound and any knowledge gained from traditional toxicity can help to better determine and assess the safety of compounds. Although the ICH guideline for safety pharmacology is in place, it is in an early stage and the actual implementation of requirements and the use of resulting data in risk/benefit decision will require time to be fully worked out and understood.

See also: Safety Testing, Clinical Studies; Toxicity Testing, Validation.

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Safety Testing, Clinical Studies

Alessandra Pagnoni

Clinical safety studies are conducted in human volunteers to determine the safety of chemicals, drugs, formulations, devices, or other products, which may come in contact with the human body. Clinical safety testing should always be conducted prior to efficacy investigations in order to determine the benefit/risk ratio of efficacy trials. When designing a study, "benefits and risks shall be balanced and shown to be in a favorable ratio" (The Belmont Report). "The rights, safety and well-being of the trial subjects are the most important considerations…." (ICH Guidelines 2.3).

The approach to clinical safety studies differentiates between drugs and cosmetics (or their ingredients), and between topical and systemic formulations. The initial safety clinical studies for systemic drugs are usually conducted in a small number of humans. In the United States, the Food and Drug Administration (FDA) categorizes these under phase I studies, which usually involve as few as 10 healthy volunteers. These may include pharmacokinetic (PK) studies and dose escalation studies to determine possible adverse events. For topical drugs, trials may include skin studies for irritation (primary and cumulative applications), allergy (repeated insult patch testing – RIPT), PK and bioavailability (dermatopharmacokinetic – DPK), phototoxicity, and photoallergy. The FDA Guidance for Photosafety Testing states that "…photoirritation and photoallergy studies in humans should be considered for all drug substances and formulation components that absorb UVB, UVA, or visible radiation (290–700 nm) and are directly applied to the [sun exposed] skin or eyes, or significantly partition to one of these areas when administered systemically..."

If the drug passes the initial safety tests, then its safety is evaluated more rigorously against a placebo within larger efficacy trials.

Safety testing for cosmetics includes usually cumulative irritation, RIPT, phototoxicity, photoallergy, and finally, exaggerated use or home use studies.

Suggested guidelines for safety testing of topical materials are explained below. Skin reactions that develop under these skin tests, with a comparison to the controls where applicable, are the bases for conclusions on safety. Skin responses can range from mild erythema to bullous or edematous reactions.

Primary irritation: This test is designed to give information on the short-term irritation potential of the formulation, and usually consists of a controlled patch test involving 15–30 subjects with a one-time (24 or 48 h) application.

Cumulative irritation: The scope of this study is to determine long-term irritation potentials under exaggerated conditions or to assess and compare the mildness of formulations. It consists of a controlled patch test involving 15–30 subjects using 14–21 repetitive 24 h applications. The FDA recommends a 21 day patch test in a 30-subject panel. For more irritating cosmetics, a 14 day application would satisfy the objective of the study.

Repeated insult patch test (RIPT): RIPT is a key investigation for evaluating the potential of a topical to induce delayed contact allergy. It consists of a repetitive patch test study involving 100–200 subjects. It is divided into an Induction (of sensitization) Phase (9×24 or 48/72 h applications for 3 weeks), a Rest Phase (10–21 days), and a Challenge Phase (single 24 or 48 h applications with evaluations at 48 and 72/96 h postapplication to a naïve site). The FDA recommends 48/72 h repeated exposures (Jordan-King design) in a 200-subject panel. For hypoallergenic claims, a 200-subject panel is also recommended. Observations at the naive site during challenge and the patterns of reactivity during the induction period provide a basis for determining if the formulation is a contact sensitizer. In general, while irritant responses occur in a large number of subjects and appear also during the induction phase, allergic responses tend to occur only in a few individuals and only at challenge, unless the subject has been presensitized to the allergen. Additionally, a contact allergen induces a reaction that tends to escalate at 72–96 h postexposure, while an irritant response tends to improve after removal of the offending agent. The exaggeration of

conditions under patch application is important since it appears that to elicit a skin sensitization response, the antigen dose should produce an adequate irritation or 'danger' signal as described by McFadden in *Contact Dermatitis*, 2000. When assessing the incidence of responses, it should be noted that a zero sensitization in a 100-subject panel simply indicates that the rate of sensitization in the population is not likely to exceed approximately 2.95% as reported by Henderson and Riley – *Certain Statistical Considerations in Patch Testing* in 1945.

Phototoxicity and photoallergy tests: Photosensitivity is a term used to describe an adverse reaction, irritant or allergic in nature, elicited by light. Most phototoxic/photoallergic agents are activated by UVA radiations, although some reactions can occur at different wavelengths. Radiations absorbed by the chemical in the skin create a photochemical reaction, which may produce direct cellular/tissue damage (phototoxicity) or may induce an immune, cell-mediated, delayed hypersensitivity reaction (photoallergy) through the creation of a photoproduct that binds to proteins and forms an allergen. Phototoxic reactions are more frequent than photoallergic ones.

Tests to study phototoxicity and/or photoallergy usually involve 25 subjects with test material bearing patches applied in duplicate: one set does not receive radiations while the second set is irradiated. In phototoxicity, patches are applied for 24 h. The irradiated patch is exposed to $16\,J\,cm^{-2}$ UVA and 0.75 MED (minimum erythema dose) UVB. All sites are evaluated at 1, 24, 48, and 72 h following exposure.

As with RIPT, photoallergy testing is conducted in three phases. The Induction Phase consists of 6×6–24 h applications (for 3 weeks), each followed by 2 MEDs UVB exposures and evaluations at 24/72 h postexposure. A second set of patches remains unexposed. This is followed by a Rest Phase of 10–21 days and then by a Challenge Phase (one duplicate 6–24 h application, followed by irradiation with $16\,J\,cm^{-2}$ UVA and 0.75 MED UVB to one set of patches and evaluations at 1, 24, 48, and 72 h postexposure). No reaction at an unirradiated site but a reaction at an irradiated site is indication of a phototoxic or photoallergic response.

Exaggerated use test: After the appropriate preliminary safety testing, the final or prototype formulations may be tested under exaggerated use condition or on compromised skin/disease state to address safety concerns. Examples of these test designs include exaggerated controlled washing over a 5 day period, facial formulation applied under controlled sweating conditions and use test on atopic skin. The panel size for these studies will depend upon the test design and type of formulation. Visual reactions as well self-assessed sensory responses are important endpoints for both exaggerated and normal Use Tests.

Use test under normal use: This is usually the last step in the safety assessment of the final formulation and it is often combined within an efficacy investigation. It simulates normal use conditions during an extended period of at-home applications (4, 12 week, etc.). It is conducted on the intended use population with a panel of 50–100 subjects per cell (cosmetic formulations).

See also: Cosmetics and Personal Care Products; Photoallergens; Risk Assessment, Human Health; Skin.

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Saint John's Wort

Molly Broderick and Teresa Dodd-Butera

- Common Names: *Hypericum perforatum*; Goat weed; Klamath; Klamath weed; Sho-ren-gyo
- Active Derivative: Hypericum, a napthodianthrone flavinoid, and several bioflavinoids
- Description: Perennial herb 1–5 ft tall with yellow flowers in various shades. The petals have black dots. The flowers are displayed in flat topped clusters
- Distribution: Native to Europe, but distributed throughout the United States and Canada. Also

found in Australia, New Zealand, and North Africa

Uses

Herbal formulation derived from the plant extract is traditionally used in the management of depression, anxiety, and insomnia, and gastritis. It appears to provide a tranquilizing effect. Medicinally, it has been used as a hepatoprotective agent as well as a diuretic. Topically it has been used as an astringent. Hypericum extracts are licensed in Germany for the treatment of depression, anxiety, and insomnia. In the United States it is considered a 'dietary supplement' and is not classified by the Food and Drug Administration as a drug. The plant extract is being investigated in clinical trials for the use in AIDS patients, as hypericin may be synergistic with another AIDS drug, AZT (3′-azido-3′-deoxythymidine or Retrovir or Ziduvodin).

Exposure Routes and Pathways

The oral and dermal routes are the most common exposure pathways.

Mechanism of Toxicity

Saint John's wort is a serotonin reuptake inhibitor and to a lesser degree appears to inhibit monoamine oxidase. The toxin is hypericin, an anthraquinone dimer, which is present throughout the plant. It also contains tannin, rutin, and flavinoids.

Acute and Short-Term Toxicity (or Exposure)

Animal

Photosensitization has occurred.

Human

Minimal toxicity data is available. Photosensitization is possible. Acute overdose may cause increased heart rate, diarrhea, fever, erythema, and pruritus.

Chronic Toxicity (or Exposure)

Animal

Minimal data available in either laboratory animal studies or controlled clinical trials. However, toxicity to livestock, especially sheep, has been reported. Common symptoms include edematous lesions of the skin, especially exposed areas.

Human

Adverse effects may include nausea, fatigue, and, confusion. Neuropathy has also been reported.

Clinical Management

Treatment is symptomatic and supportive. Concerns have been noted in the literature about the potential for adverse interactions with other drugs, especially antidepressants and sympathomimetics.

See also: Fluoxetine.

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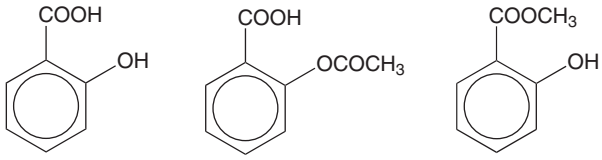
Salicylates

Alexander B Baer and Christopher P Holstege

- Chemical Abstracts Service Registry Number: CAS 50-78-2
- Synonyms: Aspirin; Acetylsalicylic acid; Acetaminosalol; Aluminum aspirin; Bismuth subsalicylate; Choline salicylate; Magnesium salicylate; Methyl salicylate; Phenyl salicylate; Potassium salicylate; Salsate; Salicylsalicylic acid; Sodium salicylate; Sodium thiosalicylate; Triethanolamine salicylate; Willow extract
- Chemical/Pharmaceutical/Other Class: Nonsteroidal synthetic derivatives of salicylic acid

- Chemical Structures:



Salicylic acid | Aspirin | Methyl salicylate

Uses

Salicylates are used for their analgesic, antipyretic, antiinflammatory, and keratolytic properties.

Exposure Routes and Pathways

Oral ingestion is the most common route of both accidental and intentional exposure to salicylates. Salicylates are also available in topical and rectal dosage forms.

Toxicokinetics

After ingestion, absorption occurs primarily from the upper small intestine via passive diffusion of unionized molecules. Serum salicylates are detected within 5–30 min after oral administration of rapidly absorbed dosage forms (aqueous solutions and uncoated or film-coated tablets). The rate of absorption may be slower with large, potentially lethal salicylate doses due to delayed gastric emptying, impaired dispersion of the drug in gastrointestinal fluids, and the possible formation of concretions. Absorption may also be delayed following ingestion with sustained release or enteric-coated preparations. Topical application of salicylic acid may result in systemic toxicity, especially in use with infants or in areas where the epidermis is disrupted. Rectal absorption is slow and unreliable.

Salicylates are metabolized principally in the liver by the microsomal enzyme system and are predominately conjugated with glycine to form salicyluric acid. Salicylates are also conjugated with glucuronic acid to form salicylphenolic glucuronide and salicylacyl glucuronide. In addition, small amounts of salicylates are hydrolyzed to form gentisic acid, which is an active metabolite and a potent inhibitor of prostaglandin synthesis. Salicylates rapidly distribute throughout extracellular fluid and into body tissues. Under normal physiologic acid–base conditions, salicylates cross the blood–brain barrier slowly because the ionized form predominates. However, systemic acidosis results in formation of the un-ionized salicylate which more easily distributes into tissues, especially the central nervous system. The volume of distribution (V_d) of salicylate at therapeutic levels is $0.2\,l\,kg^{-1}$, with ~80% of salicylate protein bound. As salicylate levels increase, the proportion bound to plasma protein decreases, and the V_d increases to $\sim 0.6\,l\,kg^{-1}$.

Salicylate and its metabolites are rapidly and almost completely excreted in the urine by glomerular filtration and by renal tubular secretion. Passive reabsorption of salicylate occurs in the distal tubules. Salicylate elimination is saturable and characterized by Michaelis–Menton kinetics where the elimination half-life is dependent on the dose. Since the pK_a of salicylic acid is 3, its renal clearance is greatly influenced by changes in urinary pH. Increasing urinary pH can significantly increase the overall salicylate elimination rate via ion trapping.

Mechanism of Toxicity

In acute salicylate toxicity, nausea, vomiting, and abdominal discomfort occur due to both local gastric irritation and stimulation of the medullary chemoreceptor trigger zone. Salicylates increase sensitivity to carbon dioxide in the medulla oblongata, thereby inducing hyperventilation, decreasing PCO_2, and causing respiratory alkalosis. A compensatory increase in the renal excretion of bicarbonate leads to the loss of potassium and sodium in the urine. A metabolic acidosis may follow due to the accumulation of organic acids. As a result, salicylate poisoning may produce a mixed acid–base abnormality consisting of both respiratory alkalosis and metabolic acidosis. In very large overdoses, salicylates uncouple oxidative phosphorylation, resulting in a failure to produce adenosine triphosphate while at the same time increasing oxygen utilization and carbon dioxide production. This results in an increase in heat production. Salicylates also interfere with glucose metabolism and gluconeogenesis. Salicylates may also profoundly decrease brain glucose concentrations despite normoglycemia.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals may manifest toxicity to salicylates with signs and symptoms similar to those seen in humans. These may include fever, hyperpnea, seizures, respiratory alkalosis, metabolic acidosis, gastric hemorrhage, and kidney damage. Methemoglobinemia has also been seen in animals following salicylate toxicity. Activated charcoal has been used in animals. Methylene blue or ascorbic acid may be utilized for the treatment of methemoglobinemia.

Human

Acute ingestions of over 150 mg kg^{-1} may result in toxic effects. Nausea and vomiting are seen early in toxicity. Tinnitus and hyperventilation also commonly occur early in toxicity. As severity of toxicity increases, intractable vomiting, hyperthermia, confusion, coma, seizures, pulmonary edema, acute renal failure, and death may occur. Hyperglycemia may be seen early, whereas hypoglycemia may occur later in toxicity. Acid–base disturbances such as respiratory alkalosis and/or metabolic acidosis may be noted. Toxic salicylate blood levels appear over 30 mg dl^{-1}. In overdose, the formation of concretions, slow absorption of enteric coated tablets, and delayed gastric emptying may delay toxic reactions and cause salicylate levels to rise over the first 12–24 h. The Done nomogram provides no value in the assessment of acute ingestions and should not be used.

Chronic Toxicity (or Exposure)

Animal

Cats are particularly susceptible to the effects of salicylate due to a lack of ability to rapidly metabolize the drug. Low doses (33–63 mg kg^{-1} day^{-1}) in cats can produce hepatic damage, central nervous system (CNS) depression, vomiting, and weight loss. In rats, high doses (300 mg kg^{-1} day^{-1}) inhibits ovulation.

Human

Patients with chronic salicylism may present with symptoms clinically similar to those seen in the acute situation. However, some patients with chronic salicylate overdose may present with CNS effects as their primary complaint, and typically have a higher morbidity and mortality than patients with acute salicylate overdose. Chronic salicylism is more often associated with pronounced hyperventilation, dehydration, pulmonary edema, renal failure, coma, seizures, and acidosis. Chronic salicylism patients will have more profound clinical effects at lower serum salicylate levels compared to patients with acute overdoses. Patients have developed toxicity with chronic salicylate serum levels as low as 15 mg dl^{-1}.

In Vitro Toxicity Data

Salicylates have been negative in Ames *Salmonella* assays for mutagenicity.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Gastrointestinal decontamination procedures should be considered in the patient with appropriate airway protection. In general, a single dose of activated charcoal should be considered in patients that have substantial ingestions. Salicylate ingestions can result in substantial delays in absorption; therefore, charcoal may be given even up to 8 h postingestion and more than one dose of charcoal may be considered to prevent further drug absorption. Careful correction of fluid and electrolyte abnormalities is essential. The clinician should insure adequate urine output, but forced diuresis should be avoided. Administration of intravenous sodium bicarbonate should be considered in patients manifesting signs and symptoms of salicylate toxicity. Hemodialysis effectively increases clearance and improves fluid/electrolyte balance. This extracorporeal method of elimination should be considered in patients with acute mental status changes, renal failure, intractable acidosis, pulmonary edema, severe fluid imbalance, or acute serum salicylate levels over 100 mg dl^{-1} or patients with chronic salicylate overdose who have symptoms and serum levels >60 mg dl^{-1}.

See also: Acetylsalicylic Acid; Gastrointestinal System.

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Salmonella

Melanie J Karst

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Description

Salmonella is a genus of gram-negative, facultatively anaerobic, rod-shaped bacteria that utilizes citrate as a sole carbon source. It is pathogenic for humans, causing enteric fevers, gastroenteritis, and bacteremia. Food poisoning is the most common clinical manifestation. Organisms within this genus are separated on the basis of antigenic characteristics, sugar fermentation patterns, and bacteriophage susceptibility. About 2200 types of *Salmonella* species are known.

Source of Exposure and Transmission

Salmonellosis in humans is contracted mainly through the consumption of raw or undercooked contaminated food of animal origin (mainly meat, poultry, eggs, and milk) although many other foods have been implicated in its transmission. The primary route by which humans acquire infection is by consumption of contaminated food of animal origin. Unlike *Salmonella enteritidis*, which is mainly associated with poultry and eggs, multidrug resistant *S. typhimurium* DT 104 can be found in a broad range of foodstuffs. The organisms pass through the food chain from primary production or through cross-contamination from food products in households or food-service establishments and institutions such as hospitals. In developed countries, human to human transmission is uncommon but can occur, notably in institutions; for instance, special-care baby units and residential homes for the elderly. Little is known about the epidemiology in developing countries but spread within hospitals and health centers has been reported.

A total of 2213 different *Salmonella* strains have been identified. *Salmonella* infects the digestive tracts of many domestic and wild animals, birds, and reptiles. They can be classified according to their adaptation to human and animal hosts:

- Group 1, for example, *S. typhi* and *S. paratyphi*, causes enteric fever only in humans and in higher primates.
- Group 2 causes disease in certain animals: *S. dublin* in cattle, *S. cholerae-suis* in pigs, but only infrequently in humans. However, when these strains do cause disease in humans, it is often invasive and can be life threatening.
- Group 3 includes the remaining strains. Typically, such strains cause gastroenteritis that is often mild and self-limiting but can be severe in the young, the elderly, and patients with weakened resistance against infectious diseases. This group includes *S. enteriditis* and *S. typhimurium*, the two most important strains for salmonellosis (transmitted from animals to humans).

Exposure Routes and Pathways

Outbreaks in the United Kingdom have been linked to poultry, a variety of meats and meat products, and unpasteurized milk. In addition to acquiring infection from contaminated food, human cases have also occurred where individuals have had contact with infected cattle. A small proportion of infected individuals may have contracted infection from pets such as cats and dogs, which can also be infected with some strains of *Salmonella*. These pets probably acquire the infection like humans, in other words through consumption of contaminated raw meat, poultry or poultry-derived products.

The evolution of specific *Salmonella* serotypes in intensive animal husbandry and subsequently in humans has been observed over the last three decades. The most recent epidemic was caused by *S. enteritidis*, which peaked in humans in 1992 in many European countries. Its current slight decline sets the scene for re-emergence of *S. typhimurium* as – epidemiologically – the most important serotype in human salmonellosis.

Dose

An infective dose may be as few as 15–20 cells depending on the age and condition of the host. The time of onset of symptoms depends on host factors, ingested dose, and strain characteristics.

Mechanism of Toxicity

The *Salmonella* organisms pass from the gut lumen and penetrate the epithelium in the small intestine where inflammation occurs. There is some evidence that an enterotoxin may be produced in some strains. *Salmonella* strains may produce a heat labile

enterotoxin related to the *Escherichia coli* heat labile enterotoxin or cholera toxin. Cytotoxins related to but distinct from those produced by *E. coli* or *Shigella* may also be produced.

Recent work indicates that a major virulence mechanism for *Salmonella* may involve type III secretion systems, which are encoded on plasmids and allows direct transfer of bacterial proteins to eukaryotic cells through a contact-dependent secretion mechanism. These effector proteins are capable of enhancing virulence and epithelial cell invasion.

Diagnosis of Human Infection/Illness

Diagnosis requires serological identification of culture isolated from a stool sample.

Nature of the Disease

The clinical course of human salmonellosis is usually characterized by acute onset of fever, abdominal pain, diarrhea, nausea, and sometimes vomiting. In some cases, particularly in the very young and in the elderly, dehydration can become severe and life threatening. Antibiotic treatment is necessary in less than 2% of the clinical cases. Serious complications occur in a small proportion of cases. The incidence is particularly high in children and the elderly, accounting for up to 60% of all reported laboratory confirmed cases. Studies in developed countries indicate that more than 80% of all salmonellosis cases occur individually rather than as outbreaks.

The onset of symptoms of *Salmonella* gastroenteritis is usually 6–72 h. Acute symptoms may last for 1–2 days or may be prolonged depending on host factors, ingested dose, and strain. Arthritic symptoms may occur 3–4 weeks after onset of acute symptoms. Symptoms are more severe in the elderly, infants, and immunocompromised individuals. *S. typhi* and *S. paratyphi* A, B, and C produce typhoid and typhoid-like symptoms in humans. Enteric fever (typhoid fever) may develop; other symptoms include anorexia, abdominal pain, malaise, myalgias, headache, cough, diarrhea or constipation, and delirium. Subsequent *Salmonella* septicemia may affect virtually every organ system.

Clinical Management

Severe forms of *Salmonella* infection may require hospitalization and isolation from other people. Patients with less severe infection and those who are recovering may be treated at home.

Antibiotics generally are not recommended unless the infection has spread from the intestines, because such medication can prolong rather than reduce the period of bacterial shedding in the intestine. Treatment involves monitoring hydration status and intravenous therapy to correct electrolyte imbalance. For individuals at high risk for invasive disease the recommended antibiotics include ampicillin, amoxicillin, trimethoprim–sulfamethoxazole, cefotaxime, and ceftriaxone.

Salmonella usually remains in the intestines for up to 5 weeks – and in some cases for many months. Be aware that some individuals can become chronic carriers of *Salmonella* bacteria and ~2% may develop chronic arthritis. Good personal hygiene and handwashing techniques would prevent the majority of transmissions. Wash hands thoroughly with warm, soapy water after visits to the restroom and before food preparation.

See also: Ecotoxicology, Genetic; Food and Drug Administration, US; Food Safety and Toxicology

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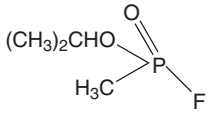
http://www.cdc.gov – CDC. Division of Bacterial and Mycotic Diseases. Salmonellosis.

Sarin

Harry Salem and Frederick P Sidell*

Published by Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 107-44-8
- Synonyms: GB; *o*-Isopropyl methyl phosphonofluoridate; G agent; Nerve gas
- Chemical/Pharmaceutical/Other Class: Nonpersistent anticholinesterase compound or organophosphate (OP) nerve agent, colorless to black liquid with no odor
- Chemical Formula: $C_4H_{10}FO_2P$
- Chemical Structure:



Uses

Sarin is a human-made nerve (gas) agent used in chemical warfare. It is an irreversible cholinesterase inhibitor.

Exposure Routes and Pathways

Casualties are caused primarily by inhalation, but can occur following percutaneous and ocular exposure, as well as by ingestion and injection. Sarin mixes easily with water, and people could be exposed by drinking contaminated water or via dermal contact with contaminated water. People could be exposed by eating contaminated food. Clothing can release sarin for ~30 min, which could lead to exposure of other people. Sarin vapor is heavier than air, and can sink to low-lying areas.

Toxicokinetics

Sarin is absorbed both through the skin and via respiration. It is more soluble in water than the other nerve agents (soman (GD) and VX); its solubility is directly related to temperature. The half-life of sarin, however, is inversely related to temperature and pH. In water the half-life of sarin is 15 min at 30°C and at pH 7.6. Nerve agents inhaled as vapors or aerosols enter the systemic circulation, resulting in toxic manifestations within seconds to 5 min of inhalation.

*The views of the authors do not purport to reflect the position of the US Department of Defense. The use of trade names does not constitute official endorsement or approval of the use of such commercial products.

Mechanism of Toxicity

Sarin and the other nerve agents are organophosphorus cholinesterase inhibitors. They inhibit the enzymes butyrylcholinesterase in the plasma, acetylcholinesterase on the red blood cell, and acetylcholinesterase at cholinergic receptor sites in tissues. These three enzymes are not identical. Even the two acetylcholinesterases have slightly different properties, although they have a high affinity for acetylcholine. The blood enzymes reflect tissue enzyme activity. Following acute nerve agent exposure, the red blood cell enzyme activity most closely reflects tissue enzyme activity. During recovery, however, the plasma enzyme activity more closely parallels tissue enzyme activity.

Following nerve agent exposure, inhibition of the tissue enzyme blocks its ability to hydrolyze the neurotransmitter acetylcholine at the cholinergic receptor sites. Thus, acetylcholine accumulates and continues to stimulate the affected organ. The clinical effects of nerve agent exposure are caused by excess acetylcholine.

The binding of nerve agent to the enzymes is considered irreversible unless removed by therapy. The accumulation of acetylcholine in the peripheral and central nervous systems leads to depression of the respiratory center in the brain, followed by peripheral neuromuscular blockade causing respiratory depression and death.

The pharmacologic and toxicologic effects of the nerve agents are dependent on their stability, rates of absorption by the various routes of exposure, distribution, ability to cross the blood–brain barrier, rate of reaction and selectivity with the enzyme at specific foci, and their behavior at the active site on the enzyme.

Red blood cell enzyme activity returns at the rate of red blood cell turnover, which is ~1% per day. Tissue and plasma activities return with synthesis of new enzymes. The rates of return of these enzymes are not identical. However, the nerve agent can be removed from the enzymes. This removal is called reactivation, which can be accomplished therapeutically by the use of oximes prior to aging. Aging is the biochemical process by which the agent–enzyme complex becomes refractory to oxime reactivation. The toxicity of nerve agents may include direct action on nicotinic acetylcholine receptors (skeletal muscle and ganglia) as well as on muscarinic acetylcholine receptors and the central nervous system (CNS).

Recently, investigations have focused on organophosphate nerve agent poisoning secondary to

acetylcholine effects. These include the effects of nerve agents on γ-amino butyric acid neurons and cyclic nucleotides. In addition, changes in brain neurotransmitters such as dopamine, serotonin, noradrenaline, and acetylcholine following inhibition of brain cholinesterase activity have been reported. These changes may be due in part to a compensatory mechanism in response to overstimulation of the cholinergic system or could result from direct action of nerve agent on the enzymes responsible for noncholinergic neurotransmission.

Human Toxicity

Toxic effects occur within seconds to 5 min of nerve agent vapor or aerosol inhalation. The muscarinic effects include ocular (miosis, conjunctival congestion, ciliary spasm), nasal discharge, respiratory (bronchoconstriction and increased bronchial secretion), gastrointestinal (anorexia, vomiting, abdominal cramps, and diarrhea), sweating, salivation, and cardiovascular (bradycardia and hypotension) effects. The nicotinic effects include muscular fasciculation and paralysis. CNS effects can include ataxia, confusion, loss of reflexes, slurred speech, coma, and paralysis.

Following inhalation of sarin, the median lethal dosage (LCt_{50}) in humans has been estimated to be $70\,mg\,min\,m^{-3}$ at a respiratory minute volume (RMV) of $15\,l\,min^{-1}$, $100\,mg\,min\,m^{-3}$ at an RMV of $10\,l\,min^{-1}$ (resting) for a duration of 0.5–2 min.

Following percutaneous exposure of bare skin to sarin vapor, the LCt_{50} has been estimated at $12\,000\,mg\,min\,m^{-3}$ for a 70 kg human. For liquid percutaneous exposure, the LD_{50} has been estimated as 1.7 g for a 70 kg human, and for intravenous injection the LD_{50} has been estimated as 1 mg for a 70 kg human.

Median incapacitation doses estimated for humans following inhalation of sarin for an RMV of $15\,l\,min^{-1}$ for a 10 min exposure are as follows: $40\,mg\,min\,m^{-3}$ for moderate incapacitation, $56\,mg\,min\,m^{-3}$ for severe incapacitation, and $72\,mg\,min\,m^{-3}$ for very severe incapacitation. The symptoms for moderate incapacitation include maximal miosis, eye pain, headache, twitching eyelids, difficulty in ocular accommodation, tightness of chest, runny nose, salivation, sneezing and coughing, anorexia, nausea, heartburn, fatigue, weakness, muscle fasciculation, anxiety, and insomnia. Severe incapacitation includes all of the above plus diarrhea, frequent urination, dysphoria, and ataxia. For very severe incapacitation, the principal effects are convulsions, collapse, and paralysis.

The minimum effective dosage for miosis in man has been estimated between 2 and $4\,mg\,min\,m^{-3}$. The permissible airborne exposure concentration of sarin for an 8 h workday or a 40 h work week is an 8 h time-weighted average (TWA) of $0.00003\,mg\,m^{-3}$.

Sarin is the nerve agent studied most thoroughly in humans. At an estimated concentration of 3–5 mg $min\,m^{-3}$ in humans, it will produce miosis, rhinorrhea, and a feeling of tightness in the throat or chest. Exposure to small amounts of nerve agent vapor causes effects in the eyes, nose, and airways. These effects are from local contact and are not indicative of systemic absorption. Small amounts of liquid agent on the skin cause systemic effects initially in the gastrointestinal tract. Lethal amounts of vapor or liquid cause a rapid cascade of events resulting, within 1 or 2 min, in loss of consciousness and convulsive activity followed by apnea and muscular flaccidity.

Although miosis is a characteristic sign of exposure to the nerve agent, rhinorrhea may be the first indication. It severity is dose dependent.

Miosis occurs from direct contact of vapor with the eyes. It may also occur from moderate to severe exposure of skin to liquid agent or from a liquid droplet near the eye. Miosis will begin with seconds or minutes following vapor exposure and may not be complete for many minutes if the exposure concentration is low. In unprotected individuals, miosis is bilateral and is often accompanied by complaints of pain, dim and blurred vision, conjunctival injection, nausea, and occasionally vomiting. On occasion, subconjunctival hemorrhage is also present.

Inhalation of nerve agent vapor causes bronchoconstriction and increased secretions of the glands in the airways, which is dose related. Small amounts of the nerve agent will produce a feeling of slight tightness in the chest to severe respiratory distress following large amounts. Large amounts will cause cessation of respiration (apnea) within minutes after the onset. Both CNS effects and peripheral effects (skeletal muscle weakness and bronchoconstriction) may contribute to the apnea.

Systemic absorption of the nerve agent will cause increased motility of the gastrointestinal tract and an increase in glandular secretions. Nausea and vomiting are early signs of liquid exposure on the skin and diarrhea may occur following large amounts of agent.

Nerve agent exposure to glands increases their secretions. These glands include lacrimal, nasal, salivary, and bronchial. Localized sweating will occur at the site of liquid agent on the skin, and after large liquid or vapor exposure generalized sweating is common.

Stimulation of skeletal muscles by nerve agents will produce muscular fasciculation and twitching large amounts of the agent will cause fatigue and muscle weakness followed by muscular flaccidity.

Large amounts of the nerve agent in the CNS will cause loss of consciousness, seizure activity, and apnea. CNS effects of smaller amounts of the agent vary and are nonspecific. However, they may include forgetfulness, inability to concentrate, insomnia, bad dreams, irritability, impaired judgment, and depression. These effects may persist up to 6 weeks.

Nerve agent exposure may cause bradycardia due to vagal stimulation or it may often cause the reverse tachycardia due to fright and hypoxia and adrenergic stimulation secondary to ganglionic stimulation. Bradyarrhythmias such as first-, second-, or third-degree heart block may also occur. Blood pressure may also be elevated because of adrenergic stimulation, but it is usually normal until the terminal decline.

Clinical Management

Management of nerve agent intoxication consists of decontamination, ventilation, administration of antidotes, and supportive therapy.

The three therapeutic drugs for treatment of nerve agent intoxication are atropine, pralidoxime chloride, and diazepam.

Atropine, a cholinergic blocking or anticholinergic drug, is effective in blocking the effects of excess acetylcholine at peripheral muscarinic sites. The usual dose is 2 mg, which may be repeated at 3–5 min intervals. Pralidoxime chloride (protopam chloride; 2-PAM CL) is an oxime used to break the agent–enzyme bond and restore the normal activity of the enzyme. Abnormal activity decreases and normal strength returns to skeletal muscles, but no decrease in secretions is seen following oxime treatment. The usual dose is 1000 mg (iv or im), which may be repeated 2 or 3 times at hourly intervals, intravenously or intramuscularly. Diazepam, an anticonvulsant drug is used to decrease convulsive activity and reduce brain damage that may occur from prolonged seizure activity. It is suggested that all three of these drugs be administered at the onset of severe effects from nerve agent exposure, whether or not seizures occur. The usual dose of diazepam is 10 mg (im).

Miosis, pain, dim vision, and nausea can be relieved by topical atropine in the eye. Pretreatment with carbamates may protect the cholinesterase enzymes before nerve agent exposure.

Supportive therapy may include ventilation via an endotracheal airway if possible and suctioning of excess secretions from the airways.

Animal Toxicity

Small doses of nerve agents in animals can produce tolerance in additional to their classical cholinergic effects. In rats, acute administration of nerve agents in subconvulsive doses produced tumors and hind-limb adduction. In animals, nerve agents can also cause behavioral as well as cardiac effects.

The cause of death is attributed to anoxia resulting from a combination of central respiratory paralysis, severe bronchoconstriction, and weakness or paralysis of the accessory muscles for respiration.

Table 1 Inhalation LCt_{50} values of sarin in various species

Species	*LCt_{50} (mg min m^{-3})*	*Exposure duration (min)*
Mouse	150	30
Rat	1500	10
Guinea pig	256	2
Rabbit	1200	10
Cat	1000	10
Dog	1000	10
Monkey	1000	10

Table 2 Acute toxicities of sarin in various species by various routes of exposure

Route of exposure/species		*LD_{50} (μg kg^{-1})*
Percutaneous		
	Mouse	1 080
	Rabbit	925
Intravenous		
	Mouse	109
	Rat	39
	Rabbit	15
	Cat	22
	Dog	19
	Monkey	22 300
Intramuscular		
	Rat	108
	Mouse	164
Intraperitoneal		
	Mouse	283
	Rat	218
Oral		
	Rat	550
Subcutaneous		
	Rat	103
	Mouse	60
	Rabbit	30
	Guinea pig	30
	Hamster	95

Signs of nerve agent toxicity vary in rapidity of onset, severity, and duration of exposure. These are dependent on the specific agent, route of exposure, and dose. At the higher doses, convulsions and seizures indicate CNS toxicity.

Following nerve agent exposure, animals exhibit hypothermia resulting from the cholinergic activation of the hypothalamic thermoregulatory center. In addition, plasma concentrations of pituitary, gonadal, thyroid, and adrenal hormones are increased during organophosphate intoxication.

The LCt_{50} values ($mg\,min\,m^{-3}$) reported following the inhalation of sarin are presented in **Table 1**. The acute toxicities by other routes of exposure in various animal species are presented in **Table 2**.

See also: Nerve Agents; Soman; Tabun; V-Series Nerve Agents: Other than VX; VX.

Relevant Websites

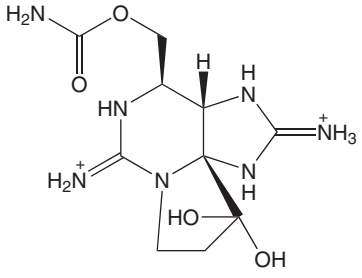
http://www.bt.cdc.gov – US Centers for Disease Control and Prevention, Agency for Toxic Substances and Disease Registry, Chemical Agents.

http://sis.nlm.nih.gov – US National Library of Medicine, Specialized Information Services, Chemical Warfare Agents.

Saxitoxin

Samantha E Gad and Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 35523-89-8
- Synonyms: Mussel poison; Clam poison; Paralytic shellfish poison; Gonyaulax toxin; STX
- Chemical/Pharmaceutical/Other Class: Complex of amino acids – a tetrahydropurine
- Chemical Formula: $C_{10}H_{17}N_7O_4$
- Chemical Structure:



Background Information

Saxitoxin is a naturally occurring toxin that is synthesized by various marine dinoflagellates. It is used in neurochemical and molecular biology research. Saxitoxin causes paralytic shellfish poisoning. It is far more potent than the classic puffer fish toxin, tetrodotoxin. Saxitoxin is one of only two naturally occurring schedule 1 chemical warfare agents (the other is ricin).

Exposure Routes and Pathways

Ingestion of shellfish containing saxitoxin is the primary route of exposure.

Toxicokinetics

Saxitoxin is readily absorbed from the gastrointestinal tract and through mucous membranes.

Mechanism of Toxicity

Saxitoxin binds to the sodium channels in the membranes of excitable cells (neurons and muscle cells) blocking synaptic transmission. Saxitoxin is connected to red tides. Saxitoxin reduces nerve conduction velocities.

Acute and Short-Term Toxicity (or Exposure)

Animal

The LD_{50} values in mice are $10\,\mu g\,kg^{-1}$ (intraperitoneal), $263\,\mu g\,kg^{-1}$ (oral), and $3.4\,\mu g\,kg^{-1}$ (intravenous). One milligram is estimated to be sufficient to kill 5500 mice.

Human

Saxitoxin paralyzes the peripheral nervous system and alters cardiac chronotrophy. Symptoms include gastrointestinal complaints and paresthesias of the face, followed by (in severe cases) muscle paralysis. The estimated lethal dose in humans is 0.3–1 mg. A single contaminated shellfish may contain 50 lethal doses. Mortality for reported cases is 5.9%, but has a

higher rate in children. Clinical effects develop over 30 min to 3 h.

Chronic Toxicity (or Exposure)

Animal

Little information is available on the chronic effects of saxitoxin in animals.

Human

There are no reports on the chronic effects of saxitoxin in humans.

Clinical Management

The gut should be decontaminated and the patient observed carefully for signs of respiratory depression. Treatment is primarily supportive. Artificial respiration may be necessary.

Environmental Fate

Saxitoxin is a naturally occurring substance in dinoflagellates and taken up by shellfish. Consumption of the shellfish leads to toxicity. Aside from the knowledge that these organisms serve as a source of exposure, the environmental fate of the chemical itself has not been studied. It is heat-stable but sensitive to strong alkali.

Ecotoxicology

Red tides (containing saxitoxin) have been known to kill fish since antiquity. Humpback whales have died shortly after consuming fish that were contaminated with saxitoxin. Thus, accumulation of saxitoxin up the food chain may occur.

See also: Neurotoxicity; Red Tide; Shellfish Poisoning, Paralytic; Toxicity Testing, Aquatic.

Further Reading

Dart RC (2004) *Medical Toxicology*, 3rd edn. Baltimore: Lippincott.

Pelligrino RG (2000) Saxitoxin. In: Spencer PS and Schaumburg HH (eds.) *Experimental and Clinical Neurotoxicology,* 2nd edn., pp 1093–1095. New York: Oxford University Press.

Relevant Websites

http://vm.cfsan.fda.gov – FDA/CFSAN Bad Bug Book.

http://www.bris.ac.uk – Saxitoxin (by Edwards N, The Chemical Laboratories at the University of Sussex at Brighton).

Scombroid

F Lee Cantrell

This article is a revision of the previous print edition article by Gaylord P Lopez, volume 3, pp. 125–126, © 1998, Elsevier Inc.

- SYNONYMS: Scombroidtoxicosis; Form of Ichthyosarcotoxicosi

Background Information

Scombroid refers to a complex of clinical symptoms associated with ingestion of improperly handled or stored fish from the Scombroidae family: mahi mahi, bluefish, Bombay duck, kahawai, kingfish, swordfish, pacific amberjack, salmon, tuna, bonito.

Exposure Routes and Pathways

The toxin (histamine) is created within the flesh of certain fish under specific environmental conditions. Histidine is present in the muscle protein of Scombrodiae. In the presence of certain bacteria, histadine gets broken down to histamine. Ingestion of the flesh of improperly handled fish causes ingestion of large amounts of histamine and development of symptoms of histamine poisoning. Histamine is not destroyed or inactivated by heating or cooking. Contaminated fish often looks and smells normal, but is periodically described as having a peppery taste. Proper refrigeration/freezing of fresh fish will dramatically reduce the risk of scombroid poisoning.

Toxicokinetics

Ingestion of histamine and saurine, when present in large amounts, results in histaminic effects. Absorption is rapid with clinical effects generally being seen within 5–90 min. The duration of untreated scombroid poisoning is generally 12–24 h.

Mechanism of Toxicity

The previously mentioned types of fish contain free histidine in their musculature. During spoilage, bacteria on the surface of the fish enzymatically convert histidine to histamine and saurine, which are responsible for the symptoms. The Food and Drug Administration considers levels of histamine >50 mg per 100 g of fish potentially toxic.

Acute and Short-Term Toxicity (or Exposure)

Human

Initial symptoms are those of a histamine reaction and typically occur within 5–90 min of ingestion. Common symptoms include dermal flushing especially of the face, neck, and upper torso, headache, nausea, vomiting, and diarrhea. Facial edema, burning of the mouth and throat, palpitations, dizziness, and rash has also been noted. Bronchospasm, urticaria, shock, and death are rare. Symptoms usually resolve within 3–24 h.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Treatment is generally symptomatic and supportive. Gastrointestinal decontamination procedures maybe considered for substantial recent ingestions. Other therapy is directed at limiting histaminic symptoms. Administration of oral or parenteral H_1 and H_2 receptor antagonists is appropriate. Inhaled beta-adrenergic agonists and epinephrine can be used for patients experiencing significant bronchospasm.

See also: Disulfiram; Fish Consumption Advisory.

Further Reading

Anon (2000) Scombroid fish poisoning – Pennsylvania, 1998. *Morbidity and Mortality Weekly Report* 49: 398–400.

Morrow JD, Margolies GR, and Rowland J (1991) Evidence that histamine is the causative toxin of scombroid-fish poisoning. *New England Journal of Medicine* 324: 716–720.

Scorpions

Gary W Everson

- SYNONYMS: *Centruroides* species; *Vejovis* species; *Hadrurus* species

Exposure Routes and Pathways

Scorpions inflict a sting and inject their venom subcutaneously with a stinger located at the end (telson) of their multisegmented tail.

Toxicokinetics

Scorpion venom may reach systemic circulation through lymphatic transport following a sting. Of those scorpions located in the United States, *Centruroides exilicauda*, found in southeastern California, Arizona, Nevada, southern Utah, and southwestern New Mexico, is an example of a scorpion that can produce significant systemic toxicity following envenomation. Onset of systemic symptoms typically occurs within 4 h of the sting. The metabolism of venom components is not well understood. Tissue distribution of venom is complex. Venom components differ among the multitude of scorpion species and thus venom distributes to different tissue sites.

Mechanism of Toxicity

Scorpion venom is composed of many different fractions that can vary among the different scorpion species. These venom fractions act at different tissue receptor sites. Local tissue reaction is a result of the inflammatory response to the injected foreign proteins and enzymes making up the venom. The venom of *Centruroides* species contains several different neurotoxins. These toxins block the transmission of nerve impulses in the central nervous system and in muscles by blocking the transport of ions through sodium and potassium channels at the cellular level. Other venom components may decrease the heart rate by causing the release of acetylcholine.

Acute and Short-Term Toxicity (or Exposure)

Human

Most scorpion stings produce some local tissue reaction that is characterized by mild to moderate

burning pain. Usually there is minimal swelling and redness. In the United States, this is the limit of the reaction following stings of *Vejovis* species, *Hadrurus* species, and several other common scorpions. Wound infection is also possible following the sting. The more poisonous *Centruroides* scorpions, represented by *C. exilicauda*, may also produce systemic symptoms following significant envenomation. However, even these scorpions often produce only pain and other localized reaction at the sting site. When systemic symptoms do develop, they include increased heart rate, hypertension, dilated pupils, sweating, and increased blood glucose. Also, salivation, tearing, diarrhea, and bradycardia may develop when parasympathetic nerve stimulation predominates from acetylcholine release. Other clinical effects may include blurred vision, nystagmus, opisthotonus, muscle fasciculations, convulsions, breathing difficulty, respiratory failure, and cardiac arrhythmias. Young children, the elderly, and those with pre-existing cardiovascular disease are at greater risk for severe systemic symptoms. Occasionally, poisonous exotic species make their way into the United States either by illegal importation or along with agricultural product shipments from abroad. Signs and symptoms following envenomation vary depending on the species of scorpion involved.

In Vitro Toxicity Data

Scorpion venom is used in a variety of research settings because of its ability to block sodium channels. A new class of toxin (tetrapandins) has recently been identified within the venom of *Pandinus imperator*. These toxins have been shown to have inhibitory effects on store-operated calcium entry in human embryonic kidney-293 cells.

Clinical Management

Basic and advanced clinical life support may be required following severe envenomation by several *Centruroides* species. However, most scorpion stings require only local wound care. Ice may be applied to the sting site for 10–15 min to help decrease pain. Acetaminophen, aspirin, or ibuprofen may be helpful for mild pain. Applying ice for long periods of time or immersing the sting site in an ice bath (cryotherapy) is not recommended since this procedure decreases blood flow at the site causing tissue damage. The majority of patients presenting with systemic symptoms can be managed at the hospital with supportive care, pain management, and observation. Careful monitoring of heart rate, blood pressure, and respiratory function are essential. Muscle spasms may respond to diazepam or calcium gluconate. Occasionally, hypertension is sufficiently severe or prolonged to require treatment. Depending on the severity of hypertension, nitroprusside, labetalol, or nifedipine may be indicated. Respiratory failure due to neuromuscular blockade is a rare but possible complication. Mechanical ventilation may be required.

C. exilicauda-specific antivenin has been available in Arizona for the treatment of severe stings of *Centruroides exilicauda* (bark) scorpions. These preparations, are not generally available outside Arizona, are not approved by the Federal Drug Administration and complete scientific data regarding their efficacy are lacking. However, anecdotal reports indicate that the antivenin has reversed symptoms associated with neuromuscular blockade within 30–60 min. The local regional poison information center may be contacted to locate antivenin and assist in determining whether clinical indications exist for its use.

See also: Acetylcholine; Animals, Poisonous and Venomous.

Further Reading

Amitai Y, Mines Y, and Aker M (1985) Scorpion sting in children. A review of 51 cases. *Clinical Pediatrics* 24: 136–140.

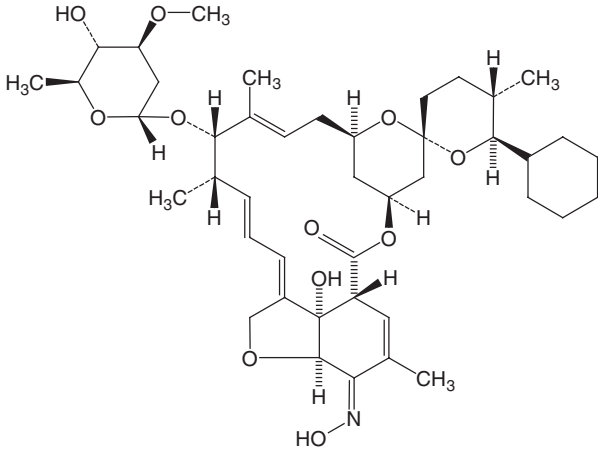
Sofer S, Shahak E, and Gueron M (1994) Scorpion envenomation and antivenom therapy. *Journal of Pediatrics* 124: 973–978.

Selamectin

Ramesh C Gupta

- Chemical Name: (5*Z*,25*S*)-25-cyclohexyl-4′-*O*-de(2,6-dideoxy-3-*O*-methyl-α-L-arabino-hexopyranosyl)-5-demethoxy-25-de(1-methylpropyl)-22,23-dihydro-5-(hydroxyimino)avermectin A_{1a}
- Chemical Abstracts Service Registry Number: CAS 220119-17-5
- Synonym: Revolution

- Chemical/Pharmaceutical/Other Class: Selamectin is a novel, semisynthetic avermectin, which has a molecular weight of 769.96. Selamectin is colorless to yellow and is flammable.
- Chemical Formula: $C_{43}H_{63}NO_{11}$
- Chemical Structure:



Uses

Selamectin is marketed as Revolution, which is a product of Pfizer, Inc. This is a topical parasiticide preparation recommended for use in dogs and cats 6 weeks of age and older. Selamectin is used to kill adult fleas and ear mites in dogs and cats. It also kills *Sarcoptes scabiei*, a mite that causes sarcoptic mange (scabies), certain ticks in dogs, and hookworms and roundworms in cats. Selamectin is also used to prevent heartworm disease and flea infestation in dogs and cats. The recommended dose is $6\,mg\,kg^{-1}$.

Exposure Routes and Pathways

Veterinarians, technicians, and pet-owners are exposed to selamectin through the dermal route.

Toxicokinetics

In dogs and cats, selamectin is rapidly absorbed from the skin into the bloodstream, where it kills heartworm microfilaria. Selamectin is excreted into the intestinal tract where it kills intestinal parasites. Finally, selamectin is selectively distributed from the bloodstream into the sebaceous glands of the skin, forming reservoirs that provide persistent efficacy against fleas, ear mites, and sarcoptic mites. Active concentrations of selamectin are found in the plasma for at least 30 days. It is excreted mostly in the feces and a small unmetabolized amount in the urine.

Mechanism of Toxicity

Selamectin binds to glutamate gated chloride channels in the parasite's nervous system, causing them to remain open. This causes chloride ions to continuously flow into the nerve cell, changing the charge of the cell membrane. The continuous flow of chloride ions blocks neurotransmission, and transmission of stimuli to muscles is prevented. Selemectin has no such effect in the mammalian nervous system, and therefore, it is much safer than common insecticides.

Acute and Short-Term Toxicity (or Exposure)

Animal

Hair loss at the site of application, vomiting, diarrhea, anorexia, lethargy, salivation, tachypnea, pruritis, urticaria, erythema, ataxia, lethargy, fever, and rare instances of death may occur with overt acute overdose.

Human

Selamectin may cause irritation to the skin and eyes. Reactions such as hives, itching, and skin redness have been observed in rare instances. Selamectin has a reported safety factor range of 279.

Chronic Toxicity (or Exposure)

Animal

In dogs and cats, seven monthly treatments of $60\,mg\,kg^{-1}$ (10 times the recommended dose) produced no adverse reactions when given to 6-week-old kittens or puppies. An exposure of $18\,mg\,kg^{-1}$ ($3\times$ dose) produced no effect on reproduction in females or males. Three monthly doses of $30\,mg\,kg^{-1}$ produced no adverse effects in ivermectin sensitive collies. There have also been rare reports of muscle spasms, seizures, ataxia, and other neurological signs.

Human

Little is known regarding effects of long-term exposure to selamectin in humans but experimental data indicate little potential for chronic toxicity.

Clinical Management

Individuals with known hypersensitivity to selamectin (Revolution) should use the product with caution. Wash hands after use and wash off any product in contact with the skin immediately with soap and water. If the product comes in contact with eyes, then

flush eyes with copious amounts of water. In case of ingestion, contact a physician immediately.

See also: Avermectins; Pesticides; Veterinary Toxicology.

Relevant Websites

http://cal.vet.upenn.edu – University of Pennsylvania.
http://www.vspn.org – Tina Wismer, ASPCA Animal Poison Control Center.

Selenium

Shayne C Gad

This article is a revision of the previous print edition article by Arthur Furst and Shirley B Radding, volume 3, pp. 127–128, © 1998, Elsevier Inc.

- Selected Compounds: Hydrogen selenide (H_2Se); Sodium selenate (Na_2SeO_4); Sodium selenite (Na_2SeO_3); Selenium chloride (Se_2Cl_2). The toxicity of compounds varies substantially
- Chemical Abstracts Service Registry Number: CAS 7782-49-2
- Synonyms: Vandex; C.I. 77805
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formulas: Se^{4+}; Se^{6+}; Se^{2-}

Uses

Selenium is used in a wide variety of industries, including electronics, glass, ceramics, glass coloring, steel, pigment manufacturing, and rubber production. Medicinally, selenium is used in antidandruff shampoos and as a dietary supplement.

Background Information

Selenium was discovered in 1817. It is an essential trace element at $\sim$0.1 ppm in diets.

Exposure Routes and Pathways

For the general population, ingestion is the primary exposure pathway; sources include dietary supplements and various foods including seafood, meats, milk products, and grains. Trace amounts are found in drinking water. Selenium is not absorbed from shampoos.

In industrial settings, inhalation may be a significant exposure pathway. Airborne concentrations of selenium are higher in the vicinity of metallurgical industries. Selenium is present in most sulfide ores and is generally a by-product of the roasting of copper pyrite.

Toxicokinetics

Selenium and most of its compounds are rather insoluble and thus not absorbed orally. Soluble selenium compounds (e.g., sodium selenate and sodium selenite) are readily absorbed (up to 90%). Blood concentrations depend on the amount of selenium ingested. After blood levels of 200–240 $\mu g\,ml^{-1}$ are obtained, homeostatic controls take over. The greatest amount of absorbed selenium concentrates in the liver and kidneys, a lesser amount in the heart and lungs, and the least in the muscles.

Selenium is an essential trace element and an integral component of heme oxidase. It appears to augment the antioxidant action of vitamin E to protect membrane lipids from oxidation. The exact mechanism of this interaction is not known; however, selenium compounds are found in the selenium analogs of the sulfur-containing amino acids, such as cysteine and methionine. Se-cysteine is found in the active sites of the enzyme glutathione peroxidase, which acts to use glutathione to reduce organic hydroperoxides.

Selenium is rapidly excreted in the urine; some is incorporated into proteins. Elemental selenium and its oxides can be methylated. Trimethyl selenium is excreted rapidly in the urine; some is exhaled.

Mechanism of Toxicity

Excess selenium results in liver atrophy, necrosis, and hemorrhage. The mechanism of toxicity is unknown but may involve redox cycling. Sulfhydryl enzymes are attacked by soluble selenium compounds.

Acute and Short-Term Toxicity (or Exposure)

Animal

The toxicity depends on the molecular form. More soluble compounds, for example, sodium selenite, are more toxic than the less soluble elemental selenium, selenium sulfide, or selenium disulfide. Selenium dioxide is highly to moderately toxic, with oral

LD_{50} values in rodents from 20 to 70 mg kg^{-1}. The dermal LD_{50} for selenium dioxide in rabbits is 4 mg kg^{-1}. Oral LD_{50} values for sodium selenite range from 14 to 7 mg kg^{-1}, whereas an LD_{50} of 138mg kg^{-1} was noted for selenium disulfide, and an LD_{50} of >6 g kg^{-1} was noted for elemental selenium.

Respiratory effects include pulmonary congestion, hemorrhage and edema, dyspnea, weakness, and asphyxial convulsions. Acute exposures can also result in altered hematocrit, liver toxicity, and hemorrhage of the kidneys.

Human

Selenium is irritating to the eyes, skin, nose, and throat. The difference between an essential dose and a toxic dose for selenium is quite narrow. Normal intake can range from 50 to 200 μg; in the milligram range, toxicity is noted. Acute selenium poisoning results in nonspecific symptoms (e.g., eye irritation and coughing) and can affect the central nervous system and lead to convulsions. Liver and spleen damage has also been noted.

Inhalation of hydrogen selenide, a gas, may produce irritation of the upper respiratory tract and reduced respiratory flow rates, which can persist for a few years.

Chronic Toxicity (or Exposure)

Animal

Livestock and other animals are particularly affected by either selenium deficiency or excess selenium. In animals with selenium-deficient diets, liver necrosis arises. In areas with deficient selenium concentrations in soil, calves and lambs develop muscle atrophy, which is referred to as either 'white' muscle disease or 'stiff' muscle disease. Selenium supplementation (often injections) prevents these symptoms.

In areas with unusually high levels of selenium in the soil, livestock develop 'blind-stagger' disease, which is characterized by loss of vision, weakness of the limbs, and possible respiratory failure. Runoff from heavily fertilized farms causes excess selenium in ponds, which results in malformation of birds.

There are two interesting paradoxes concerning selenium. The first is that excess selenium is toxic; however, at lower levels it is a protective agent against the toxicity of cadmium, methylmercury, arsenic, copper, and thalium. The second paradox involves carcinogenicity. The US National Cancer Institute found selenium monosulfide (administered orally) to be carcinogenic in rodents; however, many epidemiological studies associate selenium intake with lower cancer rates in humans. Moreover, in the laboratory, selenium somewhat negates the carcinogenic action of carcinogenic aromatic hydrocarbons, acetylaminofluorene, and azo dyes, and it protects against spontaneous mammary tumors in various species of rodents.

Selenium is teratogenic in chickens and sheep; the evidence for humans is equivocal.

Human

Toxic manifestations of selenium poisoning include decaying and discoloring of teeth, gastrointestinal tract distress, skin lesions, and loss of hair and nails. In some cases, the skin on the fingertips and toes peels constantly. Excess selenium is metabolized to the dimethyl derivative, which is volatile and produces the 'garlic' or 'rotten' breath characteristic of selenium toxicity. Target organs are the respiratory tract, liver, kidneys, blood, skin, and eyes. Threshold limit value (TLV) = 0.2 mg m^{-1}.

Clinical Management

Currently, there are no antidotes of choice for selenium toxicity. Ethylenediaminetetraacetic acid and BAL (British antilewisite; 2,3-dimercaptopropanol) should not be used because they may enhance selenium toxicity. Treatment is symptomatic (e.g., cardiopulmonary). Often, supplemental oxygen is needed. Corrosive selenious acid (in gun-bluing solution) should be treated similar to other agents that cause esophageal burns.

Environmental Fate

Although selenium occurs naturally in the environment, it also can be released by both natural and manufacturing processes. As an element, selenium cannot be created or destroyed. However, forms of selenium can be transformed (changed) in the environment. Weathering of rocks to soil may cause low levels of selenium in water or it may cause it to be taken up by plants and naturally released into the air. Volcanic eruptions are suspected of contributing to selenium in air, and soils in the areas around volcanoes tend to have enriched amounts of selenium.

More commonly, selenium enters the air from burning coal or oil. Most of the selenium in air is bound to fly ash and to suspended particles. The elemental selenium that may be present in fossil fuels forms selenium dioxide during combustion (burning). Selenium dioxide can then form selenious acid with water or sweat. Selenium anhydride is released

during the heating of copper, lead, and zinc ores when there is selenium in them. Hydrogen selenide decomposes rapidly in air to form elemental selenium and water, thus eliminating the danger from this compound for most people, except those who are exposed to it in their workplace.

Airborne particles of selenium, such as in coal ash, can settle on soil or surface water. Disposal of selenium in commercial products and waste could also contribute to selenium levels in soil. But the amount of selenium released to soil from fly ash and hazardous waste sites has not been measured. The forms and fate of selenium in soil depend largely on the acidity of the surroundings and its interaction with oxygen. In theory, at equilibrium with no oxygen present, deep-soil selenium may be present as elemental selenium. In the absence of oxygen when the soil is acidic, the amount of biologically available selenium should be low. Elemental selenium that cannot dissolve in water and other insoluble forms of selenium (such as selenium sulfide and heavy metal selenides) are less mobile and will usually remain in the soil, posing less of a risk for exposure. Active agricultural or industrial processes may increase the amount of biologically available selenium by decreasing the acidity of the soil and increasing the oxygen and the soluble selenium compounds. Selenium compounds that can dissolve in water are very mobile. For example, selenates and selenites are water-soluble, and thus mobile, so there is an increased chance of exposure to them. Irrigation drainage waters may result in increased selenium entering the surface water. Other factors that may affect the rates at which selenium moves through the soil are temperature, moisture, time, season of year, concentration of water-soluble selenium, organic matter content, and microbiological activity.

Ecotoxicology

Selenium is implicated in the poisoning of birds in enclosed saline lakes. There is some evidence that selenium can be taken up in tissues of organisms ('bioaccumulate') and possibly increase in concentration ('biomagnify') in aquatic organisms as it is passed up through the food chain. Selenium concentrations in aquatic organisms have been a problem as a result of irrigation runoff in some dry areas of the United States. It is important to remember that selenium's behavior in the environment is largely affected by its surrounding conditions and by how it interacts with other compounds.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) TLV – time-weighted average (TWA) for hydrogen selenide is $0.05\,mg\,m^{-3}$. The ACGIH TLV – TWA for selenium and its compounds is $0.2\,mg\,m^{-3}$. Symptoms of overexposure include headache, chills, fever, bronchitis, garlic breath, gastrointestinal disturbance, and dermatitis.

See also: Metals; Veterinary Toxicology.

Further Reading

Goyer RA, Klaassen CD, and Waalkes MP (1995) *Metal Toxicology.* San Diego, CA: Academic Press.

Hamilton SJ (2004) Review of selenium toxicity in the aquatic food chain. *The Science of the Total Environment* 326(1–3): 1–31.

Whanger PD (2004) Selenium and its relationship to cancer: An update dagger. *British Journal of Nutrition* 91(1): 11–28.

Relevant Websites

http://risk.lsd.ornl.gov – Toxicity Summary for Selenium (from the Risk Assessment Information System).

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Selenium.

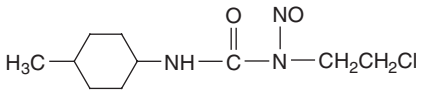
Semustine

Roberta Turci

- Chemical Abstracts Service Registry Number: CAS 13909-09-6
- Synonyms: Methyl-CCNU; *trans*-Methyl-CCNU; Lomustine, methyl; Methyl CCNU; MeCCNU; Me CCNU; Me-CCNU; NSC-95441; NSC 95441; NCI-C04955 Urea, *N*-(2-chloroethyl)-*N*′-(4-methylcyclohexyl)-*N*-nitroso-, *trans*-(9CI); Urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-, *trans*-(8CI); 1-(2-Chloroethyl)-3-(*trans*-4-methyl-

cyclohexane)-1-nitrosourea; 1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea

- Chemical/Pharmaceutical/Other Class: Nitrosoureas; Alkylating agents; Anticancer drugs
- Chemical Formula: $C_{10}H_{18}ClN_3O_2$
- Chemical Structure:



Uses

Semustine is an investigational drug used as an antineoplastic. It is the methyl analog of lomustine, a cytotoxic alkylating agent of the nitrosourea group, and has been used mainly for treatment of brain tumors, gastrointestinal tract adenocarcinomas, primary liver cancer, Hodgkin's disease and non-Hodgkin's lymphoma, melanoma, cervical cancer, head and neck cancer, breast and lung carcinomas. Occasionally, it is used in the treatment of solid tumors such as lung, carcinoma, colorectal, and breast carcinomas in combination with other anticancer agents.

Exposure Routes and Pathways

Cancer patients are exposed during chemotherapy. Semustine is available in 10, 50, and 100 mg capsules. The recommended adult oral dose ranges from 125 to 200 mg m^{-2}, given as a single dose every 6 weeks, as directed by the investigational study. Dosage may vary depending on the type of cancer and body weight and size of the individual. When used simultaneously with other antineoplastic drugs, the dose is usually reduced by 25–50%.

Health-care personnel preparing and administering anticancer therapies may be occupationally exposed to semustine by inhalation, dermal contact, or accidental ingestion.

Toxicokinetics

Semustine is well absorbed from the gastrointestinal tract following oral administration. It is rapidly metabolized and a number of active metabolites have been identified. Semustine as such is not detectable in either plasma or urine. The major route of elimination is urinary excretion. Up to 60% of a dose is eliminated renally within 48 h.

Peak plasma levels are produced within 1–6 h. The metabolites are reported to possess prolonged plasma half-lives, and can rapidly penetrate the blood–brain barrier, producing significant concentrations within 30 min in the cerebral spinal fluid. Small amounts may be excreted in feces and via the lungs as carbon dioxide.

Mechanism of Toxicity

Semustine produces interstrand cross-linking in DNA, generates carbonium ions, and may inhibit several vital enzymatic processes. Alkylation and carbamoylation by semustine metabolites interfere with synthesis and function of DNA, RNA, and proteins.

As all nitrosoureas, this agent rapidly and spontaneously decomposes into two highly reactive intermediates: chloroethyl diazohydroxide and organic isocyanate. This can result in a reaction that inactivates specific DNA repair enzymes. Semustine is cell cycle nonspecific.

Acute and Short-Term Toxicity (or Exposure)

Semustine is toxic by inhalation, if swallowed or absorbed through skin. It is irritating to the eyes, upper respiratory tract, and skin. Target organs are kidneys and bone marrow.

The more common side effects in cancer patients undergoing chemotherapies are decreased white blood cell count with increased risk of infection, decreased platelet count with increased risk of bleeding, nausea, and vomiting. Fetal abnormalities are observed if pregnancy occurs while taking this drug. Tiredness is a less common effect. Rare side effects are sores in mouth or on lips, liver problems, kidney problems, blurred vision or change in vision, scarring of lung tissue.

Animal

Ingestion of semustine affects behavior in mice. The LD_{50} in mice was 49.9 mg kg^{-1}. Prior to death, changes in motor activity were observed.

The lowest published lethal dose after oral or intravenous administration in dogs was 25 or 14 mg kg^{-1}, respectively. Hypermotility, diarrhea, agranulocytosis, and body temperature decrease were shown after intravenous exposure. Similar effects were observed in monkeys.

Human

Delayed myelosuppression, as evident by thrombocytopenia and leucopenia is a dose-limiting factor of semustine therapy. The nadir for thrombocytopenia and leucopenia is ~4–8 and 6 weeks, respectively, following administration. Myelosuppression tends to be cumulative with repeated doses. For this reason, the second or third dose is reduced by 25–50%.

Nausea and vomiting are frequently observed ~4–6 h after ingestion. Delayed nephrotoxicity, including renal failure, is often observed, especially in children. It seems to be total cumulative dose-related. About 25% of adults receiving semustine 1400 mg m^{-2} develop renal abnormalities.

Chronic Toxicity (or Exposure)

Animal

According to an International Agency for Research on Cancer (IARC) report, there is limited evidence of carcinogenicity in experimental animals. Semustine was tested for carcinogenicity by intraperitoneal injection in Sprague–Dawley and Swiss mice, together with a large number of other anticancer agents. The incidence of tumors increased in male rats, whereas the incidence of leukemia and lymphosarcomas in female mice increased only slightly. When administered by intravenous injection, semustine induced lung tumors in rats. It was not teratogenic in mice, although embryo viability was impaired. Semustine given to male mice caused temporary inhibition of spermatogenesis.

Human

Among the nitrosoureas, semustine has proved to be the most nephrotoxic compound. This has been a factor limiting more widespread use. Toxicity appears to be dose-dependent. Evidence of renal damage is often not apparent until 18–24 months following the completion of therapy. When it occurs, renal failure is usually progressive and irreversible. Nephrotoxicity is commonly heralded by increased serum creatinine levels, uremia, and proteinuria.

Semustine is a mutagen and a human carcinogen (group 1 according to IARC), based on sufficient evidence of carcinogenicity in humans. Adjuvant treatment with semustine has been evaluated in patients with gastrointestinal cancer, and a cumulative risk for the onset of acute nonlymphocytic leukemia (ANLL), of 4% at 6 years was observed. This percentage was not affected by concomitant radiotherapy or immunotherapy. A strong dose–response relationship was described, giving a relative risk of almost 40-fold among patients who had received the highest dose.

In Vitro Toxicity Data

Semustine was tested for *in vitro* effects on sister chromatid exchanges (SCE), cellular kinetics, and chromosome aberrations. Increase in SCE values was highly significant for all the concentrations tested (ranging from 1 to 10 mg l^{-1}). It also delayed cell cycle progression. Inhibition of DNA synthesis resulted in increased frequency of chromosomal aberrations. Therapeutic semustine concentrations had genotoxic effects on human peripheral blood lymphocytes and leukocytes studied *in vitro*.

Clinical Management

After inhalation exposure, the victim should be moved to fresh air. If not breathing artificial respiration should be given. If breathing is difficult, oxygen should be given.

After accidental ingestion, mouth should be washed out with water provided the person is conscious. A physician must be consulted.

Other Hazards

Hazardous combustion or decomposition products include carbon monoxide, carbon dioxide, hydrogen chloride gas, and nitrogen oxides.

Exposure Standards and Guidelines

According to the European Unit directives, semustine is classified as T (toxic).

Risk phrases: 45 46 23/24/25 36/37/38 (May cause cancer. May cause heritable genetic damage. Toxic by inhalation, in contact with skin and if swallowed. Irritating to eyes, respiratory system, and skin).

Safety phrases: 53 22 26 36/37/39 45 (Avoid exposure – obtain special instructions before use. Do not breathe dust. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)).

According to the US NTP 10th Report on Carcinogens (2000), semustine is known to be a human carcinogen.

OSHA regulates semustine under the Hazard Communication Standard and as a chemical hazard in laboratories. OSHA publication "Work practice guidelines for personnel dealing with cytotoxic (antineoplastic) drugs" and its revisions are among regulations in connection with occupational exposure to semustine in health care settings.

Miscellaneous

Semustine is a light yellow powder, stable under normal conditions. It should be protected from moisture and is incompatible with strong oxidizing agents and strong bases. It is slightly soluble in water

($<1\,\mathrm{mg\,ml^{-1}}$), and soluble in ethanol, acetone, and DMSO. Semustine is not available commercially. It has been only used in investigational studies, often producing low response rates.

See Also: BCNU (Bischloroethyl Nitrosourea); LD_{50}/LC_{50} (Lethal Dosage 50/Lethal Concentration 50); Methylnitrosourea.

Further Reading

Kintzel PE (2001) Anticancer drug-induced kidney disorders. *Drug Safety* 24: 19–38.

Relevant Website

http://www.cancer.org – American Cancer Society.

Sensitivity Analysis

Virginia Lau

Sensitivity analysis is a method used to evaluate the impact of a single variable or a group of variables on the results from a model calculation. Sensitivity analysis may be used to determine which parameters in a calculation have the greatest influence on the results such that greater emphasis is placed on characterizing these parameters. Moreover, the results from these analyses may be used to identify ways to improve the overall predictive capability of the model by reducing the uncertainty in the parameters that have the greatest influence on the outcome. Sensitivity analysis may be applied to the risk assessment process in order to identify those variables that dominate risk estimates as well as those that are relatively unimportant.

Sensitivity analyses are typically conducted at two levels: local and global. The local analysis generally assesses the effect of small perturbations of a single variable on the calculation. The primary rationale for using this technique is that the anticipated response to these slight changes is assumed to be nonlinear for at least one parameter in the calculation. Thus, increasing a specific parameter value by 10% is not predicted to produce a corresponding 10% increase in the result for all the parameters. The model is considered sensitive to a particular variable when small variations in the value produce large changes to the model predictions. There are several methods for calculating the effect of changes in inputs on model predictions. Perhaps the simplest method is to produce slight changes in a single variable in relative terms based on a fractional change from the base case value using deterministic single-point estimates while keeping the remaining parameters constant. Incrementally changing each parameter value by a set percentage essentially normalizes any differences between parameters that may occur due to deviations in units. A general case is to produce a $\pm 10\%$ change in the single variable to determine the resulting change in the output. Any arbitrary percentage may be used as long as the value is consistently applied to all the variables in the simulation. The results from the local sensitivity analysis may be used to determine places where additional parameters need to be better defined.

In mathematical terms, the local sensitivity analysis is analogous to determining the partial derivative for the calculation with respect to each parameter. Although the local sensitivity analysis normalizes differences between parameters such that the calculation is not sensitive to unit differences, this method does not address the effect on the results of using the full range of possible values for each parameter. This is an important limitation of conducting a local sensitivity analysis since the analysis is likely to produce misleading results for complicated calculations where parameter values have large uncertainties. For instance, a 10% increase above the default adult body weight in a standard risk calculation will produce a corresponding 10% reduction in risk since the individual has more mass available to equally distribute the pollutant concentration. Thus, the conclusion may be drawn that the risk calculation is not extremely sensitive to changes in body weight; however, this is not completely true since body weights are known to vary by as much as 20% from the default value. By accounting for this uncertainty, the actual risk may decrease by as much as 20%, which may be significant in certain cases. To resolve this problem, a global sensitivity analysis may be performed that explicitly evaluates the parameters in calculations where the uncertainty is large. It should be noted that the local sensitivity analysis is fairly accurate in cases in which the uncertainties are expected to be small.

The purpose of a global sensitivity analysis is to assess the effect a single parameter has on the results over the full range of possible values for that parameter. The range of values is often based on the frequency distribution assigned to the variable. Global sensitivity analysis identifies the parameters that contribute the most to the overall variance of the result by evaluating the uncertainty for each parameter individually. This approach generally follows the same Monte Carlo method used in uncertainty analysis to quantify uncertainty associated with all parameters defined in a calculation. The Monte Carlo method involves choosing values from a random selection scheme drawn from probability density functions based on a range of data that characterize the parameter of interest. A simple method of performing a global sensitivity analysis is to run the Monte Carlo simulation by incorporating the uncertainty for a single parameter and keeping all other values constant. However, this usually leads to a labor-intensive task if the calculation is complex and involves numerous parameters. Instead, the simulation may be performed by entering all the uncertainties for each parameter and somehow assigning the variances in the results to key parameters that are anticipated to be the most sensitive. Statistics such as regression analysis, partial correlations, fractional factorials, or partial rank correlations are often used to estimate which parameter is the most sensitive based on the variance of the result. This approach has the advantage that the same Monte Carlo simulations used to estimate uncertainty can be used to estimate the global sensitivity of the model predictions to the inputs and parameters used in the assessment.

See also: Hazard Identification; Risk Assessment, Ecological; Risk Assessment, Human Health; Risk Characterization; Risk Communication; Risk Management; Uncertainty Analysis.

Further Reading

Frey HC and Patil SR (2002) Identification and review of sensitivity analysis methods. *Risk Analysis* 22(3): 553–578.

Sensitization Testing *See* Toxicity Testing, Sensitization.

Sensory Organs

Lewis Nelson

Due to their complexity, the special sense organs are both protected from and susceptible to toxic insult. Most toxic substances are excluded from the inner milieu of the eye and ear by specialized blood vessel barriers, selective membrane channels, or controlled pH gradients. However, some toxins elude these protective mechanisms and consistently produce toxic effects. Although classification is imperfect, differentiation must be made between agents capable of producing acute toxicity (single exposure) from those only toxic after chronic exposure. Although most of these agents are capable of producing multiple toxic effects in humans, the sensory organs' effects are often the most unique, identifiable, or disabling.

The Eye

The eye may be our most highly specialized organ. As an outgrowth of the central nervous system (CNS), it is susceptible to many of the same toxins as is the brain. However, several toxins stand out in their ability to produce nearly isolated ocular toxicity. In order to understand ocular toxicity, certain ophthalmologic principles need to be reviewed.

Vision has been called the 'vital sign' of the eye. Normal visual acuity implies intact light transmission through the optic system (cornea, lens, and vitreous humor) to the retina (the light sensing organ). The retina converts the light into nerve impulses which are sent to the occipital cortex via the optic tracts. Abnormalities in any of these components may diminish visual acuity or produce blindness.

Cornea

The cornea is the clear, external layer of the eye over the visual axis. The most common toxicologic disorder of the cornea results from irritant or caustic injury and results in edema (swelling) or erosion of the surface cells (abrasion). Patients with only mild corneal edema or erosion may experience 'halos' around bright objects and pain. This typically heals rapidly

over a few days with no demonstrable functional defects. With more severe injury, inspection of the external surface of the eye should reveal obvious injury to the cornea and surrounding ocular structures. Healing is imperfect and may result in extensive scarring and visual loss that may be amenable to surgical therapy.

Conjunctiva

The remainder of the exposed surface of the eye is covered by a thin mucosal layer known as conjunctiva. Mild irritation results in dilation of small blood vessels in the conjunctiva ('bloodshot'), pain, and a foreign body sensation. Chemosis, or swelling of the conjunctiva, is the typical response to more severe irritant or caustic exposure. Vision is not generally affected unless ocular perforation occurs or if scarring distorts the shape of the globe.

Pupil

The pupil is the window into the internal eye. The iris, or the colored border of the pupil, opens and closes to vary the amount of light admitted through the pupil into the eye. The pupillary size and reactivity are very useful clinical parameters by which toxic exposures are assessed. Control of pupillary size is complex and is dependent on the interaction of the opposing sympathetic and parasympathetic nervous systems. The pupil enlarges with sympathetic stimulation and constricts with parasympathetic stimulation. Conversely, sympathetic system blockade results in small pupils (miosis), while parasympathetic blockade produces large pupils (mydriasis). Light hitting the retina reduces sympathetic nervous signaling to the pupil and results in pupillary constriction. The opposite occurs in dark situations. Cholinergic agents, such as organophosphorus cholinesterase inhibitors, produce miosis (**Table 1**).

Lens

The lens is deep within the eye and is responsible for focusing an image of an object on the retina. Lens opacification (cataracts) may produce similar visual abnormalities as corneal damage but is often undetectable without careful inspection of the inside of the eye. Amiodarone, a commonly used antiarrhythmic agent, produces deposits or spotting in the cornea.

Table 1 Agents associated with pupillary changes

Miosis
Cholinergics: Organophosphorus pesticides, pilocarpine, nicotine
Opioids
Sedative-hypnotics
Clonidine
Mydriasis
Anticholinergics: atropine, diphenhydramine
Cocaine, amphetamines
Sedative or opioid withdrawal

Vitreous Humor

The majority of the eye is filled with a viscous, clear substance known as vitreous humor. The vitreous humor is rarely directly altered by toxic exposure, but bleeding into the vitreous may be a secondary effect of a systemic toxin such as an anticoagulant.

Retina

The retina is a true neural structure and as such is highly complex and not fully understood. It is responsible for converting light and colors into neural signals. Retinal toxicity results in blurry vision, often described as 'walking in a snowstorm'. Retinal toxicity may be due to a direct effect on the retinal cells or it may occur indirectly through a reduction in blood flow or oxygen delivery to the retina. Although medical or surgical restoration of sight is routinely available for corneal, lenticular, or vitreal damage, therapeutic interventions are much more limited for patients with retinal toxicity.

Brain

In addition to a normal eye, vision requires an intact circuit to and from the brain itself. The optic nerve (cranial nerve II), for example, carries retinal information to the occipital cortex in the posterior aspects of the brain. In addition, information concerning pupil size, direction of gaze, simultaneous movement of the eyes (conjugate gaze), and focus clarity is relayed from the brain back to the eye through multiple nerves. Ethambutol, an antituberculous medication, affects the optic nerves (optic neuropathy) whereas organic mercury compounds have been historically associated with toxic effects on the occipital cortex (cortical blindness).

Specific Ocular Toxins

Many agents are capable of producing visual loss in humans. **Table 2** lists selected agents capable of producing chronic toxicity to various portions of the eye. In the following sections, several common or important toxins are discussed.

Caustics and Irritants Acid and alkali burns to the eye often result in severe corneal injury and require aggressive intervention. It is often difficult to predict at the outset the amount of damage any given

Table 2 Agents associated with chronic visual changes

Corneal
Metals
Amiodarone
Chlorpromazine
Cataracts (Lens)
Dinitrophenol
Corticosteroids
Retinal injury
Carbon disulfide
Quinine
Vincristine
Neurologic (optic nerve, brain)
Ethambutol
Lead
Methylmercury

chemical will inflict on the eye. Generally, agents that have extreme pH, particularly alkali, and those which are solid produce the greatest corneal damage. Irritants, such as hydrocarbons and detergents, tend to be less problematic, producing primarily irritation and corneal erosion, but exceptions abound. The clinical effects of caustics range from corneal and conjunctival irritation to ocular perforation and destruction. Immediate treatment should consist of copious irrigation with water or saline. Measurement of the pH of the ocular surface is useful to guide therapy, but chemical neutralization should never be attempted due to the potential for additional irritant or thermal injury. Even with immaculate initial care, severe caustic injuries may produce corneal scarring and visual loss.

Methanol Of all substances consistently reported to produce direct ocular toxicity in humans, methanol is most frequently responsible. Methanol is locally available as a gasoline additive and as windshield cleaning fluid and is used widely in industry as a solvent. Several well-documented epidemic poisonings have occurred in the recent past resulting from the consumption of methanol in place of ethanol, and isolated cases are common. Ocular symptoms often take several hours to develop and are actually due to the metabolite of methanol, formic acid, formed by alcohol dehydrogenase (ADH). Formic acid also produces profound systemic acidosis which may be fatal. Management consists of inhibiting the metabolism of methanol by providing ADH with ethanol, its preferred substrate. Fomepizole, an ADH inhibitor, is equally as effective and significantly easier to use than ethanol, but it is more costly. Following either antidote, the remaining methanol, which is poorly excreted without metabolism, is generally removed by hemodialysis.

Quinine The antimalarial agent quinine is derived from the bark of the cinchona tree along with several other alkaloids and salicylate (aspirin). Many of these agents produce similar toxic features (cinchonism) in patients with excessive intake, but only quinine produces blindness. Cinchonism consists of abdominal pain and vomiting, ringing in the ears (tinnitus), and confusion. Visual loss after quinine overdose is due to direct retinal toxicity, although until recently it was believed to be due to spasm of the arterial blood supply to the retina. Treatment is difficult, but limited evidence suggests charcoal hemoperfusion may be beneficial (hemoperfusion is similar to hemodialysis, except in place of a semipermeable membrane to filter the toxin from the blood, charcoal is used to bind the toxin).

Agents Capable of Indirect Retinal Toxicity

Indirect retinal toxins produce retinal ischemia or reduced oxygen delivery by the blood. Cocaine, amphetamines, and ergot alkaloids (used in the treatment of migraine headaches) may produce retinal ischemia by reducing the caliber of the retinal arteries and thereby reduce blood flow. Many of these toxins vasoconstrict by stimulating the α-adrenergic receptors on peripheral arteries. Treatment focuses on reducing the vasoconstrictive effect of the primary toxin and may include sedatives (e.g., diazepam), direct acting vasodilators (e.g., nitroglycerine) or α-adrenergic antagonists (e.g., phentolamine). Foreign bodies, such as talc, introduced by use of impure intravenous drug can result in embolization (mechanical blockade) of the retinal arteries with resultant ischemia. Retinal ischemia can also occur in patients with poor retinal blood flow due to hypotension (low systemic blood pressure). Toxic causes of hypotension reported to induce blindness include calcium channel blockers, β-adrenergic blockers, and nitrates. Additionally, by preventing hemoglobin, the main oxygen transport protein in the blood, from binding and delivering oxygen, carbon monoxide can produce retinal ischemia.

Occupational Exposures

Although occupational inhalation of methanol may rarely produce ocular toxicity, the vast majority of occupational eye toxicity results from exposure to irritant chemicals. Highly water-soluble gases, such as ammonia or hydrogen sulfide, produce immediate pain and tearing upon exposure. Gases that are poorly soluble in the water of the eye only produce irritation after prolonged exposure (e.g., phosgene and ethylene oxide).

The Ear

The ear is nearly as complex as the eye and is also an outgrowth of the CNS. The ear converts sound waves into neural impulses which are transmitted to the brain for processing. Unlike the eye, toxins produce adverse effects only at limited sites in the ear. Like the eye, however, free entry of drugs into the inner ear is prevented by a selective filtering mechanism.

External Ear and Ear Canal

While the external ear and ear canal serve as a pathway for the entrance of sound into the internal ear, they are infrequently affected by toxic exposure. Caustics and irritants are the only agents commonly producing toxicity at this level.

Middle Ear

Several tiny bones in the middle ear amplify and convert sound waves from the eardrum into fluid waves in the inner ear. There are no significant toxic exposures affecting the middle ear.

Inner Ear

Two functions are served by the inner ear: hearing (cochlear system) and balance (vestibular system). The cochlea is capable of converting fluid waves into neural impulses. The cochlea is a fluid-filled, snail-shaped organ containing specialized nerve endings known as hair cells. Vibrations transmitted by the middle ear to the cochlear fluid cause movement of the hair cells, triggering signal production which is carried by the auditory nerve (cranial nerve VIII) to the brain. The electrolyte content of the fluid within the inner ear is closely regulated by specialized transport systems. The kidney contains a nearly identical system, which it uses to regulate the electrolyte composition of the blood, explaining why ototoxic agents are frequently also nephrotoxic.

Vestibular System

The vestibular system serves to balance the body. Dysfunction results in ataxia (incoordination), nystagmus (abnormal eye movements), and spatial disorientation. Few toxins produce isolated vestibulotoxicity, and most patients demonstrate hearing abnormalities concurrently.

Specific Otic Toxins

Aminoglycoside Antibiotics The aminoglycoside antibiotics are well known for their toxic side effects, renal and inner ear toxicity. Destruction of the hair cells of the cochlea produces hearing loss, beginning with high frequencies and progressing toward the lower frequencies. All of the aminoglycosides have the potential for such toxicity, but the relative toxicities differ. Newer techniques of administration such as bolus dosing may prove to reduce the frequency of ototoxicity. Aminoglycosides are also vestibular toxins, and such toxicity often precedes hearing loss.

Salicylates Aspirin and other salicylic acid derivatives are used as analgesic and antiinflammatory agents. Tinnitus, or high-frequency ringing in the ears, is the most common sign of toxicity and is variably accompanied by hearing loss. The ability of aspirin to cause ototoxicity was so widely known that in the early part of this century, tinnitus was used as a clinical marker for therapeutic dosing. Aspirin-induced tinnitus, which is almost always reversible, is probably due to interruption of the normal metabolic processes of the sensory hair cells. Several structurally unrelated nonsteroidal antiinflammatory agents are capable of producing toxic symptoms similar to aspirin, suggesting involvement of the prostaglandin system.

Diuretics The toxic effect of diuretics on the inner ear is related to the alteration of the fluid contained within. Changes in the electrolyte composition of the inner ear fluid causes swelling of the structures of the inner ear and tinnitus. However, this cannot be the only toxic mechanism since symptoms may occur immediately upon large exposure, at which time the fluid composition has not yet been altered.

Occupational Exposures

Surprisingly little research has been performed on the otic effects of chemicals on workers. However, several widely used chemicals are known to be ototoxic. However, the combination of toxin exposure and noise may be additive or synergistic in the production of hearing loss. This has made investigation of the isolated toxic effects on exposed workers difficult (**Table 3**).

Olfaction

The sense of smell is our most sensitive special sense. We are able to detect the odor of certain chemicals in the parts per million range. However, some chemicals are undetectable altogether, and others cause olfactory fatigue, in which exposure to a substance reduces our ability to detect its odor (e.g., hydrogen sulfide). Even more interesting, certain people are unable to detect certain odors, while others can

Table 3 Agents associated with hearing loss

Occupational
Bromates
Carbon disulfide
Carbon monoxide
Lead
Mercury
Styrene
Toluene
Trichloroethylene
Xylene
Drugs
Aminoglycosides
Diuretics
Chemotherapeutic agents
Salicylates

Table 4 Agents associated with smell disorders

Occupational exposures
Acrylate and derivatives
Cadmium dust
Carbon disulfide
Formaldehyde
Hydrogen sulfide
Solvents (volatile hydrocarbons)
Drugs
Antithyroid medications: methimazole, methylthiouracil
Antihypertensives: beta blockers, captopril, enalapril
Levo-dopa
Opioids: morphine, codeine
Cigarette smoking
Cocaine insufflation

Table 5 Agents associated with taste disorders

ACE inhibitors: captopril, enalapril
Carbamazepine
Chemotherapeutic agents
Cigarette smoking
Diuretics
Levo-dopa
Phenylbutazone
Metallic taste
Allopurinol
Ciguatoxin
Coprinus mushrooms
Disulfiram
Ethambutol
Heavy metals
Lithium
Metronidazole
Methotrexate
Penicillamine
Penicillin
Tetracycline

detect the same odor at tiny concentrations (e.g., detecting cyanide's almond odor seems to be genetically determined). The most common toxic olfactory insult is cigarette smoking, and this must be considered in all patients with olfactory or taste dysfunction.

Olfactory receptors, numbering about 20 million per nares, are receptor ends of neurons that form the olfactory nerve (cranial nerve I). This nerve sends projections to many parts of the brain, possibly explaining why smells often elicit profound emotional responses or memories. In addition, irritant receptors exist within the nasal cavity, which are unrelated to smell. This is sometimes called the 'common chemical sense' and connects to the brain via the trigeminal nerve (cranial nerve V). This differential recognition of irritants from odors is clinically useful. Patients complaining of dysfunctional odor recognition should have normal recognition of ammonia and other pungents. Failure to recognize irritant effects raises the possibility of malingering.

There are several syndromes of altered sense of smell (dysosmia). Hyposmia or anosmia is the reduction in intensity or complete lack of the ability to smell, respectively. Troposmia is the distortion of an odor compared to a previous exposure. Phantosmia is the perception of an odor when there is none present.

Few agents are acutely toxic to the olfactory receptors. Hydrogen sulfide, as mentioned earlier, causes rapid olfactory fatigue, and the normal 'rotten egg' odor quickly vanishes allowing prolonged exposure to this potentially fatal mitochondrial toxin. Occupational exposure to several solvents and metals has been associated with olfactory dysfunction (**Table 4**).

Gustation

Disorders of taste, like that of smell, are generally of limited toxicologic interest. The sensation of taste, like smell, is receptor mediated. Most abnormalities of gustation involve detection of abnormal tastes, not reduction in overall function of the sense.

Taste receptors reside within taste buds on the tongue, the larynx, and the palate. There are four primary taste sensations: sour, sweet, bitter, and salty. By mixing these primary taste sensations, the brain can identify many specific tastes (analogous to primary color mixing). Impulses from the taste buds are carried through the facial, glossopharyngeal, and vagus nerves (cranial nerves VII, IX, and X, respectively) to the brain. Taste is modified by the presence of odor, and in the absence of olfactory ability, taste is virtually eliminated.

A metallic taste is often noted with exposure to metals or metal-containing compounds, tetracycline, mushrooms (e.g., Coprinus sp.), snake venom, and others. Metal fume fever, a febrile immunologically mediated reaction to metal oxides volatilized during welding, also produces a metallic taste. An abnormal

garlic sensation is experienced after exposure to dimethylsulfoxide, organophosphate insecticides, and arsenic. Such an abnormal taste sensation is likely due to cross-recognition of certain chemical agents by specific taste receptors (**Table 5**).

See also: Acids; Aminoglycosides; Behavioral Toxicology; Corrosives; Eye Irritancy Testing; Metals; Methanol; Occupational Toxicology; Organophosphates; Physical Hazards; Quinine; Salicylates.

Further Reading

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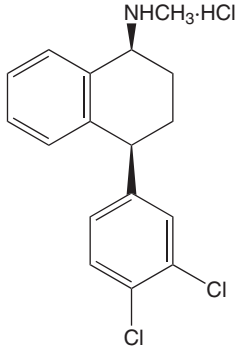
Mott AE and Leopold DA (1991) Disorders of taste and smell. *Medical Clinics of North America* 75: 1321–1353.

Schusterman DJ and Sheedy JE (1992) Occupational and environmental disorders of the special senses. *Occupational Medicine* 7: 515–542.

Sertraline Hydrochloride

Bruce Ruck

- Chemical Name: (1*S*-*cis*)-4-(3,4-Dichlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-1-naphthalenamine hydrochloride
- Chemical Abstracts Service Registry Number: CAS 79559-97-0
- Synonym: Zoloft
- Chemical/Pharmaceutical/Other Class: Antidepressant agent; Selective serotonin reuptake inhibitor
- Chemical Formula: $C_{17}H_{17}NCl_2 \cdot HCl$
- Chemical Structure:



Uses

Sertraline hydrochloride is used in the management of depression, obsessive–compulsive disorder (OCD), panic disorder, posttraumatic stress disorder (PTSD), premenstrual dysphoric disorder (PMDD), and social anxiety disorder.

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposure to sertraline hydrochloride. Sertraline is available as an oral tablet and as an oral concentrate (liquid).

Toxicokinetics

Pharmacokinetic data is derived from ingestion of doses considered to be in the therapeutic range; hence, no data are included on the pharmacokinetics of sertraline after overdose.

After oral doses of 50–200 mg, sertraline reaches a maximum plasma concentration in ~4.5–8.4 h. Administration of the oral concentrate results in a higher area under the curve (AUC) and C_{max} compared to the oral tablet. Peak absorption may be delayed with large ingestions. When food was administered with therapeutic doses of tablet formulation, both the peak and total plasma levels (AUC) increased; however, the time to reach peak plasma concentration decreased. With repeated dosing, a steady-state plasma level should be achieved within 7 days.

Sertraline's bioavailability is reduced by extensive first-pass metabolism. The principal metabolite of this first-pass metabolism is *N*-desmethylsertraline. *N*-Desmethylsertraline has a half-life of 62–104 h and is substantially less active than the parent compound. Sertraline and its metabolite *N*-desmethylsertraline both undergo further biotransformation to nonactive compounds. Clearance of sertraline may be faster in children than in young adults and slowest in the elderly.

Sertraline is highly protein bound (~98%). Its volume of distribution is estimated at $20\,l\,kg^{-1}$.

Mechanism of Toxicity

Sertraline is a potent and highly selective serotonin reuptake inhibitor (SSRI) that increases the availability of this neurotransmitter in the synaptic cleft. Sertraline has minimal effects on norepinephrine and dopamine reuptake. It shows no significant affinity for adrenergic, cholinergic, γ-aminobutyric acid, dopaminergic, histaminergic, serotonergic, or benzodiazepine receptors. Sertraline has no effects on monoamine oxidase.

Acute and Short-Term Toxicity (or Exposure)

Human

Sertraline has a relatively low risk of toxicity. It is less sedating and has fewer cardiovascular effects than the tricyclic antidepressants. It has a high therapeutic index, which is consistent with other serotonin uptake inhibitors.

In general, ingestions of up to 4500 mg have been tolerated without significant toxicity. However, one patient ingesting 2500 mg had a fatal outcome. Patients may develop symptoms including nausea, vomiting, drowsiness, tachycardia, dilated pupils, slurred speech, and ataxia. Therapeutic and toxic plasma concentrations have not been well defined.

Sertraline has the potential to cause 'serotonin syndrome'. Most commonly, this syndrome occurs when two or more drugs capable of enhancing serotonin activity are used concomitantly. This syndrome can occur in the overdose situation.

Manifestations of serotonin syndrome include: altered mental status, restlessness, myoclonus, hyperreflexia, diaphoresis, shivering, tremor, incoordination, and/or fever.

Chronic Toxicity (or Exposure)

Animal

Mice receiving 10–40 $\mathrm{mg\,kg^{-1}\,day^{-1}}$ developed increased rates of hepatic adenomas compared to unexposed controls.

Human

Therapeutic chronic use of sertraline has reportedly caused visual defects, cardiac toxicity, gastrointestinal irritation, renal pathology, and loss of appetite. However, causality of these effects still requires confirmation.

Like other SSRIs, sertraline should not be used within 2 weeks of discontinuing monoamine oxidase inhibitors (MAOIs) and MAOIs should not be started for at least 2 weeks after stopping sertraline.

In Vitro Toxicity Data

Studies using RBL-2H3 cells demonstrated an increase in mRNA and protein levels of tryptophan hydroxylase due to sertraline.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Gastric decontamination with activated charcoal should be considered for substantial recent ingestions. Treatment recommended after decontamination is symptomatic and supportive. There is no antidotal therapy. Because patients taking an overdose of sertraline may also have access to other medications/chemicals, the patient should be evaluated and treated as appropriate for other substances that may have been ingested.

It should be noted that the abrupt discontinuation of an SSRI can cause a 'withdrawal' syndrome. This syndrome is often referred to as 'discontinuation syndrome'. SSRI discontinuation syndrome is often manifested by symptoms of fatigue, gastrointestinal complaints (nausea, vomiting, diarrhea, cramping), shortness of breath, memory impairment, dizziness, insomnia, chills, headache, eye discomfort, tinnitus, ataxia, and abnormal sensations (e.g., 'electric shocks', skin tingling sensations, and involuntary movements).

See also: SSRIs (Selective Serotonin Reuptake Inhibitors); Tricyclic Antidepressants.

Further Reading

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Klein-Schwartz W and Anderson B (1996) Analysis of sertraline-only overdoses. *The American Journal of Emergency Medicine* 14: 456–458.