Seveso Disaster, and the Seveso and Seveso II Directives

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The Seveso Disaster

The Seveso disaster began on July 10, 1976 at the Industrie Chimiche Meda Società Azionaria (ICMESA) chemical plant in Meda, Italy. This event became internationally known as the Seveso disaster, after the name of the most severely affected community. An increase in pressure due to an exothermic reaction in a 2,4,5-trichlorophenol-production reactor caused the rupture disk of the safety valve to burst. About 3000 kg of chemicals were released into the air. The release included 2,4,5-trichlorophenol, used in the manufacture of herbicides, and possibly up to 30 kg of the dioxin TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin). Dioxin first came to widespread public notice during the Vietnam War, when it was identified as a component of the defoliant Agent Orange. Dioxin has also been considered to be the most toxic human-made substance.

The chemicals released into the air from the chemical plant near Milan in northern Italy were carried southeast by the wind towards Lake Como. The dioxin cloud contaminated a densely populated area about 6 km long and 1 km wide downwind from the site. The four most impacted municipalities included Seveso (a 1976 population of 17 000), Meda (19 000), Desio (33 000), and Cesano Maderno (34 000). Two other municipalities, Barlassina (6000) and Bovisio Masciago (11 000), were subject to postaccident restrictions. Health monitoring was extended to a further five municipalities. The entire affected area is part of the Brianza, one of the wealthiest and most industrialized regions of Italy. The economy of this area at the time of the disaster depended on small workshops and industries, mainly engaged in manufacturing furniture.

The Seveso disaster had a traumatic effect on the minds of the local populations because its seriousness was recognized only gradually. People elsewhere in the world also experienced heightened concern about chemical exposures and risks and the need for tighter regulation of hazardous chemical manufacturing. The accident was not immediately noticed since no one was at the plant when it happened. One day later, ICMESA managers informed local authorities of the escape of a "cloud of herbicide that causes harm to agriculture," stating that "in all likelihood the aerosol mixture which escaped consists of sodium trichlorphenate, caustic soda, and solvent, but possibly other toxic substances as well." They requested the authorities to warn the population, and samples were sent by courier for examination to a company, Givaudan SA, in Switzerland. Givaudan SA, once one of ICMESA's main customers, had taken over ICMESA as a subsidiary in 1969.

Two days after the disaster, nearby residents were warned not to eat any vegetables from their gardens. Four days after the disaster, the Technical Director of Givaudan in Geneva informed the Technical Director of ICMESA that the samples contained traces of TCDD. Authorities were told much later about the TCDD. The Seveso disaster resulted in the highest known TCDD exposure to residential populations, and has possibly been the most systematically studied dioxin contamination incident in history.

The first sign of human health problems was burn-like skin lesions, appearing on children after the accident. Beginning in September of 1976, chloracne, a severe skin disorder usually associated with dioxin, broke out on some people who were most exposed. Authorities began an investigation five days after the accident, when large numbers of local animals such as rabbits began to die. After a linkage to dioxin was made, over 700 hundred people living closest to the plant were evacuated within 3 weeks after the accident. In all, up to 2000 people were treated for dioxin poisoning. Approximately 4% of the local farm animals died, and $\sim$80 000 additional animals were killed to prevent contamination from filtering up the food chain.

The Seveso disaster areas were divided and subdivided based on soil contamination levels. Zone A, the most contaminated area with more than 50 μg of TCDD per square meter and covering 110 ha, was completely evacuated and fenced-off with entry prohibited. Zone A was later turned into a park, the Seveso Oak Forest. In the next-most contaminated areas, zone B (between 5 and 50 $\mu g\,m^{-2}$) and zone R (below 5 $\mu g\,m^{-2}$), farming as well as consumption of local agricultural goods and meats were strictly prohibited.

Professor Paolo Mocarelli of the University of Milano-Bicocca's Hospital of Desio was put in charge of a laboratory setup two weeks after the accident to test people for health problems. Dr. Mocarelli's laboratory has conducted neurological, obstetric, and other tests that have surpassed 1 million in number, and Dr. Mocarelli decided to save one sample of blood from each person just in case it would be possible to measure very low levels of TCDD in small blood samples someday. This became possible in 1987, and Dr. Mocarelli has worked with the US

Centers for Disease Control and Prevention to analyze the thousands of samples and to try to associate the levels with health effects. Reconstruction of the disaster using the samples taken over time has helped clarify how long dioxin stays in the human body, and the different effects it has on children and adults.

One toxic effect of the Seveso dioxin exposure was on reproduction. In the first 7 years after the accident, a very high proportion of females (46 females compared to only 28 males) were born to parents who were exposed to the chemical cloud. This was the first time a chemical had been observed to change the sex ratio, implicating dioxin as a hormone disrupter. TCDD is associated with increased fetal loss and reduced birth weight in animal studies.

The Seveso Women's Health Study (SWHS) is a retrospective cohort study of people who resided in the most contaminated areas, zones A and B. Serum samples collected near the time of the explosion were analyzed for TCDD. Also analyzed were pooled serum samples collected in 1976 from females who resided in the "unexposed" zone to assess concurrent background exposures to other dioxins, furans, and polychlorinated biphenyls (PCBs). The youngest children had the highest TCDD levels, which decreased with age at explosion until $\sim$13 years of age and were essentially constant thereafter. The zone of residence and age were the strongest predictors of TCDD level. Chloracne, nearby animal mortality, location (outdoors versus indoors) at the time of explosion, and consumption of homegrown food were also related to serum TCDD levels. The serum pools from the 'unexposed' zone residents had TCDD concentrations and average total toxic equivalent (TEQ) concentrations that suggested that the background exposure to dioxins, furans, and PCBs unrelated to the explosion may have been substantial. Therefore, the early SWHS studies that considered only TCDD exposure may have underestimated health effects due to total TEQ concentrations.

The early part of the SWHS looked at the relation of pregnancy outcome to maternal TCDD levels measured in serum collected shortly after the explosion. Ninety-seven pregnancies (10.9%) ended as spontaneous abortions. TCDD was associated with a nonsignificant adjusted decrease in gestational age and a 20–50% nonsignificant increase in the odds of preterm delivery. The exposed population also reported symptoms of immune system and neurological disorders; however, studies found no link to dioxin. Increases in some forms of cancer found in the exposed population have suggested a link between dioxin and some cancers.

Further research on the children of victims of the disaster is being conducted, as is research focusing on dioxin's long-term carcinogenic properties. For example, 25 years after the Seveso disaster, human milk from mothers in Seveso was found to have TCDD concentrations more than twice as high as those in central Milan and elsewhere in the areas near Seveso. This suggests that breastfed infants in Seveso might have appreciable amounts of TCDD in their body fat; however, the health consequences remain to be determined.

In addition to monitoring victims and offspring of the accident, another type of monitoring that continues concerns the Seveso Oak Forest's two large concrete tanks lying beneath the surface. These tanks are the resting place of the top 40 cm of soil removed after the disaster, and also are the final resting place of the contaminated animals that were slaughtered, and the factory (disassembled brick by brick by workers in protective suits) and other buildings exposed to the cloud. The area around the tanks is monitored for leaks, and the soil is said to now have lower dioxin levels than in average areas. The area is now a place where families can gather and animals have returned to the park and adjacent nature reserve.

After the Seveso disaster, investigation of the potential emission sources in the area and studies of people not exposed to the cloud indicated that combustion of wood residues from furniture factories might be an additional and perhaps substantial local source of dioxins, furans, and PCBs.

The Seveso Directive

In 1982, the European Union's Council Directive 82/501/EEC on the major-accident hazards of certain industrial activities, also known as the Seveso Directive, was adopted. The Directive was mostly designed to promote information flow and created the requirement that each Member State (i.e., each country belonging to the European Union) appoint a Competent Authority to oversee safety issues. The Seveso Directive was amended twice, following major accidents at the Union Carbide chemical factory in Bhopal, India in 1984 (a leak of methyl isocyanate caused thousands of deaths), and at the Sandoz chemical warehouse in Basel, Switzerland in 1986 (fire-fighting water contaminated with mercury, organophosphate pesticides and other chemicals caused massive pollution of the Rhine River and the death of hundreds of thousands of fish). Both amendments, broadened the scope of the Directive, in particular to include the storage of dangerous substances.

The Seveso Directive covered all European Union Member States, and held them responsible for ensuring that the relevant national institutions do what is required for adequate risk management. The entire Directive was also driven by a concern for prevention, including those parts that relate to post-accident activities. For example, terms such as 'industrial activity, manufacturer, major accident, and dangerous substances' were defined, the types of production, operations, and storage activities that are subject to regulation were described, and the dangers that are anticipated were noted.

Member States were required to ensure that manufacturers identify existing major accident hazards, and that they adopted all appropriate safety measures, including information, training, and equipment for workers. Further, Member States must set up Competent Authorities that will take responsibility for receiving such a notification, examining the information provided, organizing inspections or other measures of control, and ensuring that off-site emergency plans are prepared. The manufacturers were also required to provide the Competent Authorities with a notification containing detailed and updated information on safety precautions and other matters. In addition, Member States were held responsible for assuring that "persons liable to be affected by a major accident were informed in an appropriate manner of the safety measures and of the correct behaviour to adopt in the event of an accident."

Article 8 of the Seveso Directive was noteworthy in its content because the safety of people outside hazardous installations was taken into account for the first time in Europe (before this, only workers might have had the right to be informed). Information that had previously been 'for experts alone' was opened-up to inspection by, and input from, the public. Another article of the Directive required Member States to take the necessary measures to ensure that the manufacturer immediately provided full and detailed information about an accident to the competent authorities; they must in turn were to ensure that all necessary measures were taken and that full analysis of the accident was accomplished whenever possible. The European Commission was put in charge of setting up a register containing a summary of major accidents that occur within the European Union, including an analysis of causes, experience gained, and measures taken to enable Member States to use this information for prevention purposes.

The Seveso Directive also led the way to similar initiatives on other issues, for example, in environmental management and public health. This included the mandating of measures to encourage improvements in occupational safety and health; minimum safety and health requirements for the workplace, measures related to biotechnology; the freedom of access to environmental information; and public information about radioactive emergencies. Beyond Europe, the Seveso Directive was important for many international organizations, for example, the World Bank, the United Nations Environment Programme, the Council of Europe, the International Atomic Energy Agency, the Office of the United Nations Disaster Relief Coordinator (UNDRO), the World Health Organization, and the International Labour Organization (ILO). Further, the Organization for Economic Cooperation and Development (OECD) has focused much attention to accident prevention and response and has published a number of recommendations, some of which are specifically addressed to public information and public participation in decision-making.

The Seveso II Directive

In 1996, Council Directive 96/82/EC on the control of major-accident hazards, also known as the Seveso II Directive, was adopted. The Seveso II Directive replaced the original Seveso Directive, and Member States had up to 2 years to bring into force the national laws, regulations and administrative provisions to comply. Important changes and new concepts included a revision and extension of the scope of the Seveso Directive, the introduction of new requirements relating to safety management systems, emergency planning and land-use planning, and a reinforcement of the provisions on inspections to be carried out by Member States. Further, Member States can maintain or adopt stricter measures than those contained in the Seveso II Directive.

The Seveso II Directive covers industrial 'activities' and the storage of dangerous chemicals, with larger quantities of a chemcial leading to more control measures. A company holding a quantity of dangerous chemical less than Seveso II's lower threshold levels is not covered by this legislation, but will be proportionately controlled by general provisions on health, safety and the environment provided by other legislation not specific to major-accident hazards. Important areas excluded from the scope of the Seveso II Directive include nuclear safety, the transport of dangerous substances and intermediate temporary storage outside establishments and the transport of dangerous substances by pipelines.

All operators of establishments coming under the scope of the Seveso II Directive need to send a notification to the competent authority, and need to

establish a Major-Accident Prevention Policy. In addition, operators of 'upper tier establishments' (i.e., holders of high levels of a dangerous chemical) need to establish a Safety Report, a Safety Management System, and an Emergency Plan.

Member States have the obligation to report major accidents to the Commission. In order to fulfill its information obligations toward the Member States, the European Commission has created the Major Accident Reporting System (MARS) database to store and retrieve accident information reported by the Member States, and a Community Documentation Centre on Industrial Risks (CDCIR) was established to collect, classify, and review materials relevant to industrial risks and safety.

In order to assist Member States with the interpretation of certain provisions of the Seveso II Directive, the European Commission in co-operation with the Member States developed documents on the preparation of a safety report, guidelines on a major accident prevention policy and safety management system, explanations and guidelines on harmonized criteria for dispensations, guidance on land-use planning, guidance for the content of information to the public, and guidance on inspections. In addition, a series of answers to frequently asked questions has been published and regularly updated. These guidance documents and the answers to frequently asked questions have no legal status, but do provide valuable guidance to industrial operators as well as enforcement authorities within the European Union.

See also: Bhopal; Dioxins; European Union and Its European Commission; International Labour Organization (ILO); Organisation for Economic Cooperation and Development.

Further Reading

Bertazzi PA, Bernucci I, Brambilla G, Consonni D, and Pesatori AC (1998) The seveso studies on early and long-term effects of dioxin exposure: A review. *Environmental Health Perspectives* 106(Suppl. 2): 625–633.

Eskenazi B, Mocarelli P, Warner M, *et al.* (2003) Maternal serum dioxin levels and birth outcomes in women of Seveso, Italy. *Environmental Health Perspectives* 111: 947–953.

Eskenazi B, Mocarelli P, Warner M, *et al.* (2004) Relationship of serum TCDD concentrations and age at exposure of female residents of Seveso, Italy. *Environmental Health Perspectives* 112: 22–27.

Fattore E, Di Guardo A, Mariani G, *et al.* (2003) Polychlorinated dibenzo-*p*-dioxins and dibenzofurans in the air of Seveso, Italy, 26 years after the explosion. *Environmental Science and Technology* 37: 1503–1508.

Landi MT, Bertazzi PA, Baccarelli A, *et al.* (2003) TCDD-mediated alterations in the AhR-dependent pathway in Seveso, Italy, 26 years after the accident. *Carcinogenesis* 24: 673–680.

Pesatori AC, Consonni D, Bachetti S, *et al.* (2003) Short- and long-term morbidity and mortality in the population exposed to dioxin after the "Seveso accident.". *Industrial Health* 41: 127–138.

Weiss J, Päpke O, Bignert A, *et al.* (2003) Concentrations of dioxins and other organochlorines (PCBs, DDTs, HCHs) in human milk from Seveso, Milan and a Lombardian rural area in Italy: A study performed 25 years after the heavy dioxin exposure in Seveso. *Acta Paediatrica* 92: 467–472.

Relevant Website

http://www.europa.eu.int – European Commission, Chemical Accident Prevention, Preparedness and Response.

Shampoo

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- Trade Names: Neutrogena; Head and Shoulders; Prell; Pert; Johnson's; Pantene
- Chemical/Pharmaceutical/Other Class: Combination of nonionic, amphoteric, and anionic surfactants

Uses

Shampoos are rinse-off products used to cleanse the hair and scalp; they are available in noncoloring and coloring formulations. Lindane shampoos are available for the treatment of lice; antidandruff formulations are also available to control the symptoms of dandruff and seborrheic dermatitis.

Exposure Routes and Pathways

Ingestion is a common route of exposure. Ocular and dermal exposures occur as well.

Toxicokinetics

There is minimal absorption of anionic, nonionic, and amphoteric surfactants. Antidandruff shampoos may contain zinc pyridinethione and selenium

sulfide. The pharmacokinetics of zinc pyridinethione have been evaluated via multiple routes (percutaneous, oral, intravenous, intraperitoneal) and in several species (rats, rabbits, monkeys, dogs).. Selenium sulfide is poorly absorbed. Peak serum levels of lindane (an ingredient in shampoos used to treat lice infestation) occur ~6 h after a single dermal application. Lindane is highly lipid soluble and is stored in adipose tissue. Lindane is metabolized in the liver to chlorophenols.

Amphoteric, anionic, and nonionic surfactants are eliminated in the urine and feces. Selenium salts are excreted in the urine. Lindane has a half-life of ~18–21 h following dermal application.

Mechanism of Toxicity

The surfactants and other adjuvants in shampoo are primarily irritants, and most dermal, ocular, or gastrointestinal toxicity is a consequence of the irritant properties.

Acute and Short-Term Toxicity (or Exposure)

Animal

In general, nonlindane shampoos do not produce toxicity. Transient irritant effects are expected, especially in the event of ocular exposure. Exposure to lindane shampoos can produce vomiting, tremors, increased salivation, and seizures. Treatment is aimed at appropriate gastrointestinal decontamination and control of seizures.

Human

Nonionic and anionic surfactants and selenium and zinc pyrithione shampoos are irritants by nature. Nausea and vomiting can occur following ingestion in large volumes. Spontaneous emesis is common. Persistent vomiting has the potential to cause fluid and electrolyte imbalance. In general, gastrointestinal irritation is self-limiting.

Acute ingestion of lindane shampoo does have the potential to cause central nervous system excitation. Toxicity can occur when children ingest one teaspoon or more of 1% lindane shampoo. Ingestion of one tablespoon or more of lindane shampoo may result in significant toxicity. Symptoms of lindane toxicity include agitation, tremors, seizures, and respiratory depression.

Chronic Toxicity (or Exposure)

Human

Chronic dermal application of 1% lindane shampoo does have the potential to cause lindane toxicity, so it is not uncommon for products to contain precautionary labeling to avoid the reapplication of lindane products within at least a few months after use.

Clinical Management

Dilution is generally all that is required in exposures to nonlindane-containing shampoos. If spontaneous emesis does not occur, then it is unlikely that a large ingestion occurred, and mild to moderate, transient gastrointestinal distress is likely to be the only sequelae. If persistent vomiting occurs, then fluid and electrolytes should be monitored.

In toxic exposures to lindane shampoos, basic and advanced life-support measures should be utilized as needed. Emesis is not recommended in oral exposures to lindane. Gastric lavage utilizing saline cathartics is recommended; milk and fatty foods should not be administered in oral lindane exposures since this may enhance absorption.

See also: Lindane; Surfactants, Anionic and Nonionic.

Relevant Websites

http://www.ctfa.org – US Cosmetic, Toiletry, and Fragrance Association (CTFA).

http://hpd.nlm.nih.gov – US National Library of Medicine, 'Household Product Database' and 'ToxTown'. The Household Products Database links several thousand US consumer brands to health effects from Material Safety Data Sheets (MSDSs) provided by the manufacturers, and allows scientists and consumers to research products based on chemical ingredients.

Shellfish Poisoning, Paralytic

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Background Information

Paralytic shellfish poisoning is a constellation of clinical effects caused by ingestion of contaminated shellfish found on the East and West coasts of the United States and Canada, the coasts around Japan, and coastal areas from southern Norway to Spain.

Implicated sources (shellfish) are mussels, clams, scallops, univalve mollusks, starfish, xanthid crabs, sand crabs, and turban shells.

Exposure Routes and Pathways

Ingestion of toxin-infected bivalve shellfish is the route of exposure. There is no reliable taste, smell, or color to detect contaminated shellfish. The toxin is not destroyed or inactivated by heating or cooking.

Toxicokinetics

The toxin is water soluble and absorbed through the oral mucosa and small intestine.

Mechanism of Toxicity

Neosaxitoxin, saxitoxin, and gongantoxin I–IV block transmission of impulses between nerve and muscle. They also block sodium channels in nerve and skeletal muscle, inhibiting the nerve and muscle action potential, thereby blocking nerve conduction and muscle contraction.

Acute and Short-Term Toxicity (or Exposure)

Animal

Shags, terns, and cormorants may develop inflammation of the gastrointestinal tract, hemorrhages in the base of the brain, and other hemorrhages.

Human

Common initial effects include numbness in the lips, tongue, and fingertips within a few minutes of ingestion. This numbness may spread to the extremities and then to the remainder of the body causing weakness and even muscle paralysis. Gastrointestinal symptoms are less common and consist of nausea and vomiting. Other symptoms include nystagmus, temporary blindness, irregular heartbeats, drops in blood pressure, headache, dizziness, difficulty in swallowing, and loss of gag reflex. Symptoms may persist for weeks.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Treatment is entirely symptomatic and supportive. Gastrointestinal decontamination with activated charcoal may be used depending upon the patient's clinical status, the history of the ingestion, and the time since the ingestion. Mechanical ventilation may be required for patients with decreased respiratory function.

See also: Red Tide; Saxitoxin.

Further Reading

Gessner BD and Middaugh JP (1995) Paralytic shellfish poisoning in Alaska: A 20-year retrospective analysis. *American Journal of Epidemiology* 141: 766–770.

Morbidity and Mortality Weekly Report (1991) Paralytic shellfish poisoning – Massachusetts and Alaska, 1990. MMWR 40: 157–161.

Shigella

Melanie J Karst

Name of the Organism

Shigella spp. (*Shigella sonnei*, *S. boydii*, *S. flexneri*, and *S. dysenteriae*).

Description

Shigella are Gram-negative, nonmotile, nonsporeforming, facultatively anaerobic, rod-shaped bacteria. *Shigella* are differentiated from the closely related *Escherichia coli* on the basis of pathogenicity, physiology (failure to ferment lactose or decarboxylate lysine), and serology. The genus is divided into four serogroups with multiple serotypes: A (*S. dysenteriae*, 12 serotypes); B (*S. flexneri*, 6 serotypes); C (*S. boydii*, 18 serotypes); and D (*S. sonnei*, 1 serotype).

Sources of Exposure/Transmission

Shigellosis, also known as bacillary dysentery, is caused by several bacteria of the genus *Shigella*. Contamination of foods is through the fecal-oral route. Fecally contaminated water and unsanitary handling of food are the most common causes of contamination. Foods that are frequently associated with shigellosis include salads (potato, tuna, shrimp, macaroni, and chicken), raw vegetables, milk and dairy products, and poultry. Any foods that require considerable handling during preparation are often involved in shigellosis.

Epidemiology

Shigellosis accounts for less than 10% of foodborne illness in the United States. Bacillary dysentery constitutes a significant proportion of acute intestinal disease in the children of developing countries. Shigellosis is endemic in developing countries where sanitation is poor. Typically 10–20% of enteric disease, and 50% of the bloody diarrhea or dysentery of young children, can be characterized as shigellosis. In developed countries, single-source, food, or waterborne outbreaks occur sporadically, while cases of endemic shigellosis can be found in some areas with substandard sanitary facilities.

Dose

An infective dose may be as few as 10 cells depending on the age and condition of the host. The time of onset of symptoms is somewhat influenced by the size of the challenge.

Mechanism of Toxicity

The disease is caused when virulent *Shigella* organisms attach to, and penetrate, epithelial cells in the intestinal mucosa. The cells then multiply intracellularly and spread to neighboring epithelial cells resulting in tissue destruction. Some strains produce enterotoxin and Shiga toxin. The hallmarks of shigellosis are bacterial invasion of the colonic epithelium and inflammatory colitis. These conditions are interdependent processes amplified by local release of cytokines and by the infiltration of inflammatory elements. Virulence involves both chromosomal- and plasmid-coded genes: including (1) siderophores that are iron-chelating compounds; (2) cytotoxins that cause cell necrosis; (3) Shiga toxins, a family of potent cytotoxins that inhibit protein synthesis and may play a role in progression of mucosal lesions; and (4) chromosomal genes that control lipopolysaccharide antigens in cell walls which may enhance cytotoxicity of Shiga toxins on endothelial cells.

Host Defenses

Inflammation, copious mucus secretion, and regeneration of the damaged colonic epithelium limit the spread of colitis and promote spontaneous recovery. Serotype-specific immunity is induced by a primary infection, suggesting a protective role as antibodies recognize the lipopolysaccharide somatic antigen. Other *Shigella* antigens include enterotoxins, cytotoxin, and plasmid-encoded proteins that induce bacterial invasion of the epithelium. The protective role of immune responses against these antigens is unclear.

Diagnosis of Human Infection/Illness

Colitis in the rectosigmoid mucosa, with concomitant malabsorption, results in the characteristic sign of bacillary dysentery: diarrhea tinged with blood and mucus. Shigellosis can be correctly diagnosed in most patients on the basis of fresh blood in the stool; however, watery, mucoid diarrhea may be the only symptom of many *Shigella* infections. This disease differs from profuse watery diarrhea, as is commonly seen in choleraic diarrhea or in enterotoxigenic

E. coli diarrhea, in that the dysenteric stool is scant and contains blood, mucus, and inflammatory cells. Any clinical diagnosis should be confirmed by serological identification of a culture isolated from stool. It is difficult to detect organisms in foods with current methods.

Nature of the Disease

The onset of symptoms of shigellosis is usually 12–50 h. The most common symptoms are abdominal pain, cramps, vomiting, and blood or mucus in stools. Infections may be associated with mucosal ulceration, rectal bleeding, and drastic dehydration. Death from Shigellosis may be as high as 10–15% with some strains. Sensitive populations such as the elderly, infants, and immunocompromised individuals are more susceptible to complications from the disease. The average duration of symptoms in untreated adults is 7 days, and the organism may be cultivated from stools for 30 days or longer.

Other complications may include; lethargy, delirium, seizure, encephalopathy, hemolytic-uremic syndrome, septicemia, Reiter syndrome, hepatitis, rectal prolapse, myocarditis, and toxic mega colon.

Clinical Management

Prevention of fecal–oral transmission is the most effective control strategy. Severe dysentery is treated with ampicillin, trimethoprim-sulfamethoxazole, or a 4-fluorquinolone such as ciprofloxacin.

Vaccines are not currently available. Dehydration is the most common complication of shigellosis. Supportive care with fluids and electrolyte replacement may be required.

See also: Food and Drug Administration, US; Food Safety and Toxicology; Gastrointestinal System.

Relevant Websites

http://vm.cfsan.fda.gov – US Food and Drug Administration. Center for Food Safety & Applied Nutrition. Foodborne Pathogenic. Microorganisms. and Natural Toxins Handbook. *Shigella* spp.

http://www.who.int – World Health Organization. Initiative for Vaccine Research (IVR). *Shigella*.

http://www.amm.co.uk – Association of Medical Microbiologists. The Facts about Shigella Infection and Bacillary Dysentery. What is dysentery?

Short-Term Exposure Limit *See* Occupational Exposure Limits.

Sick Building Syndrome

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Sick building syndrome (SBS) is a term used to describe office worker discomfort and medical symptoms related to buildings and pollutant exposures, work organization, and personal risk factors. A wide range of definitions exists. Symptoms commonly considered integral parts of the syndrome are listed in **Table 1**. In recent years, with increased understanding, odors have generally been dropped from the list and chest symptoms have been included under mucous membrane irritation.

The problem may be viewed from the perspectives of (1) medicine and health sciences, to define symptoms related to work indoors and their associated pathophysiologic mechanisms; (2) engineering, based on design, commissioning, operations, and maintenance strategies and difficulties; and (3) exposure assessment, the formal measurement of specific pollutants.

Health and People

Since the mid-1970s, increasingly voiced office worker discomfort has been studied in formal ways

Table 1 Sick building syndrome

Mucous membrane irritation	Eye, nose, and throat itching and irritation
Central nervous system symptoms	Headaches, fatigue, difficulty concentrating, lethargy
Chest tightness and asthma-like symptoms (without true wheezing)	
Skin itching and irritation	
Odors	
Diarrhea	

including field epidemiologic studies using buildings or workstations as the sampling unit to identify risk factors and causes, population-based surveys to define prevalence, chamber studies of humans to define effects and mechanisms, and field intervention studies.

Cross-Sectional and Case–Control Studies

Approximately 30 cross-sectional surveys have been published and were reviewed by Mendell in 1993. Many of these have included primarily 'nonproblem' buildings, selected at random. These consistently demonstrate an association between mechanical ventilation and increasing levels of symptoms. Additional risk factors have been defined in several case–control studies. **Table 2** presents a grouping of widely recognized factors.

Many of these factors overlap. For some, pathophysiologic explanations exist. Women are considered more likely to voice discomfort at any given level of exposure and are exposed, on average, to higher levels of pollutants, such as volatile organic compounds (VOCs) and particulates, associated with symptoms.

Factor and principal components analyses of questionnaire responses in cross-sectional surveys have explored the interrelationship of various symptoms. Consistently, symptoms related to a single organ system have clustered together more strongly than symptoms relating different organ systems. That is, eye irritation, eye tearing, eye dryness, and eye itching all appear to correlate very strongly, and little benefit is obtained from looking at multiple symptoms.

Table 2 Risk factors for and causes of the sick building syndrome

Personal	Atopy (allergies, asthma, eczema) seborrheic dermatitis
	Work stress
	Gender
	Lower job status and pay
	Increased tear-film break-up time
Work activities	More time spent at photoduplication
	Carbonless copy paper
	More time at video display terminals
	Increasing amounts of time spent at workstation
Building factors	Mechanical ventilation
	Inadequate maintenance
	High-fleecing surfaces (high surface area surfaces such as carpets and drapes)
	Carpets
	Recent renovation
	Inadequate operations strategies

Data suggest that irritation among office workers does not represent a single distribution, but that susceptible subpopulations exist. Experimental studies, in chambers, show that atopic individuals usually have much lower irritation thresholds for agents commonly found indoors, even when their atopy is not active. In addition, the presence of tear-film instability, from Sicca complex, aging, or other underlying causes, poses an increased risk for irritation.

Controlled Exposure Studies

Animal testing to determine irritant properties and thresholds has become standard. A consensus method, American Society for Testing and Materials E, is widely regarded as the basis. This method has been used to develop structure–activity relationships, to demonstrate that more than one irritant receptor may exist in the trigeminal nerve, and to explore the interaction of multiple exposures. Recently, it has been used to demonstrate the irritating properties of office equipment offgassing.

In keeping with this method, several approaches have been developed to document methods and dose–response relationships for irritation in humans. This work suggests that, at least for 'nonreactive' compounds such esters, aldehydes, ketones, alcohols, carboxylic acids, aromatic hydrocarbons, and pyridine, the percentage of vapor pressure saturation of a compound is a reasonable predictor of its irritant potency. Specific physical properties of molecules predict overall irritation potential. This work is based on the identification of irritant thresholds for homologous series of specific agents. Quantitative structure–activity relationships derived from such work suggests a reasonable model to explain mucosal irritation.

Controlled exposure studies of volunteers in stainless steel chambers have been performed. Most have involved exposure to one specific mixture of VOCs; for example, the work of Molhave and Nielsen in 1992. These studies consistently document relationships between symptoms and increasing exposure levels. Office workers who perceived themselves as 'susceptible' to the effects of usual levels of VOCs indoors demonstrated some impairment on standard tests of neuropsychological performance. Healthy volunteers, on the other hand, demonstrated mucous membrane irritation and headaches at exposures in the range of 10–25 $\mathrm{mg\,m^{-3}}$ but no changes on neuropsychological performance. Recently, office workers demonstrated similar symptoms after simulated work in environments where pollutants were

generated from commonly used office equipment. Animals, using a standardized test of irritant potency, reacted similarly.

Population-Based Studies

At least three population-based studies have been published in Sweden, Germany, and the United States. The questionnaires differed considerably, and the studies do not allow prevalence estimate comparisons. Nevertheless, between 20% and 35% of respondents were thought to have complaints.

Mechanisms

A number of potential mechanisms and objective measures to explain and examine symptoms within specific organ systems have been identified. None of these have a high predictive value for the presence of disease and are not suitable for clinical diagnostic use. They are useful in field and laboratory investigations. These mechanisms and measures were reviewed by Doty and co-workers in 2004.

Both allergic and irritant mechanisms have been proposed as explanations for eye symptoms. More rapid tear-film break-up time, a measure of tear film instability, is associated with increased levels of symptoms. 'Fat-foam thickness' measurement and photography for documentation of ocular erythema have also been used. Some authors attribute eye symptoms at least in part to increased individual susceptibility based on those factors. In addition, office workers with ocular symptoms have been demonstrated to blink less frequently when working at video display terminals. Conjunctival staining with fluorescent dyes is a common clinical test for conjunctivitis sicca.

Nose

Both allergic and irritant mechanisms have been proposed as explanations for nasal symptoms. Measures that have successfully been used include nasal swabs (eosinophils), nasal lavage or biopsy, acoustic rhinometry (nasal volume), anterior and posterior rhinomanometry (plethysmography), and measures of nasal hyperreactivity (visual, using a dental prosthesis as a head fixative, and using an ear surgery microscope to measure distances and swelling).

Central Nervous System

Neuropsychological tests have been used to document decreased performance on standardized tests both as a function of controlled exposure and as a function of symptom presence.

Engineering and Sources

Beginning in the late 1970s, the National Institute for Occupational Safety and Health responded to requests for help in identifying causes of occupant discomfort in buildings. Although no standard investigative protocol was used, the primary cause of problems was attributed to ventilation systems ~50%), microbiological contamination (3–5%), strong indoor pollution sources (tobacco, 3%; others, 14%), pollutants entrained from the outside (15%), and the remainder unknown. On the other hand, Woods and Robertson published two well-known series of engineering analyses of problem buildings, documenting on average three problems that could be the source (**Table 3**).

The current professional ventilation standard (ASHRAE 62-89) suggests two approaches to ventilation: a ventilation rate procedure and an air quality procedure. The former provides a tabular approach to ventilation requirements: office buildings require $20\,ft^3$ of outside air per occupant per minute to maintain occupant complaint rates of environmental discomfort at below 20%. This assumes relatively weak pollution sources. When stronger sources are present, the same rate will provide less satisfaction. For example, when smoking is permitted at usual rates (according to data from the early 1980s), ~30% of occupants will complain of environmental discomfort. The second approach requires the selection of a target concentration in air (e.g., particulates, VOCs, and formaldehyde), information on emission rates (pollutant per time per mass or surface), and

Table 3 Defined engineering problems in series of problem buildings

Problem category	*Physical cause*	*Frequency*	
		Woods	*Robertson*
System design	Inadequate outdoor air	75	64
	Inadequate distribution	75	46
Equipment	Inadequate filtration	65	57
	Inadequate drain lines and pans	60	63
	Contaminated ducts and liners	45	38
	Humidifier malfunction	20	16
Operations	Inappropriate control strategies	90	–
	Inadequate maintenance	75	–
	Thermal and contaminant load charges	60	–

derives the ventilation requirements. Although this is an intellectually much more satisfying procedure, it remains elusive because of inadequate emissions data and disagreement on target concentrations.

In the past, odors were included under the etiologic list of SBS. A recent publication by Boswell and co-workers provide at least an overview of common odor sources. The single largest source was plumbing, such as dried-up traps (16%), followed by maintenance supplies (14%), renovations (11%), and ventilation (8%).

Pollutants

Environmental scientists have generally defined exposure and health effects on a pollutant-by-pollutant basis. In indoor environments these include multiple air pollutants (i.e., 20–50 different VOCs, including formaldehyde and other aldehydes), microbial products (including spores, cell fragments, viable organisms, and secretion products), and reactive agents such as ozone, fibers, and others. The American Thoracic Society defined six important categories listed in **Table 4**.

Environmental criteria have been established for many of these, but the utility and applicability of such criteria for indoor environments is controversial for at least four reasons. For example, the goals of the threshold limit values often do not include preventing irritation, a primary concern in indoor environments with requirements for close eye work at video display terminals. For most of the pollutant categories, the problem of interactions, commonly termed the 'multiple contaminants problem', remains inadequately defined. Even for agents that are thought to affect the same receptor, such as aldehydes, alcohols, and ketones, no prediction models are well established. Finally, the definition of 'representative compounds' for measurement is unclear. That is, pollutants must be measurable, but complex mixtures vary in their composition. It is unclear whether the chronic residual odor annoyance from environmental tobacco smoke correlates better with nicotine, particulates, carbon monoxide, or other pollutants. The measure 'total volatile organic compounds' is meanwhile considered an interesting concept but is not considered for practical purposes because the various components have such radically different effects. Particulates found indoors may differ in composition from those found outdoors because filter sizes affect entrained concentrations and indoor sources may differ from outdoor sources.

Finally, emerging data suggest that reactive indoor pollutants may interact with other pollutants and lead to new compounds. For example, Wechsler has shown that ozone, either from office machines or entrained from outdoors, may interact with 4-phenylcyclohexene and generate adlehydes.

Table 4 Principal pollutant categories (American Thoracic Society)

Bioaerosols
Combustion
Environmental tobacco smoke
Radon
Volatile organic compounds
Fibers

Primary Etiologic Theories

Volatile Organic Compounds

Buildings have always relied on general dilution strategies for pollutant removal, but designers have assumed that humans were the primary source of pollutants. Emissions from 'solid materials' (e.g., particle board desks, carpeting, and other furniture), from wet products (e.g., glues, wall paints, and office machine toners), and personal products (perfumes) have been recognized as contributors to a complex mixture of very low levels of individual pollutants as described by Hodgson and co-workers.

Several studies suggest that the presence of reactive VOCs, such as aldehydes and halogenated hydrocarbons, is associated with increasing levels of symptoms. Offices with higher complaint rates showed greater 'loss' of VOCs between incoming and outgoing air than did offices with lower complaints. In a prospective study of schools, short-chain VOCs were associated with symptom development. In another survey, higher personal samples for VOCs using a screening sampler that 'overreacts' to reactive VOCs such as aldehydes and halogenated hydrocarbons were associated with higher symptom levels. In that study, women had higher levels of VOCs in their breathing zone, suggesting another potential explanation for the increased rate of complaints among women. VOCs might adsorb onto sinks, such as fleecy surfaces, and be reemitted from secondary sources. The interaction of ozone and relatively nonirritant VOCs to form aldehydes is also consistent with this hypothesis. Although many individual agents are usually present, they are present only at concentrations well below those needed to cause irritation.

Modeling the irritant effects of such complex mixtures, such as that reported by Alarie and co-workers, has led to the development of a quantitative structure–activity relationship for irritation. Under

normal conditions, with usual sources and standard ventilation rates, concentrations should not reach irritant levels. On the other hand, in the presence of unusual sources (renovation, unusual office conditions) or inadequate ventilation, and especially in the setting of reactive species coexposure, concentrations are very likely to reach irritating levels.

Bioaerosols

Bioaerosols grow in moisture films on surfaces. Several studies have suggested that bioaerosols may contribute to occupant discomfort through several different mechanisms: irritant emissions; release of fragments, spores, or viable organisms leading to allergy; and secretion of complex toxins. Several studies suggest a relationship between symptoms indoors and airborne endotoxin levels. The endotoxin concentrations on cooling coils themselves are better predictors of irritation than airborne bacteria or endotoxin measurements. This suggests that some bacterial component, possibly endotoxin, perhaps in combination with other bioaerosols, plays an important role in generating symptoms and that moisture and growth on the coils rather than in the air or ductwork plays a role in generating symptoms. Clearly, heating, ventilating, and air-conditioning systems may be sources for microorganisms. Fundamentally, the presence of moisture always raises the suspicion of potential cause. Bulk moisture incursion, through roof defects, wall penetration, below-grade seepage, or internal leaks, may generate problems. The presence of moisture barriers in perimeter walls also presents an opportunity for condensation. Especially in the setting of negative pressure in the building, pulling moisture towards such barriers (such as vinyl wall paper), bioaerosols may grow and cause problems. They have also been noted in building construction materials (as a result of improper curing) and in office dust. The presence of sensitizers in the office environment, such as dust mites or cat danders brought in from home on clothing, presents another interesting exposure though not likely one of substantial importance.

Psychosocial Aspects of Work

In all studies in which it has been examined, 'worker stress' was clearly associated with SBS symptoms. Workers' reactions to job pressures, such as task conflicts, and outside pressures, such as spousal or parental demands, may lead to the subjective experience of 'stronger' irritation. At times, such experiences may result at least in part from poor supervisory practices. In addition, the persistence of irritants leading to subjective irritation may reinforce work stress.

Conclusion

The SBS is a phenomenon experienced by individuals, usually seen in groups, associated with engineering deficiencies, and likely caused by a combination of pollutants representing a variety of pollutant categories. As with all 'disease', a component of personal psychology serves as an effect modifier contributing to varying degrees of symptom intensity at any given level of distress.

See also: Behavioral Toxicology; Dose–Response Relationship; Exposure Assessment; Mixtures, Toxicology and Risk Assessment; Multiple Chemical Sensitivities; Neurotoxicity; Pollution, Air Indoor; Psychological Indices of Toxicity; Respiratory Tract; Sensory Organs.

Further Reading

Abraham MH, Andonian-Haftvan J, Cometto-Muniz JE, and Cain WS (1996) An analysis of nasal irritation thresholds using a new solvation equation. *Fundamental and Applied Toxicology* 31: 71–76.

Alarie Y, Schaper M, Nielsen GD, and Abraham MH (1998) Structure–activity relationships of volatile organic chemicals as sensory irritants. *Archives of Toxicology* 72: 125–140.

Boswell RT, DiBerardinis L, and Ducatman A (1994) Descriptive epidemiology of indoor odor complaints at a large teaching institution. *Applied Occupational and Environmental Hygiene* 9: 281–286.

Cone J and Hodgson MJ (eds.) (1989) Building-associated illness and problem buildings. *State-of-the-Art Reviews in Occupational Medicine* 4: 575–802.

Doty RL, Cometto-Muniz JE, Jalowayski AA, *et al.* (2004) Assessment of upper respiratory tract and ocular irritative effects of volatile chemicals in humans. *Critical Reviews in Toxicology* 34: 1–58.

Hodgson MJ, Levin H, and Wolkoff P (1994) Volatile organic compounds and the sick-building syndrome. *Journal of Allergy and Clinical Immunology* 94: 296–303.

Lockey R and Ledford D (eds.) (1994) Symposium on the sick-building syndrome. *Journal of Allergy and Clinical Immunology* Suppl. 2, Pt. 2: 214–365.

Mendell MJ (1993) Non-specific symptoms in office workers: A review and summary of the literature. *Indoor Air* 4: 227–236.

Menzies D, Popa J, Hanley JA, Rand T, and Milton DK (2003) Effect of ultraviolet germicidal lights installed in office ventilation systems on workers' health and well-being: Double-blind multiple crossover trial. *Lancet* 362: 1785–1791.

Middaugh DA, Pinney SM, and Linz DH (1992) Sick building syndrome: Medical evaluation of two work

forces. *Journal of Occupational Medicine* 34: 1197–1204.
Molhave L and Nielsen GD (1992) Interpretation and limitations of the concept "Total volatile organic compounds" (TVOC) as an indicator of human responses to exposures of volatile organic compounds (VOC) in indoor air. *Indoor Air* 2: 65–77.
Selzer J (ed.) (1995) Illnesses associated with indoor environments. *State-of-the-Art Reviews in Occupational Medicine* 10: 1–245.
Wechsler CJ (1992) Indoor chemistry: Ozone, volatile organic compounds, and carpets. *Environmental Science and Technology* 26: 2371–2377.
Woods JE (1989) Cost avoidance and productivity in owning and operating buildings. *Occupational Medicine* 4(4): 753–770.
World Health Organization (WHO) (1987) *Air Quality Guidelines for Europe*. Copenhagen: WHO Regional Office for Europe.

Silent Spring

Michael A Kamrin

Introduction

In the period following World War II, there was a great increase in industrial production in the developed world. Not only were existing products manufactured in larger numbers, but also a large number of new synthetic chemicals were developed. These included innovative structural materials, such as plastics, new and more effective pharmaceuticals and more effective pest control chemicals. Many of these new compounds rapidly gained widespread use in the developing as well as the developed world.

This increase in industrial production was soon accompanied by concerns about the environmental consequences of the effluents from production facilities as well as the new chemicals themselves. Graphic pictures alerted the public to the problems of decreasing air quality, and increasing amounts of waste effluents reaching surface waters. These included images of thick smoke bellowing from factories and fires in rivers, such as the Cuyahoga. The problem was international in scope as was illustrated by the discovery of serious human health impacts around Minamata Bay, Japan due to ingestion of fish contaminated with methylmercury formed from effluents emitted by an industrial facility on the bay.

Complementing this boom in industrial production was an great increase in the use of agricultural chemicals, both fertilizers and pesticides. In addition to an increase in the amounts applied, the types of pesticide chemicals used changed to those that were long lasting, such as the organochlorines. The most prominent example of this class of pesticides is DDT.

There were two main types of concerns about these persistent pesticides – one was their impact on birds and wildlife and the other their impact on human health. The former was illustrated by the reports in the late 1950s that DDT usage was linked to the decimation of the robin population on the Michigan State University campus. The latter concern was illustrated by the great cranberry scare that occurred right before Thanksgiving in 1958 when it was thought that pesticide contamination of cranberries would lead to cancer in people who ate them. It was also the year that the Congress passed the so-called Delaney Amendment that forbid the use of any food additive that might be linked to cancer in laboratory or epidemiological studies.

Silent Spring

It is against this background that *Silent Spring*, written by Rachel Carson, was published in 1962. The book was a polemic that focused on the impacts or potential impacts of pesticides on both humans and their environment. It was a call for people to be much more aware of the seriousness of the problems caused by these synthetic chemicals and to take actions to minimize and/or eliminate their use. The author recommended that chemical control of pests be replaced by biological controls and that persistent chemicals, such as DDT, be taken off the market.

The impact of this book was enormous as it seemed to coalesce the diverse and growing concerns of the public about damage to the environment and public health by industry. It contributed strongly to the rise and expansion of the environmental movement in the mid- to late 1960s and to the establishment of a number of environmental protection laws and policies in the United States and elsewhere in the 1970s. A very important result of this environmental movement was the creation of the US Environmental Protection Agency. In addition to catalyzing organizational change, it also led to specific actions that were called for in *Silent Spring*, particularly the banning of DDT use in the United States – which occurred in 1972.

While the focus of *Silent Spring* was pesticides, the environmental movement that grew out of it was

much broader and had the goal of limiting the use and disposal of a wide variety of industrial chemicals. Signal events, such as Love Canal, led to efforts in particular directions other than limiting pesticide use. In the case of Love Canal, this direction was the clean-up of hazardous wastes from the past. However, efforts to force a reduction in pesticide usage also continued unabated.

These efforts have led to increased reliance on a combination of methods for pest control including both chemical and biological controls, a technique known as integrated pest management (IPM). The increasing use of IPM has led to a decreasing use of pesticides. The continuing public concern about pesticides and other chemicals used in food production has been the impetus for a growing organic food movement. At least in the United States, a significant number of people are willing to pay a premium for foods that are certified as having been grown without the use of pesticides or other commercial chemicals.

Indirectly, this movement has also led to the development of agricultural biotechnology, a field that focuses on altering crop plants to reduce the need for pesticide applications. This includes research to develop plants that produce their own natural pesticide as well as crop plants that are resistant to synthetic pesticides. Plantings of bioengineered crops have rapidly increased in recent years and a majority of some crops grown in the United States are products of this technology.

Summary

The publication of *Silent Spring* was a seminal event in the environmental movement in the United States and, later, abroad. Prior to the book there was slowly increasing public recognition of environmental problems due to industrial effluents and use of certain synthetic chemicals. Afterwards, the environment became an overriding issue to many Americans and an environmental movement arose that is still going strong. While there are still questions about the wisdom of some of the recommendations that were made in *Silent Spring*, there is no question that the book has led to a different way of looking at our environment and the effects of some aspects of human progress on this environment.

From the toxicological perspective, it is clear that much of the research that has been performed in the past four decades has resulted from concerns that were raised in Silent Spring. These include studies of the adverse effects on humans and other organisms of pesticides and other chemicals in our environment as well as basic research on mechanisms of toxicity. It is evident that this type of research will continue as questions still remain about well known as well as newly discovered chemicals in our environment.

See also: DDT (Dichlorodiphenyltrichloroethane); Pesticides.

Further Reading

Carson R (1962) *Silent Spring*. New York: Fawcett Crest.
Marco GJ, Hollingworth RM, and Durham W (1987) *Silent Spring Revisited*. Washington, DC: American Chemical Society.
vanEmden HF and Peakall DB (1996) *Beyond Silent Spring: Integrated Pest Management and Chemical Safety*. London: Chapman and Hall.

Silica, Crystalline

Kent E Pinkerton and Randal J Southard

- Representative Chemicals: Quartz; Cristobalite; Stishovite; Tridymite; Coesite
- Synonym: Silicon dioxide
- Chemical Formula: SiO_2 – Crystalline silica, also called 'free silica', is defined as silicon dioxide (SiO_2). This chemical formula represents a very stable form of silicon, wherein the Si is completely polymerized through Si–O bonds in three dimensions
- Chemical Structure: Crystalline silica represents a form of silica, which is in a highly organized, framework pattern. The term 'crystalline' refers to the orientation of SiO_2 molecules in a fixed pattern as opposed to random molecular arrangement defined as amorphous, noncrystalline, or short-range order. The oxygen and silicon atoms of silicon dioxide are arranged in a three-dimensional pattern repeated indefinitely in three directions, forming the crystalline structure

Uses/Occurrence in Nature

The most common hazard for exposure to crystalline silica occurs with sandblasters who use sand for cleaning of surfaces, thus generating dust clouds of freshly fractured crystalline silica. Other occupations include farm labor where mineral dusts are generated

in field preparation and processing of crops, particularly in arid to semiarid regions where irrigation is required for crop production. Although silica is only one constituent of mineral dust, it can represent up to 15% of the respirable dust present in agricultural settings because quartz is so abundant in most soils. The proportion of quartz in respirable dust will thus be related to soil mineralogy and the relative abundances of the sand, silt, and clay fractions in the soil. Other compounds in which crystalline silica may be found include gravel, slate, diatomaceous earth, concrete, mortar, plaster, refractory materials, pottery clay, limestone, shale, bricks, and abrasives. The three most common crystalline forms of silica encountered are cristobalite, tridymite, and quartz.

Quartz is the most common crystalline form of silica encountered in nature. Quartz is present as alpha and beta (high temperature) forms. Alpha quartz is the most common form, and is found in large quantities in rocks and soils worldwide. That quartz is among the most abundant minerals in many, if not most, soils is a reflection of its chemical stability and resistance to weathering. In fact, quartz is so prevalent that the term 'quartz' is often used in place of crystalline silica. Coesite and stishtovite are formed at high pressure (e.g., meteorite impact craters), whereas tridymite and cristobalite form at high temperature (e.g., volcanic rocks). Other than alpha quartz, all of these forms are metastable at earth surface temperatures and pressures and will slowly convert to alpha quartz given enough time. Microcrystalline varieties of silica also include small grains of this material, possibly combined with amorphous silica. Tripoli, flint, chalcedony, agate, onyx, and silica flour are examples.

Background Information

Silicosis is the oldest known occupational lung disease. Ancient Greeks were familiar with lung disease in quarry workers (Hippocrates) and the fact that respirators could prevent the disease (Pliny). Agricola (1566) described disease in stone cutters as later did Ramazini (1713). By 1917, the US Public Health Service identified sand blasters and foundry workers to be at high risk of silicosis. As the twentieth century progressed, silicosis was the reference to which newer diseases were compared.

Exposure Routes and Pathways

Exposure routes are primarily by inhalation. The greatest hazards for exposure to crystalline silica are typically found in the workplace. When crystalline silica becomes small enough (i.e., $<10\,\mu m$ in diameter), these materials can become aerosolized and are able to enter the respiratory tract where they can deposit along the tracheobronchial tree or into the deep recesses of the lung where gas exchange takes place (i.e., alveoli). Bulk crystalline silica is defined by the size of the individual particles. In soils terminology, for example, 'sand' is composed of grains 50–2000 μm in diameter, 'silt' is in the 2–50 μm range, while 'clay'-size particles are less than 2 μm in diameter. Crystalline silica is considered respirable or inhalable when particles are less than 10 μm in diameter (i.e., in the silt and clay sizes).

Mechanism of Toxicity

Although a number of theories exist to explain the potential mechanism of toxicity to crystalline silica, the primary cause is described as membrane damage occurring to cells that ingest these tiny particles. It is thought that once crystalline silica is ingested into a cell, free radical oxygen species can be generated from the surface of the particle leading to lipid peroxidation and membrane damage followed by release of lysosomal contents and lysis of the cell, resulting in cell death. This process creates a vicious cycle where crystalline particles are taken up again into other cells that will undergo the same sequence of events. Although the precise events to drive this cell injury process are unclear, it has been observed that freshly fractured surfaces of crystalline silica more readily generate free radicals to damage the membrane of cells taking up these particles, thus producing greater and more rapid cell injury. This may explain, in part, the hazardous condition created in sandblasting where silica particles may be further fractured in the cleaning process. The acute toxicity of exposure to crystalline silica for both animals and humans follows a similar mechanism with rapid ingestion of particles into cells, primarily alveolar macrophages, and subsequent damage to lipid membranes and the lytic death of these cells. This repetitive process results in inflammatory events leading to the influx of numerous macrophages into the alveolar air spaces.

Pulmonary silicosis occurs by way of breathing these particles over short to long periods of time. The underlying mechanism for this disease involves the ingestion of silica particles by macrophages in an attempt to remove them from the lungs. However, silica particles produce membrane damage and death to these cells. The repetitive process of uptake and release from macrophages leads to the further release of hydrolytic enzymes and mediators that stimulate the influx of inflammatory cells and laying down of collagen by fibroblasts in an abnormal pattern to

produce diffuse interstitial lung disease or pulmonary fibrosis. Fibrotic changes in the lungs are a reflection of a prolonged injury process to macrophages with the accompanying deposition of collagen by fibroblasts to bring about scar tissue formation. This scarring process leads to the loss of alveolar airspace with excessive amounts of collagen fibers forming wherever quartz particles have been deposited and/or translocated in the lungs. The pattern of scarring associated with silicosis is typically found to be more prevalent in the upper lobes of the lung in a nodular pattern, leading to the complete obliteration of alveolar air spaces in affected sites.

Acute and Short-Term Toxicity (or Exposure)

In both animal and human studies, the toxicity of crystalline silica is manifested acutely as an inflammatory process with the influx of a large number of macrophages into the alveolar airspaces of the lungs.

Chronic Toxicity (or Exposure)

Chronic toxicity of crystalline silica in both animals and humans results in a patchy nodular disease known as pulmonary silicosis. Both animal and human studies demonstrate the persistence of lung inflammation associated with excess collagen deposition to form nodular as well as diffuse fibrotic lesions throughout the lungs. The disease process of silicosis is incurable and nonreversible. the disease progress, breathing becomes labored and more difficult, and can result in death in extreme cases. Symptoms of silicosis include cough, shortness of breath, wheezing, and repeated chest illnesses. The diagnosis of a chronic disease due to silicosis is determined through pulmonary function tests, chest X-rays, and history of occupational exposure to silica.

In addition to the disease process of silicosis, inhalation of crystalline silica has been associated with other diseases such as bronchitis and tuberculosis. There is also some indication of an association with lung cancer.

In Vitro Toxicity Data

Crystalline silica is toxic to cells *in vitro*, and it is commonly used as a positive control material in cytotoxicity testing in cell culture systems.

Exposure Standards and Guidelines

The US Occupational Safety and Health Administration mineral dust standards for occupational exposure to crystalline silica depend on the actual composition of the sample.

As outlined in the 1974 Center for Disease Control/National Institute for Occupational Safety and Health publication, *Occupational Exposure to Crystalline Silica*, employees who are exposed to free silica must be apprised at the beginning of their employment of the hazards, relevant symptoms, appropriate emergency procedures, proper conditions and precautions for safe use or exposure. The following warning is required to be posted in both English and the predominant language of non-English-speaking workers potentially exposed to free silica dust:

> WARNING!
> CONTAINS FREE SILICA
> DO NOT BREATHE DUST
> May Cause Delayed Lung Injury (Silicosis)

Further Reading

Criteria for a Recommended Standard for Occupational Exposure to Crystalline Silica, HEW Publication No. (NIOSH) 75-120. Washington, DC: Center for Disease Control, National Institute for Occupational Safety and Health, 1974.

Drees LR, Wilding LP, Smeck NE, and Senkayi AL (1989) Silica in soils: Quartz and disordered silica polymorphs. In: Dixon JB and Weed SB (eds.) *Minerals in Soil Environments*, 2nd edn. Madison, WI: Soil Science Society of America.

Goldsmith DF, Winn DM, and Shy CM (eds.) (1996) *Silica, Silicosis, and Cancer: Controversy in Occupational Medicine*. New York: Praeger.

Relevant Websites

http://geology.usgs.gov – Crystalline Silica Primer, Branch of Industrial Minerals, US Department of the Interior, Washington, DC, 1978.

http://www.osha.gov – The US Occupational Safety and Health Administration mineral dust standards for occupational exposure to crystalline silica depend on the actual composition of the sample. Interested readers may consult Table Z-3 in the website.

Silver

Shayne C Gad

This article is a revision of the previous print edition article by Arthur Furst and Shirley B Radding, volume 3, p. 144, © 1998, Elsevier Inc.

- Representative Chemicals: Silver chloride (AgCl); Silver nitrate ($AgNO_3$); Silver cyanide (AgCN)
- Chemical Abstracts Service Registry Number: CAS 7440-22-4
- Synonym: Plata
- Chemical/Pharmaceutical/Other Class: Precious metals
- Chemical Formula: Ag^+

Uses

Silver is used extensively in jewelry, eating utensils, coins, batteries, and dental amalgams. Silver solutions are used as antiseptics, astringents, and germicides. In some domestic water purifiers, silver is used to remove chlorine and kill bacteria. It has also been used in hair dyes. Medicinal use includes silver nitrate eye drops for newborns (a legal requirement in some states) and use as an antimicrobial on some implantable medical devices. The main industrial use of silver is in the form of silver halide for the photographic industry. Silver halide is photosensitive, making it an ideal coating for photographic plates.

Background Information

Silver is one of the earliest known metals. Silver has no known physiologic of biologic function, though colloidal silver is widely sold in health food stores.

Exposure Routes and Pathways

Ingestion and inhalation are possible routes of exposure; dermal absorption of silver is unlikely. Silver is not a normal constituent of foodstuff. Very little, if any, silver is detected in domestic drinking water; however, some domestic water-purifying systems contain silver.

Toxicokinetics

Approximately 10% of ingested silver is absorbed. Inhaled silver can be absorbed from the lungs. Silver can be absorbed across oral mucosa. Once absorbed, silver tends to precipitate in various tissues, as the affinity for sulfide by silver is immense. Silver tends to complex with sulfhydryl groups on macromolecules. It is carried by globulins in the serum and forms complexes with the serum proteins, mainly albumin, which accumulate in the liver. Silver is excreted in the feces (primarily) and urine.

Mechanism of Toxicity

While specific mechanisms of toxicity are unclear, high affinity for sulfhydryl groups on proteins could lead to alteration of a number of cellular processes.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute toxicity of metallic silver and water-soluble compounds is moderate. The oral LD_{50} in mice for colloidal silver was $100\,mg\,kg^{-1}$ and relatively similar for the water-soluble compounds silver nitrate ($50–129\,mg\,kg^{-1}$) and silver cyanide (LD_{50} in rats, $125\,mg\,kg^{-1}$). Silver nitrate appears much less toxic in rabbits by the oral route ($800\,mg\,kg^{-1}$). The insoluble silver oxide was reported to exhibit an LD_{Lo} of $>2\,g\,kg^{-1}$ in rats.

Human

Acute oral exposure to silver nitrate has led to irritation and corrosion in the gastrointestinal tract, abdominal pain, diarrhea, vomiting, shock, convulsions, and death in humans. Silver or silver nitrate can lead to respiratory irritation with inhalation exposures. Silver nitrate is highly irritating to the skin, mucous membranes, and eyes. Insoluble silver compounds (e.g., silver chloride, silver iodide, and silver oxide) are relatively benign.

Chronic Toxicity (or Exposure)

Animal

With selenium or Vitamin E deficient diets, repeated exposure to silver (76 ppm in the drinking water for 52 days) in rats elicited hepatic necrosis and ultrastructural changes in the liver indicative of oxidative damage. This toxicity may be related to a silver-induced selenium deficiency and impairment of synthesis of the enzyme glutathione peroxidase. Dietary supplementation with selenium or Vitamin E prevented such changes. Mice exposed to silver nitrate in the drinking water for 4 months exhibited

silver-containing deposits in the central nervous system and reduced motor activity.

Human

Workers chronically exposed to silver have experienced industrial argyria, an occupational disease characterized by discoloring of the skin. Blue–gray patches are noted on the skin and possibly the conjunctiva of the eye or the mucous membranes. Long-term exposure can result in extensive skin discoloration, mainly on the parts of the body that are exposed to light (e.g., the face). Light may decompose the silver complex, resulting in extremely fine silver that gives the skin a metallic sheen. In some cases, the dark patches turn black. Chronic bronchitis has been reported following medicinal use of colloidal silver. Potential symptoms of overexposure are blue–gray eyes, nasal septum, throat, and skin. The discoloration can be permanent.

Clinical Management

Clinical management is supportive and there no known active treatment. Administration of table salt will help precipitate soluble silver as the insoluble silver chloride. British antilewisite (2,3-dimercaptopropanol) has not proven useful.

Environmental Fate

Silver exists in four oxidation states (0, 1+, 2+, and 3+). Silver occurs primarily as sulfides with iron, lead, tellurides, and with gold. Silver is found in surface waters as sulfide, bicarbonate, or sulfate salts, as part of complex ions with chlorides and sulfates and adsorbed onto particulate matter. Silver is released through natural processes, for example, erosion of soils. Sources of atmospheric contamination arise from processing of ores, steel refining, cement manufacture, fossil fuel combustion, and municipal waste incineration. Of anthropomorphic release, over 75% was estimated to be from disposal of solid waste. Ore smelting and fossil fuel combustion can emit fine particulates that may be transported long distances and deposited with precipitation. The major source of release to surface waters is effluent from photographic processing. Releases from the photographic industry and from disposal of sewage sludge and refuse are the major sources of soil contamination with silver. Silver can leach into groundwater; acidic conditions increases leaching. Silver can be bioconcentrated in fish and invertebrates.

Ecotoxicology

The no-observed-effect concentration of silver nitrate in a 28 day toxicity test using a marine invertebrate (*Americamysis bahia*) was $34\,\mu g\,l^{-1}$. The 96 h LC_{50} value was $260\,\mu g\,l^{-1}$. In a 21 day toxicity study using the freshwater invertebrate *Daphnia magna*, $5\,\mu g\,Ag\,l^{-1}$ (as silver nitrate) under static conditions, 20% mortality was noted (0% in controls). Silver caused a significant reduction of reproductive performance (14% decrease in the number of neonates). Silver caused a 65% decrease in whole body sodium concentration and a 60% increase in whole body Na^+,K^+-ATPase activity.

Silver nitrate ($10\,\mu g\,l^{-1}$) elicited a 35% reduction in whole body sodium and increases in daily mortality in developing rainbow trout. Exposure to $0.1\,\mu g\,l^{-1}$ silver led to reduction in growth.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value (TLV) time-weighted average is $0.1\,mg\,m^{-3}$ for silver metal; the TLV is $0.01\,mg\,m^{-3}$ for soluble silver compounds.

See also: Metals.

Further Reading

Dart RC (2004) *Medical Toxicology*, 3rd edn., pp. 1459–1461. Baltimore: Williams & Wilkins.

Relevant Websites

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

http://cira.ornl.gov – Toxicity Summary for Silver (from the Oak Ridge National Laboratory).

Sister Chromatid Exchanges

David A Eastmond

Sister chromatid exchanges (SCEs) are reciprocal exchanges of segments of chromatids: chromatids are the subunits of chromosomes, as visualized in metaphase, which become daughter chromosomes upon completion of cell division. Sister chromatid exchanges were discovered by J.H. Taylor in the late 1950s in experiments using the pulsed uptake of ^{3}H-labeled thymidine (TdR) in growing *Vicia faba* root tips, followed by autoradiography, to define the pattern of DNA replication in chromosomes.

Before Taylor's experiments, it was thought that one chromatid might be composed of newly synthesized DNA and the other of preexisting DNA. However, Taylor found that the DNA replicated semiconservatively, that is, in the first metaphase after [^{3}H]TdR incorporation (M_1) each chromatid was ^{3}H-labeled, which demonstrated that the chromatid was duplex, containing both preexisting and newly synthesized DNA. Furthermore, in the second division after ^{3}H incorporation (M_2) one chromatid was labeled and the other was unlabeled, that is, both the preexisting and the newly synthesized DNA in each daughter chromosome had served as a template for the next round of DNA replication, again resulting in two sister chromatids. Taylor also noted that occasionally in M_2 one otherwise labeled chromatid had an unlabeled segment and, when this occurred, the corresponding segment of the otherwise unlabeled chromatid was labeled with [^{3}H]TdR, indicating a reciprocal exchange of segments between the two sister chromatids, that is, a sister chromatid exchange.

The development of SCE assays for genetic toxicology research and testing did not occur until the early 1970s, a time when a plethora of approaches were identified for assessing the potential genetic hazards of chemical exposure. Rather than using [^{3}H]TdR and autoradiography to visualize SCEs, the defined approaches are usually based on the more precise and efficient incorporation of bromodeoxyuridine (BrdU), an analog of thymidine, in two rounds of replication followed by Giemsa, or fluorescence-plus-Giemsa, staining of the chromosomes. Because of semiconservative DNA replication, the chromatids are equally stained in M_1 chromosomes, and the M_2 chromosomes possess one chromatid that is half-BrdU-substituted and one fully substituted chromatid that is stained more lightly than the other. SCEs are revealed in M_2 chromosomes by an alternating or 'harlequin' pattern of darkly and lightly staining chromatid segments.

This approach has also been used to reveal chemical- and concentration-related delays in the progression of cells through the cell cycle as a preliminary test for selecting exposure conditions for chromosomal aberration assays. The objective of such preliminary tests is to define exposure conditions and harvest times that will yield sufficient numbers of first division, M_1, cells for cytogenetic analysis. This is beneficial because a high percentage of chromosomally damaged cells are often unable to progress to the second and following metaphases.

In vitro SCE assays are routinely conducted in cultured Chinese hamster ovary (CHO) cells or human lymphocytes, and assessments of SCEs in human lymphocytes have been used for human population monitoring. Following *in vivo* exposure, SCEs are usually visualized in bone marrow cells from mice implanted with BrdU-containing tablets (or pumps). Such SCE assays have been used to test several hundred chemicals and have been shown to be highly sensitive and, in comparison to conventional assays for chromosomal aberrations, to be more rapid, less subjective, and capable of detecting effects at lower dose levels.

SCE assays would, therefore, appear to be uniquely suited for inclusion in initial batteries of tests to assess genotoxicity. However, while this was initially perceived to be the case, the use of SCE assays for genotoxicity testing has been greatly reduced for several reasons. First, it was found that the use of BrdU (or [^{3}H]TdR) can induce SCEs; thus, there was concern that when SCE frequencies were elevated following chemical exposure, synergistic effects were being measured, which might not be as appropriate for risk assessment as the measurement of direct effects. Second, although there is strong evidence that SCEs result from misreplication of a damaged DNA template, probably from recombination at a stalled replication fork, there was uncertainty concerning whether to classify SCE assays as cytogenetic tests, as a measure of the repair of DNA damage, or as an independent category of test. Third, alarm was expressed when common chemicals such as NaCl (i.e., table salt) were found to be positive in *in vitro* SCE assays. It was subsequently shown that in *in vitro* assays, particularly in the presence of exogenous metabolic activation, such false-positive results could be eliminated if exposure conditions are monitored

and adjusted to preclude acidic pH shifts and high osmolality.

However, the most significant reason for an absence of regulatory requirements for the routine use of SCE tests and their discontinuation by industry was the outcome of a National Toxicology Program (NTP) comparison of the concordance of results from four *in vitro* tests with results from rodent carcinogenicity bioassays. Specifically, the NTP studies conducted by Tennant and colleagues found that, although few positive results were obtained for noncarcinogens in the *Salmonella typhimurium* reverse mutation assay (Ames test) or in the test for chromosomal aberrations in CHO cells, an unacceptably high number of false-positive results were obtained in the *in vitro* SCE assay.

Thus, SCE tests have been largely discontinued by industry and are recommended by regulatory agencies on a limited basis. However, this assay continues to be used as a research tool and in some regulatory settings. For example, despite its poor concordance, the SCE assay continues to be used by the NTP, in part because SCE techniques are sufficiently similar to those used for *in vitro* chromosomal aberration assays so that the two tests can efficiently be used in parallel by cytogenetic testing laboratories.

Similarly the measurement of SCEs for population monitoring has also diminished in recent years as a result of several prospective studies whose objective was to determine if cytogenetic assays had predictive value for future cancer risk. In these studies, no correlation was seen between SCE levels and future cancer risk whereas good correlations were seen between the frequency of chromosomal aberrations and the subsequent development of cancer in the study groups.

Recent evidence indicates that SCEs are primarily formed as the result of homologous recombination so that SCE frequencies represent a measure, not only of mutagen exposure but also of the efficiency of DNA repair. As a result, a direct correlation between SCEs and cancer incidence may not be expected. In spite of the recent developments, there continues to be uncertainty about the underlying mechanisms by which SCEs are formed, and how DNA damage or disturbances in DNA synthesis stimulate SCE formation. Based on our current understanding, SCEs are probably best regarded as a general indicator of mutagen exposure rather than as a specific measure of mutagenic effects.

See also: Ames Test; Analytical Toxicology; Aneuploidy; Carcinogen–DNA Adduct Formation and DNA Repair; Chromosome Aberrations; Developmental Toxicology; Dominant Lethal Tests; Host-Mediated Assay; Molecular Toxicology – Recombinant DNA Technology; Mouse Lymphoma Assay; Toxicity Testing, Mutagenicity.

Further Reading

Hagmar L, Bonassi S, Stromberg U, *et al.* (1998) Chromosomal aberrations in lymphocytes predict human cancer: A report from the European Study Group on Cytogenetic Biomarkers and Health (ESCH). *Cancer Research* 58: 4117–4121.

Latt SA, Allen J, Bloom SE, *et al.* (1981) Sister-chromatid exchanges: A report of the GENE-TOX program. *Mutation Research* 87: 17–62.

Natarajan AT (2002) Chromosome aberrations: Past, present and future. *Mutation Research* 504: 3–16.

Perry PE (1980) Chemical mutagens and sister-chromatid exchange. In: de Serres FJ and Hollaender A (eds.) *Chemical Mutagens: Principles and Methods for their Detection*, vol. 6, pp. 1–39. New York: Plenum.

Sonda E, Sasaki M, Morrison C, *et al.* (1999) Sister chromatid exchanges are mediated by homologous recombination in vertebrate cells. *Molecular Cell Biology* 19: 5166–5169.

Tucker JD, Auletta A, Cimino MC, *et al.* (1993) Sister-chromatid exchange: Second report of the Gene-Tox program. *Mutation Research* 297: 101–180.

Skeletal System

M Joseph Fedoruk and Tee L Guidotti

This article describes the structure and function of the musculoskeletal system and provides an overview of the categories of toxic effects that can affect this body system. The article is divided into two principle parts, which form the main components of the musculoskeletal system: bone and skeletal muscle.

Bone

Bone, a form of connective tissue, composes the skeletal system. The skeletal system provides mechanical support for the body and protects internal organs such as the brain and heart, which are contained in

skull and the chest wall cavity, respectively. The human skeleton is composed of 206 bones that vary in size and shape and include flat, trabecular, and cuboid bones. The body size and shape are determined by the skeletal system.

Bone serves other functions. It is a dynamic tissue that plays a vital role in mineral homeostasis and is a reservoir for several essential minerals including calcium, phosphorus, magnesium, and sodium. Bone houses the delicate bone marrow that forms blood from hematopoietic cells. Bone is an extremely vascular tissue and receives up to 10% of the cardiac output.

Joints form the sites where bones come together or articulate. Joints are classified by the type of tissue that lies between the bones. Joints with fibrous tissue between the articulating surfaces are called fibrous joints and include the sutures of the skull. Cartilaginous joints are united by hyaline cartilage and are classified into primary and secondary cartilaginous joints. Primary cartilaginous joints do not allow any movement.

Bone is composed of live cells interspersed in an organic matrix. Inorganic elements or minerals (65%) are deposited into this organic matrix (35%), which makes bone one of the few tissues that normally mineralize. The principal inorganic element in bone is calcium hydroxyapatite ($Ca_{10}(PO_4)OH_2$), which accounts for $\sim$99% of the calcium and 80% of the stores of these respective minerals in the body. Calcium hydroxyapatite provides bone with strength and hardness. The organic matrix provides a degree of elasticity to bone.

The cellular elements of bone include osteoprogenitor cells, which are pluripotential cells derived from mesenchymal tissue. Osteoprogenitor cells produce offspring cells that can differentiate into osteoblasts. Osteoblasts are responsible for the formation of the organic matrix of the bone into which the mineral elements can be deposited. Groups of several hundred osteoblastic cells coordinate activities to facilitate the formation of the organic matrix. The organic matrix is principally composed of type I collagen (90%) and several other noncollagenous proteins, including (1) osteocalcin, which serves to translate mechanical stresses or signals into local bone activity; (2) osteonectin, a calcium-binding protein; (3) osteopontin, a protein that facilitates cell adhesion; (4) cytokines; and (5) growth factors, which help control cell proliferation, mineralization, and metabolism. Osteoblasts have several different types of receptors including those for hormones (e.g., parathyroid hormone and estrogen) as well as other receptors for cytokines and growth factors.

Osteoblastic activity initiates the process of mineralization. Unmineralized bone is known as osteoid. Minerals are deposited in specific holes that are located between collagen fibrils produced by the osteoblast. The architecture of the fibrils is designed to withstand external stress. Mineralization begins shortly after the formation of the secreted matrix. This process occurs in osteons, also referred to as Haversian systems, and is completed in several weeks. Blood vessels penetrate bond through channels known as Haversian canals.

Osteoblasts which have become encased in bone are called osteocytes. The bony covering is not complete and osteocytes maintain communication with other cells and the general circulation through a network of tunnels located in the bony matrix, which are called canaliculi. Osteocytes play several roles in body homeostasis, including maintaining normal levels of serum calcium and phosphorus.

Bone tissue also contains osteoclasts, which are multinucleated cells that are derived from the hematopoietic (granulocyte–monocyte) cell line located in bone marrow. Osteoclasts are primarily responsible for bone resorption and they secrete enzymes and hydrochloric acid that break down collagen matrix and help dissolve the bone. The area where osteoclast cell membrane lies adjacent to bony tissue is known as a Howship's lacunae. The osteoclast cell membrane that lies in close proximity to bone can contain numerous villous extensions and form a ruffled border. These areas are also known as resorption pits. The plasmalemma border of the osteoclast cell in this region forms a specialized seal with the underlying bone to prevent the release of enzymes and hydrochloric acid. This process also results in the release of growth factors previously deposited in bone by osteoblasts, which are responsible for maintaining the process of regenerating new bone.

Bone is developed by two methods. Membranous development involves bone formation directly from cartilaginous tissue. Osteoblasts directly deposit calcium and other mineral in this mesenchymal-derived tissue. The skull and portions of the clavicles (collar bone) are formed by this method and, at birth, portions of the membrane persist in the skull and are referred to as 'soft spots'.

The other method of bone formation involves endochondral ossification and is not completed until the 18th year or later. The long bones of the limbs are formed by endochondral ossification. Mesenchymal-derived cartilaginous tissue, which is formed during early fetal development, contains chondrocytes and provides a model for future bone. By the eighth week of gestation, this cartilaginous tissue undergoes a series of changes, which initiates the process of bone

formation. The center of this cartilage undergoes degradative changes that involve mineralization and later resorption by osteoclast-type cells. This process moves up and down the cartilaginous tissue and is accompanied by the ingrowth of blood vessels and osteoprogenitor cells, which will become new bone-forming cells. The remnants of the mineralized cartilage, also known as the primary spongiosa, serve as a framework for new bone deposition. Similar changes occur in the epiphyses of the bone. These changes produce an area of cartilage that lies between two centers of bone formation which is known as the growth plate.

The growth plate chondrocytes undergo proliferation, growth, degradation, mineralization, and resorption and provide the support structure for new bone formation. Bones can increase in length and width through this process. Endochondral ossification occurs near the base of the articular cartilage at the joints.

Bone undergoes continual remodeling through bone resorption and formation. The balance between formation and resorption determines the mass of bone during growth of the skeleton. In childhood, bone formation predominates. Peak bone mass is reached in early adulthood. During adulthood, ~10–15% of the skeletal mass undergoes remodeling and resorption yearly and this process remains balanced. The amount of resorbed bone starts to exceed the amount of newly deposited bone by the third or fourth decade.

The osteoblast and osteoclast can be considered to be the basic multicellular units of bone. The osteoblast plays an important role in mediating local osteoclast activity through the release of chemical messengers. The principal factors responsible for stimulation of bone resorption, such as parathyroid hormone, interleukin-1 (Il-1), and IL-6, have minimal effects on osteoclasts, but osteoblasts have receptors for these substances.

Increased resorption of bone relative to new bone formation leads to osteoporosis. Osteoporosis is characterized by a net reduction in the mass of bone with no significant decrease in the ratio of mineral components to organic matrix. The bone can be thought of as having increased porosity. Osteoporosis starts to occur in both sexes at the age of 46–50 years. Trabecular bone loss probably occurs earlier. On an average 0.7% of bone is lost on an annual basis. Bone loss accompanies aging for several reasons. Aging is associated with decreased activity of osteoprogenitor cells, decreased synthetic capability of osteoblasts, and the lessened biologic activity of growth factors contained in the organic bone matrix. Diminished physical activity associated with aging acts to reduce bone growth since exercise acts to stimulate the new bone formation. Bone loss occurs rapidly in astronauts who are in a weightless environment, with bed rest, or with the immobilization or paralysis of an extremity. Bone growth is stimulated by skeletal loading and muscle contraction associated with resistive exercises such as weight training.

Postmenopausal women are vulnerable to osteoporosis, which largely involves trabecular bones including the spinal vertebrae. Estrogen deficiency plays a major role since estrogen replacement reduces the rate of bone loss. The mechanism for this effect has not been fully characterized but decreased estrogen resulted in increased IL-1 secretion from blood monocytes. IL-1 stimulates osteoclastic activity and bone resorption. Other risk factors include excessive alcohol consumption and smoking.

Bone is a target tissue for several xenobiotics. Several metals can effect the development of bone. Radiographs of bone in children with significant lead exposure can reveal lead lines, which are areas of increased bone density in the metaphyseal bone region. Lead lines are characteristically seen in rapidly growing tubular bones including the distal femur and proximal tibula and fibula (knee joint), but the vertebral bodies and iliac wing can also be affected. This effect has been attributed to the action of lead on the remodeling of calcified cartilage in the zone of provisional calcification of the metaphyseal bone. Bismuth and yellow phosphorus can also produce similar metaphyseal bands.

Chronic ingestion of high concentrations of fluoride can produce fluorosis, whose clinical picture can include osteosclerosis. Osteosclerosis is a painful condition characterized by an increased density in the bones. This is thought to occur because hydroxyapatite is replaced with fluorapatite. Fluoride also accumulates in ligaments where X-rays can demonstrate increased bone density; mineral deposits in ligaments, tendons, and muscles; and periosteal outgrowths. Osteosclerotic changes have been observed among aluminum workers with fluoride exposures and among persons with prolonged use of water containing high concentrations of fluoride.

Hypervitaminosis A and D have also been associated with bone abnormalities. Vitamin D can cause resorption of calcium from bone. Chronic vitamin D intoxication may result in increased mineralization on bone and metastatic calcifications including joints, periarticular, and the kidney. Excessive vitamin D intake can cause demineralization of bone resulting in multiple fractures from very slight trauma.

Osteomalacia and likely osteoporosis among Japanese woman has been linked with ingestion of

cadmium-contaminated food, including shellfish. This painful condition, known as 'itai-itai byo' (ouch-ouch disease), has occurred primarily in postmenopausal multiparous women. Chronic exposure to cadmium has been associated with microfractures, osteomalacia, radiological decreases in bone density, and disturbances in calcium metabolism. One possible mechanism to account for this finding is increased serum parathyroid hormone and decreased serum vitamin D levels from cadmium-induced renal damage.

The mineral structure of bone also incorporates metals and metalloids that resemble calcium, including lead and a variety of elements some isotopes of which emit alpha radiation, including strontium-90, uranium-235, and plutonium-239. Bone acts as an important storage depot for these elements and the high local concentration in bone is responsible for the high risk of bone marrow effects and of bone cancers from α-emitting radionuclides.

Skeletal Muscle

Skeletal muscle is a major component of body tissue and accounts for 40–50% of the body weight. Skeletal tissue is composed of specialized striated cells, which function to convert chemical energy to mechanical work. Skeletal muscle plays a central role in body metabolism and serves as a source of body heat and a storage depot for energy-rich compounds, protein, and intracellular ions (e.g., potassium). It also contains up to 80% of the body water content. In contrast to cardiac and smooth visceral muscle tissue, skeletal muscle is under voluntary control.

Skeletal muscle is composed of individual muscle fibers or cells that are contained in connective tissue. The muscle fibers are composed of hundreds or thousands of myoblasts. Muscle fibers are consequently multinucleated cells that have lengths of up to 10 cm and diameters ranging from 10 to 100 μm. They seldom are as long as the length of muscle which they compose and form interlocking irregular polygons. The size of muscle fiber is influenced by several factors. Proximal large muscles have large-diameter fibers, while smaller distal muscles contain more smaller diameter fibers. Physical activity can increase the size of muscle fibers in both sexes, although men of comparable age have larger fibers than woman. Children have smaller fibers.

Striated skeletal muscle fibers are bound together by collagenous connective tissue to form individual muscles. The connective tissue covering a muscle is known as the epimysium. This forms a resilient elastic sheath covering that separates the muscle from surrounding structures such as tendons and bone. The connective tissue extends into muscle fibers and separates groups of individual muscle fibers or fasiculi. This connective tissue is known as the perimysium. Each muscle cell is surrounded by connective tissue known as the endomysium. This collagenous membrane combined with the adjacent muscle cell membrane is termed the sarcolemma. This tissue serves to maintain a framework for striated muscle cells. As long as the connective tissue remains intact, skeletal muscle can regenerate following injury and grow in the pattern provided by this connective tissue.

The muscle cell membrane is termed the plasmalemma. The cytoplasm of the muscle cell is filled with myofilaments, which form the myofibrils. Myofibrils are composed of sarcomeres, which consist of longitudinally directed thin and thick filaments and perpendicularly disposed z bands that are α-actin filaments. The myofibrils form the contractile apparatus of the muscle.

The sarcolemmal membrane has invaginations which run parallel to the z bands. These invaginations are also known as the T system and are involved in the release of calcium into the cell. The release of calcium leads to a contraction of the myofibrils. Sarcoplasm accounts for $\sim$40% of the volume of the fiber and contains glycogen, mitochondria, and lipid vacuoles.

There are two principal types of skeletal muscle fibers in humans: type 1 and type 2 fibers. They can be thought of as corresponding to red and white muscle. Type 1 fibers, or dark fibers, have more myoglobin and have the capacity for maintaining sustained force and weight bearing. They contain large numbers of mitochondria and maintain activity through sustained aerobic glycolysis. Type 2 fibers, or white fibers, are important in performing sudden and rapid movements. They have abundant glycogen but scant mitochondria and are not able to maintain sustained activity because they accumulate lactic acid. Strength training increases the number and size of type 2 fibers. Aerobic training involves hypertrophy of type 1 fibers.

A number of pathological processes can affect skeletal muscle. Since individual muscle fiber is formed by numerous myoblasts, any injury and pathological changes may only affect a small part of a muscle fiber. This has clinical significance since biopsy of a small segment of muscle may provide a nonrepresentative sample of muscle for assessment of a myopathy. Handling of specimens can be difficult and lead to artefactual lesions from fractures rising from the processing of the muscle. In general, the reactions of muscle are not specific to any disease or toxic agent.

Skeletal muscle can undergo atrophy due to several factors. Loss of innervation from anterior horn cell or a peripheral neuropathy can affect type 1 and type 2 fibers. This is characterized by a diminution of synthesis of myosin and actin and a decrease in size and resorption of the myofibrils; the cells, however, remain viable. Type 2 fiber atrophy can occur in several types of situations including inflammatory disorders involving muscle (e.g., polymyositis and polymyalgia rheumatica), metabolic disorders, corticosteriod myopathy, Cushings disease, and general cachectic states. Disuse of the muscle associated with inactivity (such as the placement of a cast) can lead to atrophy of muscle fibers, especially type 2 fibers. The cellular mechanism of the atrophy is poorly understood but could involve an increase in the rate of protein degradation, a reduction in protein synthesis, or a combination of both factors.

Hypertrophy, an increase in size of muscle cells, can occur in response to several situations. This is largely caused by an increase in the number of myofibrils. Hypertrophy is seen in more slowly progressive muscular dystrophies, a heterogeneous group of inherited muscle disorders (e.g., Becker's muscular dystrophy). Clinically they result in muscle wasting and weakness. Becker's muscular dystrophy is an X-linked disorder. Endocrine disorders including hypothyroidism and an increase in growth hormone or acromegaly can also be associated with hypertrophy.

Necrosis is the other principal muscular pathological process that muscle fibers can undergo. Usually the entire muscle fiber does not undergo necrosis. Segmental necrosis is the term used to describe necrosis confined to a segment of variable length of fiber rather then the entire fiber. The clinical spectrum of persons with systemic necrotizing myopathy typically includes proximal muscle limb weakness and elevated serum creatine kinase. Myoglobinuria can sometimes also be observed.

There are several potential causes for segmental necrosis of muscle fiber. The cause of this type of necrosis is not well understood but could be due to effects on the plasma membrane or the outer boundary of the muscle fiber. Aminocaproic acid (an antifibrinolytic medication used in the treatment of a subarachnoid hemorrhage), clofibrate (used to treat hyperlipedemia), emetine (found in ipecac syrup), cardiac glycosides, heroin, and phencyclidine have been associated with necrotizing myopathies.

Select drugs injected by the intramuscular route can produce focal necrotizing myopathic changes. Paraldehyde, chlorpromazine, and a number of antibiotics have produced this type of reaction.

Interference with homeostasis of mitochondrial DNA has been linked to segmental necrosis. Medications associated with this type of effect include zidovidine, which is used to treat HIV. Electron microscopic findings include marked increases in mitochondrial enlargement with vacuolation. Chemicals that block aerobic metabolism (e.g., 2,4-dinitrophenol) have been used to experimentally produce a mitochondrial myopathy characterized in some instances by segmental necrosis.

Several drugs that share the chemical property of being a large cationic amhiphillic molecule that has a hydrophobic and hydrophilic region with a primary or substituted amine group with a net positive charge have been shown to produce a necrotizing myopathy. The mechanism of action is that these drugs interfere with lysosomal digestion and lead to autophagic degeneration and accumulation of phospholipds. Autophagic membrane-bound vacuoles containing membranous debris and curvilinear bodies with short, curved membrane structures with light and dark areas are seen. Drugs in this class include chloproquiin, vincristine, colchicine, and amiodarone.

Corticosteroids have produced necrotizing muscle changes. Severity is variable and not always associated with the steroid level or therapeutic regimen but is most likely to occur in persons taking over 40 mg of prednisone per day. Pathologically, degeneration of type 2 fibers is often seen.

Myopathies can also occur as a result of secondary effects. Hypokalemia has been associated with myopathy. Myopathy has been observed in persons consuming large quantities of licorice extract, in persons taking diuretic and some other medications, and in persons with purgative abuse.

Myopathies can be associated with immunologically based reactions that have features of polymyositis or dermatomyosotis. The clinical features of eosinophylia–myalgia syndrome include the abrupt onset of muscle pain. This syndrome was thought to be due to a contaminant in l-tryptophan introduced by the manufacturing process. Certain medications are associated with necrotizing myositis, which has similar features. Acute rhabdomyolysis is a severe form of necrotizing myopathy.

See also: Blood; Cadmium; Eosinophilia–Myalgia Syndrome; Lead; Radiation Toxicology, Ionizing and Nonionizing; Tissue Repair; Vitamin D.

Skin

Peter Robinson

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Introduction

Skin is the largest organ in the body. As the primary interface between the body and its external environment, it serves as a living, protective envelope that prevents the entry of foreign chemicals and microbes, as well as preventing the evaporative loss of body fluids and heat. Although skin is an effective barrier, it is not a complete one in that it is becoming increasingly apparent that skin is an important portal of entry for chemicals into the body. Not all chemicals penetrate equally well, however, and an important part of the study of the toxicology of the skin is to understand and predict the differential penetration of potentially toxic materials into the skin, and through the skin into the general systemic circulation. We need to understand how the structure of the skin interacts with different chemical species in order to limit their entry into the body. We also need to understand how the skin itself reacts to the presence of toxic chemicals, and how this manifests itself in terms of various familiar local skin reactions.

Not long ago, studies in skin toxicology were primarily concerned with developing methods to produce and evaluate irritation and allergic reaction in both animal and human skin. However, significant recent advances in tissue culture techniques, cellular and molecular biology, and the understanding of toxicokinetic principles have enormously expanded our horizons in studies of skin function and toxicity, and we are just beginning to appreciate some of the more novel, but important, biochemical, physiological, and metabolic capabilities of this organ.

The purpose of this entry is to provide a general overview of cutaneous toxicology. Current knowledge of the etiology and mechanisms of skin toxicity will be summarized and some of the more obvious and typical skin responses to toxic insults will be described. Furthermore, current concepts regarding skin absorption and metabolism will be discussed and, together, it is hoped that a review of these topics will provide a better understanding of the toxicology of the skin.

We will not be discussing dermal exposure in any great detail, although this is, of course, crucial when incorporating the principles of dermal toxicity outlined here into an overall assessment of the hazards and risks of chemicals in the environment or in the workplace. In many cases, in fact, the uncertainties associated with quantifying dermal exposure may drive the overall uncertainty in the risk assessment. For example, exposure to chemicals in the soil may be a common pathway for many environmental contaminants, particularly for children and agricultural workers, but the contact of soil with the skin, both in terms of the overall amount and the exposed surface area is very difficult to quantify. Added to that, the availability of chemicals adsorbed to the soil particles for dermal absorption in the complex environment of the skin surface is also fraught with uncertainty. Similar situations exist for dermal exposure of chemicals from consumer products, household surfaces, fabrics, etc. These limitations should be borne in mind in what follows.

Skin Structure and Function

Mammalian skin can be described as a multilayer heterogeneous organ that forms the external covering of the body. It is the largest organ in the body and is continuous with the lining of orifices that open onto the body surface. In an adult man, the skin has a total surface area of $\sim 2\,m^2$ and in most places it is no more than 2 mm in thickness, yet it can account for 10–20% of total body weight. The basic structure of mammalian skin can be divided into three main components: (1) a superficial lining of epithelial cells, the epidermis, supported by (2) a subepithelial connective tissue stroma and vasculature, the dermis, which in turn is supported on (3) a layer of subcutaneous fat of varying thickness, called the hypodermis. Impregnated within the epidermis and dermis are specialized 'adnexa', which include hair follicles, sebaceous glands, sweat glands, and a complex neural network (**Figure 1**).

The epidermis, which develops from the embryonic ectoderm, comprises ~5% of full-thickness skin by weight. It is avascular and is composed primarily of keratinocytes. Based mostly on structural criteria, the epidermis can be subdivided into several layers. The basal layer consists of germinative cells, which retain the capability to undergo cell division and are extremely metabolically active. The daughter cells from the dividing basal layers migrate upward and undergo terminal differentiation to form the next two viable cell layers of the epidermis, the spinus and granular cell layers. During this process, the keratinocytes become flatter and lose many of their cytoplasmic organelles. Their nuclei condense and this is

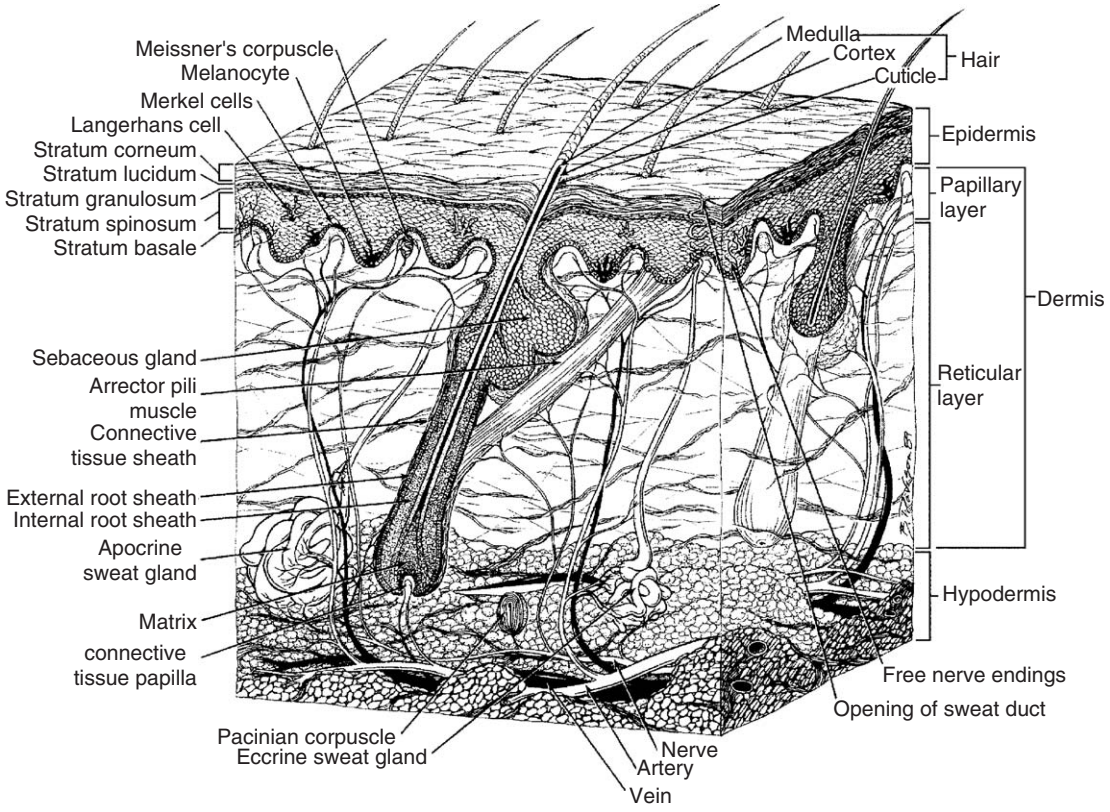

Figure 1 A composite representation of the structure of the integument found in typical skin in various regions of the body. (Reproduced from Hobson DW (ed.) (1991) *Dermal and Ocular Toxicology: Fundamentals and Methods*. Boca Raton, FL: CRC Press, with permission from CRC Press.)

accompanied by the appearance of granules that ultimately form keratin filaments. The endproduct of this terminal differentiation process is the strateum corneum, the outermost layer of the epidermis. The cells of this layer are essentially flat, anucleuated, and devoid of any metabolic activity. These cells are eventually sloughed off to be replaced by terminally differentiating cells from the basal layer. This process of differentiation and outward migration in the epidermis is continuous. The average turnover time varies greatly from species to species: in humans, it has been estimated to be about 28 days, but there is considerable variation, depending on anatomical site and disease state.

In addition to keratinocytes, the epidermis contains several 'dendritic' cell types. Langerhan's cells, which account for $\sim$5–10% of all cells found in the epidermis, are bone marrow mesenchyme-derived cells. These cells are involved in antigen recognition and processing during induction of immune responses in skin. Melanocytes are melanin-synthesizing cells of neural crest origin. These cells are found adjacent to the basal cells and supply them with melanin, the principal pigment in skin, localized in specialized organelles, called melasomes. Merkel cells are the third type of dendritic cell found in the epidermis. They are of neuroectoderm origin and are believed to have a neuroendocrine function in the skin.

The basal lamina, with its characteristic ridge-shaped appearance, forms the epidermal–dermal junction and is the interphase that separates the epidermis from the underlying dermis. The dermis, which originates from the embryonic endoderm, consists of connective tissues and covers the internal organs of the body in a strong, flexible envelope. This envelope can be divided into two anatomical layers – the thin outer papillary layer and the thick inner reticular layer. The papillary layer consists of loose connective tissue, whereas dense connective tissue is found in the reticular layer. The elasticity and strength of this envelope are due to the constituent materials forming the dermal matrix. This matrix contains fibrous proteins, such as collagens, elastin, and reticulin, which are embedded in an amorphous material known as the ground substance, consisting of proteins and the glycosaminoglycans chondroitin A sulfate and hyaluronic acid. Fibroblasts are the most important and most numerous cell types found in the dermis. They are motile, capable of mitosis, and are responsible for the synthesis and secretion of

the proteins and fibers that make up the bulk of connective tissue. In addition to fibroblasts, the dermis also contains a variety of cells scattered throughout this tissue, including macrophages, lymphocytes, adipocytes, and mast cells. The mast cells are of special interest and are most numerous in areas adjacent to skin appendages (hair follicles), blood vessels, and nerves. Their function in skin homeostatis is unclear; however, it appears mast cells are involved in the pathogenesis of some inflammatory conditions. They are generally indistinguishable from fibroblasts, except they have special intracellular granules containing histamine, heparin, and other vasoactive agents that are released in response to certain chemical irritants.

Within the dermis, there are a number of epithelial structures, known collectively as cutaneous adnexa or epidermal appendages. They are all various types of extensions of modified epidermal cell structures into the dermis. The pilosebaceous units are located over the entire surface of the body, consisting of hair follicles and their associated sebacous glands, together with the accompanying arrector pili muscle, capillary plexus, and nerve fibers. The hair follicles are composed of three layers: the inner root sheath of keratinized cells, which form the hair shaft; the outer root sheath, which is a continuation of the epidermis; and the connective tissue layer. This latter layer merges with the papillary layer of the dermis and is continuous with the dermal papilla, located at the base of the hair follicles. The dermal papilla contains the germinative epithelial cells that give rise to hair proper through a complex process of growth, differentiation, and regression. Hair follicles undergo a continuous cycle of growth, called anagen, where new hair shafts are formed, followed by a short period known as catagen, where mitotic activity of the germinative cells in the dermal papilla ceases and the papilla atrophies. At telogen, the resting phase of hair cycle, the hair produced during anagen remains anchored to the skin, and at the next anagen phase, the newly synthesized hair replaces this hair. The sebaceous glands develop from the infundibulum of the hair follicle and are found surrounding the hair follicles. They contain differentiating cells that are active in lipid synthesis. The lipids, which form droplets in the fully differentiated cells, are released into the sebaceous ducts and onto the surface of the skin as sebum. This release of sebum is accompanied by the total disintegration of the lipid-laden cells. Sebum, a complex mixture of lipids, has antibacterial and waterproofing functions on the skin's surface.

Eccrine and apocrine glands, the sweat glands, are distributed throughout the skin. They are situated deep within the dermis and are simple coiled tubular structures composed of a long coiled secretory tubule and a long connecting excretory duct that traverses the epidermis and opens directly onto the surface of the skin. The eccrine glands are responsible for producing and secreting aqueous sweat (i.e., water and salts) and participate in thermoregulation. The apocrine glands secrete a viscous material containing proteins, pheromones, sugars, and ammonia. Their function in humans is unclear, but in animals they are believed to be associated with communication, probably acting in a sex-attractant or territorial marker role. The mammary glands are merely enlarged and modified apocrine glands. In man, the apocrine glands are found only in specific body regions, such as the axilla, the areola, the pubis, the perianal region, the eyelids, and the external auditory meatus. They secrete odorless secretions that are decomposed by surface bacteria to form characteristic odiferous products. Due to the combined secretions of the sweat glands and the sebaceous glands, the outer surface of the skin is generally always coated by an acidic film (pH 4–6), composed of various lipids, including triglycerides, phospholipids, and esterified cholesterol, together with salts and water. This film is frequently colonized by certain species of bacteria (e.g., *Micrococciae* and *Corynebacterium*) and the overall composition of this film may vary, depending on such factors as the disease state of the skin or occlusion. Any changes in the composition of the film will have dramatic effects on the makeup of the microflora present on the skin surface.

The dermis is separated from the underlying fiscia of muscle by the subcutis. The subcutis is a layer of adipose tissue of varying thickness, which, in humans, depends on the body region, sex, age, and nutritional status. The extensive dermal vasculature arises from the subcutis and consists of networks of vascular plexuses that are found in the transitional zone of the dermis and subcutis, adjacent to and surrounding the adnexa (eccrine sweat glands, hair follicles, and sebaceous glands). Arterioles branch out from these areas, forming anastomoses generally immediately under the epidermis, in both the reticular dermis and the papillary dermis. The dermal blood supply is usually substantially greater than that required merely to nourish the skin and skin blood flow can vary by orders of magnitude; thus, the dermal vasculature has an additional role in thermoregulation by controlling the dissipation of heat to the body surface.

Intertwined with the dermal vasculature is a complex network of nerve plexuses consisting of both encapsulated and free nerve endings. These sensory and sensorimotor nerves ramify throughout the skin

and are of extreme importance in the perception sensory stimuli. The skin also has a plexuses of lymphatics that drains into the regional lymph nodes.

This is a generalized and simplified description of mammalian skin. The basic architecture of skin is similar in all mammals. However, there are substantial species differences for aspects such as thickness, blood supply, and types or amounts of the various adnexa, as well as regional differences within each species. The most obvious species difference is hair follicle density in the skin. In lower mammals (rodents, dogs, cats, etc.), hair density is relatively high, whereas hair coat covering in humans and pigs is typically rather sparse. Skin thickness is another parameter that shows extensive species and regional differences, varying from a few micrometers to several millimeters thick (**Table 1**). Furthermore, in humans, the predominant sweat gland is the eccrine sweat gland, which opens directly onto the surface of the skin, whereas in animals the apocrine sweat glands, emptying into hair follicles, predominate. In general, it can be said that the density and distribution of the adnexal structures provide the basis for species differences in skin structure and function while also contributing to the regional differences of skin anatomy noted for each particular species. Such species differences are important considerations when using experimental animals in percutaneous penetration experiments, either *in vivo* or *in vivo*, and extrapolating the results to man.

Table 1 Comparative epidermal thicknesses

Species	*Epidermis* (mm)	*Stratum corneum* (mm)
Mouse	9.7±2.3[a]	3.0±0.3
Rat	11.6±1.0	4.6±0.6
Rat	32.1±1.3[b]	18.4±0.5
Rabbit	15.1±1.4	4.9±0.8
Monkey	17.1±2.2	5.3±0.4
Dog	22.5±2.4	8.6±1.9
Cat	23.4±9.9	4.3±1.0
Cow	27.4±2.6	8.1±0.6
Horse	29.1±5.0	7.0±1.1
Pig	46.8±2.0	14.9±1.9
Pig	65.8±1.8[c]	26.4±0.4
Human	46.9±2.3[d]	16.8±0.7
Human	(60–120)[e]	(20–25)

[a] Mean±s.e. ($n=6$); histologically determined in skin from the ventral abdomen.
[b] Mean±s.e. ($n=9$); histologically determined in skin from the back.
[c] Mean±s.e. ($n=35$); histologically determined in skin from the back.
[d] Mean±s.e. ($n=16$); histologically determined in skin from ventral abdomen.
[e] Range; histologically determined using skin taken from the ankle.

Percutaneous Absorption

General Concepts

Mechanisms of Percutaneous Absorption In order to understand drug and chemical toxicity in the skin, the process whereby the various responsive cell types within skin are exposed to these agents must be examined. Percutaneous, or 'via the skin', absorption may be defined as the translocation of surface-applied agents through the various layers of the skin to a location where they can enter systemic circulation via the dermal microvasculature and lymphatics or remain in the deeper layers of the skin. Based on our current knowledge, the important steps involved in skin absorption have been identified as the partitioning of the compound from the delivery vehicle to the stratum corneum, transport through the stratum corneum, partitioning from the lipophilic stratum corneum into the more aqueous viable epidermis, transport across the epidermis, and uptake by the cutaneous microvasculature with subsequent systemic distribution. This process, therefore, is the sum of the penetration and permeation of a chemical into and through the different strata of the skin.

Assessment of this process following topical application of drugs and environmental chemicals is becoming an increasingly important aspect of both toxicological and pharmaceutical investigations. Relative to toxicology, the ultimate aims of skin absorption studies are to identify and quantify the potential cutaneous toxicity, estimate the relative risk, and develop the appropriate strategies to minimize this risk resulting from topical exposure. In contrast, percutaneous absorption studies in transdermal delivery are designed primarily to assess and manipulate the rates of transport of drugs across the skin and ultimately to determine if such rates are sufficient to achieve the desired exposure and provide optimal therapeutic response. The aim is to identify or design the therapeutic compound and its vehicle and/or its delivery system with the appropriate properties for commercial development. Closely associated with these studies are investigations that are designed to assess the cutaneous and systemic bioavailability and bioequivalence of the compounds under development. Thus, depending on one's perspective, the focus of skin absorption studies may be to increase penetration or to reduce absorption.

The rate-limiting barrier to skin absorption is generally considered to be the outermost layer, the nonviable stratum corneum. Consequently, the skin is frequently thought of as a passive, inert barrier and percutaneous absorption of chemicals was thought to be dominated by laws of mass action and physical diffusion. This reduction of percutaneous absorption

to diffusion equations and mass transfer coefficients has overshadowed any considerations of the possible contribution of biochemical factors that may influence the percutaneous fate of topically applied substances. In general, the diffusional theories and the assumption that the skin is merely a physical barrier persist, despite the fact that the skin is an organ active in many essential biochemical and physiological functions. Moreover, for certain lipophilic chemicals, it is clear that the stratum corneum is no barrier at all. The lipid-rich stratum corneum and skin appendages may act as a reservoir for topically applied lipid materials, thus functioning more as a sponge, capable of absorbing a quantity of material that is limited only by the solubility of the substances in the sebaceous and intrinsic epidermal lipids. For such lipophilic chemicals the viable epidermal membrane may be the more important barrier.

Skin appendages, which include sebaceous glands, hair follicles, and sweat glands, are often regarded as channels that bypass the stratum corneum barrier as such, they are generally thought to facilitate the dermal absorption of topical agents. Because they occupy only a small fraction of the skin's surface area in humans (0.01–0.1%), their overall effect on the extent of percutaneous absorption will be minimal for most compounds. Moreover, it is often overlooked that these appendageal structures are not open pores through the skin, but are usually plugged with hair shafts, dead cells, sebum oils, stratum corneum lipids, and/or aqueous salt solutions (sweat). Thus, this pathway is probably only important immediately after a substance is applied to the skin as a rapid shunt. The bulk of skin absorption takes place via the diffusion processes described previously and later. However, the significance of this follicular pathway in skin absorption remains to be experimentally assessed. The correlation between permeation and hair density of the different rat skin phenotypes (haired, fuzzy, and hairless) tends to support the hypothesis that the transfollicular pathway may be the more dominant route for the skin absorption of certain highly lipophilic chemicals such as polycyclic aromatic hydrocarbons and coal tars. In addition, these appendages may be important for highly polar molecules, which generally penetrate the stratum corneum very slowly, if at all (see the discussion of the 'polar pathway' in the next section).

Systemic and Local Skin Effects: Exposure Considerations Even without exploring directly the mechanisms of dermal absorption, external dermal exposure has different implications for compounds that may produce systemic effects (such as cancer or toxic effects in remote tissues) and for those that act locally in the skin itself. In the latter category, contact sensitization and irritation are two areas of concern (as well as skin cancer). Whether one is considering local or systemic effects (together with the dermal penetration potential of the compound) has a profound impact on what measures of applied dose are most relevant to consider in an exposure analysis. A few highly simplified examples will make this clear.

First consider a freely or moderately penetrating compound applied to the skin in a leave-on formulation. If there is little loss via evaporation or rub-off, it is reasonable to assume 100% absorption in such cases. For systemic effects, the most relevant exposure parameter is the total applied dose, regardless of how it is applied (all of which is absorbed and becomes available to the systemic circulation). On the other hand, if a local skin effect is indicated, the relevant parameter is likely to be the local concentration in the skin itself, or some other measure of local skin exposure. This is more likely to be related to the applied dose per unit area of the skin, rather than the total applied dose. In broad terms, this is the reason why the 'dose per unit area' is crucial in the elicitation of contact sensitization responses to allergens.

Now consider a poorly penetrating dermally applied compound, or one that is applied for such a short time T that only a small fraction of the applied dose is absorbed. In this case, it is the concentration c of the material in the formulation (as well as other factors that determine the flux across the stratum corneum, such as the dermal penetration coefficient K_p – see below), rather than the total applied amount of material, that determines the amount absorbed both into and through the skin. In simplest terms, the total amount Q absorbed into the systemic circulation is given by the equation:

$$Q\ (\text{mg}) = K_p\ (\text{cm h}^{-1}) \times c\ (\text{mg ml}^{-1}) \times T\ (\text{h}) \times A\ (\text{cm}^2)$$

where A is the applied surface area of skin. In terms of external exposure, the important dose parameter in this case is the product $c \times A$. (This in turn is equivalent to the total dose D divided by the film thickness h). In other words, both the applied concentration and the exposed surface area are relevant for systemic effects. Unlike the case of a rapidly penetrating compound, the total dose alone is not sufficient to characterize exposure: also needed is an additional parameter (such as the film thickness) that indicates how the dose is distributed over the skin surface. In addition, the amount absorbed also depends on the exposure time T.

The case is again different for local skin effects. In this case, it is the amount absorbed per unit area of skin that again is most relevant for local effects. From the equation given above, $Q/A = K_p \times c \times T$. Thus, the most relevant exposure parameter is the applied concentration c (as well as the exposure time T). In this case, at least to a first approximation, how the material is distributed over the skin surface is not as important as the nature of the material itself (i.e., its concentration).

Thus, to a first approximation, the most relevant exposure parameters for a specific situation is given in **Table 2**.

Mathematical Models Diffusion principles have been traditionally recognized as the most important determinants in skin absorption. Thus, Fick's law of diffusion provided the mathematical basis for early kinetic descriptions of percutaneous absorption. Fick's law simply states that

$$J_s = K_p \Delta c_s$$

where J_s is net flux of substance s ($\mu g\,cm^{-2}\,h^{-1}$), K_p is the permeability constant ($cm\,h^{-1}$), and Δc_s is the concentration gradient of s across the diffusion membrane ($\mu g\,cm^{-3}$). The validity of applying mass diffusion principles to skin absorption rests on at least two main assumptions. First, penetration of chemicals into and permeation within the various layers of skin are passive diffusional processes. Second, the diffusional resistance of the skin layers can be formulated into one or more equations describing diffusion of small particles or molecules across thin layers or barriers, and the layers or barriers must simulate as closely as possible the behavior of dilute polymeric solutions.

Because diffusional resistance of the outermost region of skin, that is, the stratum corneum, is generally far greater than that of other cutaneous substructures, models of skin absorption are frequently simplified to involve diffusion across only one layer, the stratum corneum. This equation forms the simplest mathematical framework describing many percutaneous absorption investigations.

The dermal penetration coefficient K_p in this simplest case depends on both the partitioning of the chemical from its vehicle (usually water) into the stratum corneum, and its diffusion through the stratum corneum. Both of these quantities can be estimated from a chemical's properties or structure. Partitioning from water into the stratum corneum can be estimated from a chemical's octanol–water partition coefficient, K_{ow}. Diffusion through the stratum corneum is dependent on the molecular volume of the chemical, which is in turn a function of its molecular weight (MW). Perhaps the most widely used expression of the dependence of stratum corneum permeability on readily available physicochemical properties is the 'Potts-Guy' equation:

$$\log K_{sc} = -2.72 + 0.71 \log K_{ow} - 0.0061\ MW$$

in which the parameters have been fitted to an empirical dataset of (usually *in vitro*) measured stratum corneum penetration coefficients K_{sc} from aqueous vehicle.

Unfortunately, percutaneous absorption is in reality a complex phenomenon involving a myriad of diffusional and metabolic processes that are proceeding either concurrently or sequentially. Consequently, theoretical models describing the overall process will be approximations and will reflect our current knowledge concerning the most relevant events. Neglecting cutaneous metabolism for the moment, but going beyond the simple stratum corneum model, the skin may be modeled as a number of serial and parallel barriers and pathways.

For compounds that have either a very low diffusion coefficient or a very low lipid–water partition coefficient, the lipid barrier of the stratum corneum is a formidable impediment to penetration through the skin. However, for such compounds it has been observed that there is no longer a correlation between skin permeation and lipid solubility; further, there also appears to be little dependence on molecular weight. It has therefore been hypothesized that such compounds make use of an alternative, low-permeability, and essentially aqueous pathway through the stratum corneum. Although direct physical evidence for such pores is lacking, the notion of a

Table 2 Relevant applied doses for systemic and local effects for rapidly and poorly penetrating materials (K_p is the dermal penetration coefficient)

	Rapidly penetrating compound	*Poorly penetrating compound*
Local skin effects predominate	Total applied dose (mg)	Concentration × surface area × exposure time (× K_p)
Systemic effects predominate	Dose per unit area of exposed skin ($mg\,cm^{-2}$)	Concentration × exposure time (× K_p)

different functional pathway (with a different (and minimal) functional dependence on K_{ow} and on MW) for such compounds is important. Estimates of the permeability coefficient K_{pol} of such a pathway for neutral molecules in human skin range from about (1 to 10) $\times 10^{-6}$ cm h^{-1}; taking into account a (weak) molecular weight dependence based on diffusion through a liquid, we obtain the following expression as representative of K_{pol} for healthy human skin:

$$K_{pol} \sim 1 \times 10^{-6}(300/MW)^{1/2}(\text{cm h}^{-1})$$

Note that as lipid solubility increases, the relative contribution of K_{pol} to overall passage through the stratum corneum progressively decreases.

Additional barriers include the viable epidermis and dermis, which can together be considered to constitute an aqueous layer beneath the stratum corneum, which may act as a significant barrier to the passage of more lipophilic materials from the surface of the skin to the systemic circulation. Finally, once they have penetrated sufficiently deeply into the skin, materials must also actually enter the circulation itself by passing through the capillary wall and partitioning into the blood (often with the help of plasma protein binding).

A schematic representation of the multicomponent structure of the skin that takes these multiple barriers/pathways into account is shown in **Figure 2**. When penetration through the stratum corneum (including the parallel polar pathways) and through the aqueous layers in series with the stratum corneum are taken into account and combined with clearance into the bloodstream, the overall dermal penetration coefficient K_p (cm h^{-1}) for such a (simplified) composite system is given (by analogy with electrical resistances in series and in parallel) by

$$K_p = \frac{1}{1/(K_{sc} + K_{pol}) + 1/K_{aq} + 1/K_{cap}}$$

where K_{sc} represents the penetration coefficient for the stratum corneum; K_{pol} represents that of the polar pathway in parallel with the stratum corneum; K_{aq} represents the permeability of the aqueous layer of the viable dermis, in series with the stratum corneum; and K_{cap} represents the effective penetration coefficient for clearance into the vasculature via the capillary network of the skin (in series with the stratum corneum and the aqueous layer). Note that K_{cap} will in principle depend on the rate of blood flow through the capillary bed of the skin. All four K parameters have units of, for example, cm h^{-1}.

As the lipophilicity of compounds continues to increase (as estimated by their octanol–water partition coefficients), dermal penetration does not increase indefinitely. In fact, it is limited by a number of factors. As we have seen above, penetration into the systemic circulation for lipophilic compounds is limited by their very modest capacities to penetrate the aqueous layers of the skin (dermis and viable epidermis), as well as their relative reluctance to enter the bloodstream. These factors set a natural upper limit to the dermal penetration coefficients for very lipophilic compounds of $\sim 10^{-1}$ cm h^{-1}. In many cases, however, it is not the penetration coefficient itself in which we are interested, but rather the flux of a compound through the skin. This flux is the product of a penetration coefficient and a driving concentration. Since the aqueous layers of the skin are the major impediment for lipophilic compounds, solubility in water plays a major role in determining the maximum flux of such compounds through the skin. Like other parameters we have

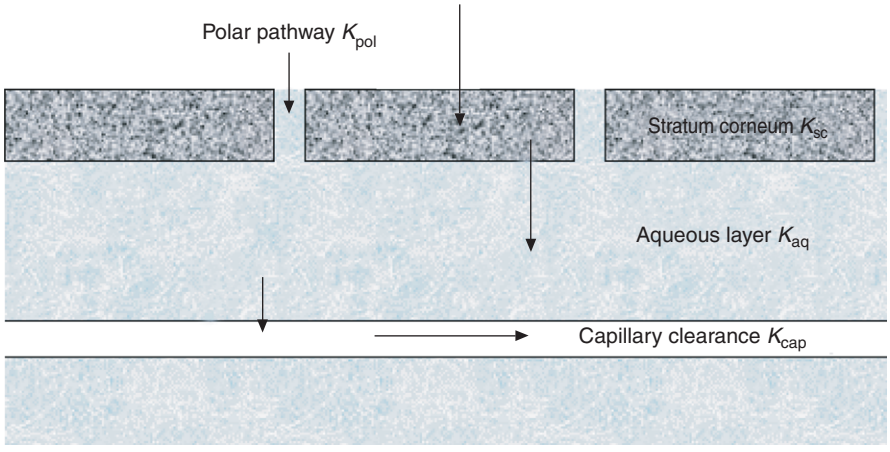

Figure 2 Schematic representation of a composite model of the skin.

been considering, water solubility (WS) depends on the physicochemical properties of compounds, particularly the octanol–water partition coefficient and molecular weight:

$$\log \mathrm{WS} = (3.25 - \log K_{\mathrm{ow}}) \times \mathrm{MW}/1000 \ (\mathrm{mg\ cm^{-3}})$$

The maximum flux $J_{\max}$ is then given by the product $K_{\mathrm{p}} \times \mathrm{WS}$. The maximum flux estimated in this way is a very useful conservative estimate of dermal penetration, particularly when the applied concentration of a compound or the precise nature of its vehicle is not known. It also applies to exposures of neat materials (liquids).

Conceptual models of percutaneous absorption which are rigidly adherent to general solutions of Fick's equation are not always applicable to *in vivo* conditions, primarily because such models may not always be physiologically relevant. Linear kinetic models describing percutaneous absorption in terms of mathematical compartments that have approximate physical or anatomical correlates have been proposed. In these models, the various relevant events, including cutaneous metabolism, considered to be important in the overall process of skin absorption are characterized by first-order rate constants. The rate constants associated with diffusional events in the skin are assumed to be proportional to mass transfer parameters. Constants associated with the systemic distribution and elimination processes are estimated from pharmacokinetic parameters derived from plasma concentration–time profiles obtained following intravenous administration of the penetrant.

These linear kinetic models and diffusion models of skin absorption kinetics have a number of features in common; they are subject to similar constraints and have a similar theoretical basis. The kinetic models, however, are more versatile and are potentially powerful predictive tools used to simulate various aspects of percutaneous absorption. Techniques for simulating multiple-dose behavior; evaporation, cutaneous metabolism, microbial degradation, and other surface-loss processes; dermal risk assessment; transdermal drug delivery; and vehicle effects have all been described. Recently, more sophisticated approaches involving physiologically relevant perfusion-limited models for simulating skin absorption pharmacokinetics have been described. These advanced models provide the conceptual framework from which experiments may be designed to simultaneously assess the role of the cutaneous vasculature and cutaneous metabolism in percutaneous absorption.

Due to the deficiencies of current experimental and analytical methods, our ability to appreciate and fully utilize these sophisticated models is limited. As noted previously, the model parameters (e.g., rate constants) are usually based on assumed partitioning phenomena or kinetic behavior. These assumptions are limited by the paucity of kinetic information provided by current experimental methods. For example, little is known about how volatility affects absorption of the applied dose and the concept of mass-balance studies following topical doses has only recently been addressed. From the perspective of absorption, the skin is a portal of entry for a variety of topically applied chemicals, a drug-metabolizing organ, and a target organ for local toxicity. When skin contact with a chemical results in local effects, pathological changes in the skin may be expected to affect its barrier properties and, hence, influence the fate of surface-applied chemicals. The integrity of the stratum corneum is therefore of primary importance. However, biochemical changes in the skin in response to topical exposure to biologically active chemicals may also influence the metabolic capabilities and metabolic status of the skin and thereby modulate the cutaneous disposition of topically applied substances.

Thus, knowledge of the processes involved in the translocation of chemicals through the skin into systemic circulation and the response of the skin to such chemicals are important aspects of skin pharmacology and toxicology. Research in this area is in its infancy and offers many opportunities. Mechanistic and functional approaches to skin absorption need to be developed. **Table 3** presents a fairly comprehensive list of the known factors affecting percutaneous absorption of topically applied drugs and chemicals. It is anticipated that future research will increase our knowledge of skin absorption, and exploitation of such knowledge would greatly facilitate the continual development of new strategies in reducing the skin absorption of hazardous industrial chemicals. Also, it would provide the basis for improving topical therapy and the transdermal delivery of drugs and prodrugs.

Metabolic Fate of Topically Applied Substances It is now generally recognized that skin is an organ capable of performing a variety of metabolic functions, including those involved in the metabolism of hormones, carcinogens, drugs, and environmental chemicals. Since skin contains enzymes capable of metabolizing xenobiotics, any chemicals that are applied to the surface of the skin will, during the course of penetration and translocation through this organ, be exposed to available biotransformation systems that are present in the skin. Consequently, the ability of the skin to function as an organ of xenobiotic

Table 3 Factors affecting percutaneous absorption

Factor	*Specific examples or other contributing factors*
Vehicle	Suspensions, emulsions, lotions, creams, ointments, pastes, PEG and PPG, demulcents, emollients, pH
Solvents	Water, acetone, ethanol, methanol, chloroform, THF
Enhancers	Dimethylformamide (DMF), DMSO, dimethylacetamide, urea, azone, 2-pyrrolidone, surfactants
Species	Skin thickness, hair density, quantity and types of glands, cutaneous vasculature, and blood flow
Application site	Epidermal and stratum corneum thickness, keratinization, blood flow, hair follicles/glands, skin condition (dermatoses, damage, hydration state, occlusion, pH)
Environment	Ambient temperature and humidity, air flow
Dose applied	Surface area, concentration, contact time, vehicle
Physicochemical	Partition coefficients, molecular weight, particle size/shape, dissolution characteristics, absolute aqueous solubility
Miscellaneous	Humans (age, sex, race); metabolic capacity of the skin

metabolism is of considerable interest, and questions are raised concerning the functional significance of skin metabolism in the percutaneous fate of chemicals. Is skin metabolism important? Can cutaneous metabolism influence dermal absorption? What, if any, is the functional significance of skin metabolism in the cutaneous and systemic disposition of chemicals, and can it be an important determinant in the development of local and systemic toxicity? What are the modulating factors that may affect skin metabolism and, consequently, the disposition of topically applied chemicals? What are the implications of skin metabolism in dermatotoxicity and dermatopharmaceutics? Indeed, will the ability of the skin to metabolize drug prove to be a desirable advantage or a confounding factor that complicates the development of novel transcutaneous therapeutic devices? Perhaps more important, what conceptual and experimental approaches are readily available for use in evaluating the functional significance of skin metabolism on the percutaneous fate of topically applied chemicals?

Numerous investigations with tissue slices, isolated cell preparations, and subcellular fractions from skin have shown this organ to possess a variety of enzyme activities including those involved in the metabolism of xenobiotics. Although the biotransformation of only two classes of compounds, steroids and polycyclic hydrocarbons, has been studied extensively in the skin, it is evident that a full complement of drug-metabolizing enzyme activities is present in the skin. These drug-metabolizing enzymes are generally thought to be associated with the epidermal cells. Recent reports have suggested that high drug metabolizing activities are also localized in the differentiated cells of the hair follicles and adjacent sebaceous glands. It is possible that the resident microorganisms of the skin may also contribute to the metabolism of the topically applied compounds. However, during the development of methods for assessing skin graft viability, it was determined that the metabolic contributions due to skin microorganisms were negligible. Furthermore, experience with metabolism in human skin preparations which have been thoroughly scrubbed with antimicrobial disinfectants support a conclusion that it is the constituent skin cells themselves, rather than surface microbes, which are responsible for the observed biotransformations. Finally, since it is known that the metabolizing activities of the skin readily respond to modulation by inducers and inhibitors, such enzyme modulation could have important implications on cutaneous absorption and disposition experiments.

Experimental Models In the past, percutaneous absorption investigations have usually concentrated on the physicochemical and biophysical factors that influence skin penetration and permeation of chemicals. There are a wide variety of experimental approaches that have been developed to assess skin absorption; however, it should be noted at the outset that currently there is no generally accepted technique. Debates and conflicting opinions continue to revolve around the various factors that are important in influencing percutaneous absorption. Consequently, the rationales by which experimental models are selected and developed are continually being modified and revised. Fundamentally, skin absorption investigations are concerned with how much, how fast, and what are the modulating factors that may influence the penetration and percutaneous fate of the topically applied agents. To answer these questions the primary methods available are based on *in vivo* and *in vitro* studies. The former is from the Latin phrase for 'in life', that is, using a living organism, while the latter means literally 'in glass', or done in the test tube. Both approaches have their advantages and limitations, as will be discussed below.

In Vivo Techniques It is generally recognized that the most reliable method for learning about skin

absorption is to measure it *in vivo* using the appropriate animal model or human volunteers. In principle, the *in vivo* approach is simple, but in practice it is often fraught with experimental and ethical difficulties, particularly when studies are conducted in man. Typically, *in vivo* studies are performed by applying the compound of interest, in a suitable vehicle, to the surface of a defined area of skin. To protect the application site, occlusive or nonocclusive covering is often placed over the treated skin area, and absorption is then monitored by various procedures. However, the techniques used to monitor *in vivo* skin absorption often assess absorption indirectly, and frequently measurements are based on nonspecific assays. The validity of the *in vivo* determination will depend, therefore, on the validity of the method used.

When a topically applied compound induces a biological response following skin absorption, the quantitation of that response may provide a basis for assessing skin absorption. Indeed, such physiological or pharmacological responses have been employed as endpoints in assessing skin absorption *in vivo*, and perhaps the most successful example is the vasoconstrictor response to topical corticosteroids. However, while these pharmacodynamic endpoints may be very sensitive and selective for defined classes of compounds, it should be noted that the parameter measured is the product of both the quantity and the potency of the compound under investigation and may not necessarily reflect the extent of skin absorption, cutaneous metabolism, or disposition.

Ideally skin absorption and metabolism should be assessed based on the analysis of the compound and metabolites of interest in the body following topical application, and such analysis should be performed using sensitive, selective, and specific assays. Although it has been possible in some select cases to determine a plasma concentration–time profile of the compound following topical application, such specific analyses in body fluids are not routinely feasible because the low absolute amount normally absorbed via the skin is often too small to quantitate. It is for this reason that radiolabeled compounds are frequently used, and the extent of absorption is typically assessed by monitoring the elimination of radioactivity in excreta over a period of several days. For small laboratory animals, the absorbed radioactivity that may be retained in the animal and not eliminated in the excreta can be determined directly by analysis of the carcass, following removal of the application site and homogenization of the appropriate tissues. However, in larger animals and in humans, such an approach is impractical, and a correction is required to adjust for such pharmacokinetic factors as absorption, distribution, metabolism, and excretion. This correction has often been made by injecting intravenously a single dose of the radiolabeled compound and monitoring radioactivity in the excreta. A correction factor can be obtained which represents the fraction of dose that would be excreted during the time course of the percutaneous absorption study if it were instantly absorbed upon topical application. This has been the standard approach by which the vast majority of *in vivo* skin absorption studies is conducted and has provided invaluable information concerning percutaneous absorption in humans.

When measurements from intravenous dosing are applied as a correction, the validity is dependent on the underlying assumption that metabolism and disposition of the applied compound are not route dependent and that pharmacokinetic behavior of the intravenous and topical doses are similar. Unfortunately, there is little or no experimental basis for substantiating this assumption, and often the pharmacokinetic profile of the compound under investigation has not been fully characterized. Kinetically, skin absorption resembles a slow infusion, but the intravenous dose for correction is often given as a single bolus injection. Subcutaneous injection or a slow intravenous infusion may be the more appropriate delivery method for correction. Moreover, the selection of the size of the intravenous dose is often not rationalized. When differences in the relative amount of radioactivity excreted in the urine and feces following intravenous and topical administration are observed, these differences may be the consequence of route of administration or they may be related to differences in extent of systemic exposure. Furthermore, when metabolites are found in the excreta following topical application, it is difficult, if not impossible, to differentiate between skin metabolism and systemic metabolism. As a result, the significance of cutaneous metabolism in skin absorption cannot be readily established from *in vivo* investigations.

More direct approaches for monitoring skin absorption have been proposed – for example, measuring the rate of disappearance of the chemical at the application site. However, the generally low permeability of the skin means that the rate of disappearance is often very slow, and the accuracy of the measurement will depend on analytical techniques that are capable of accurately quantifying minute differences. Reliable results can only be obtained with chemicals that are rapidly absorbed and/or easily quantitated analytically. The main use of this technique is monitoring the loss of radioactivity from the skin surface, but it should be appreciated that

measurements using high-energy emitters whose transmission range may be similar to or greater than the thicknesses of the skin could result in erroneous estimates of skin absorption. Other methods, such as those based on histochemical and fluorescence techniques, are highly specialized and cannot be used with all compounds. Another approach involves correlating the extent of percutaneous absorption with the reservoir function of the stratum corneum, as measured by tape-stripping the application site and extracting to determine the amount taken up by the stratum corneum after a short exposure period (30 min).

In most toxicological and pharmacological investigations, the dose administered is precisely defined and dose–response relationships are usually carefully evaluated. In percutaneous absorption studies, however, this is not always the case. A great deal of absorption information in the literature may be of questionable validity since the dose applied was frequently not clearly defined or reported, even though the extent of skin absorption is usually reported in terms of a percentage of the dose applied. Dose application in skin absorption studies conducted *in vivo* is relatively straightforward. The compound of interest is prepared in an appropriate vehicle that may be liquid or semisolid, and an appropriate amount of this preparation is then applied uniformly onto the surface of the skin. Uniformity of application is important but often difficult to assess and is generally assumed without supporting evidence. Furthermore, very little is known concerning the potential influence of local toxicity on cutaneous metabolism and skin absorption and it is suggested that whenever possible a 'no-effect' level of the compound should be used in these types of studies.

Defining the amount of the topical dose applied that is available for absorption is particularly challenging when the compound under investigation is volatile or semivolatile as in the case of solvents and insect repellents. Following topical application, some of the applied dose will penetrate the skin and be absorbed. At the same time, some fraction will evaporate slowly from the surface of the skin and be lost, unavailable for percutaneous absorption. It has been demonstrated that the rate of evaporation, and consequently the relationship between evaporation and skin penetration, can influence the quantity of chemical absorbed dermally. The extent of evaporation from the skin surface is a function of the dose applied, airflow, and temperature at the skin surface. The extent to which these variables may be controlled or monitored can have a major impact on the results of *in vivo* skin absorption studies. Furthermore, consideration of the evaporative loss of the applied dose will be particularly important when surface disappearance or stratum corneum concentrations are employed as methods for assessing *in vivo* skin absorption.

Vehicle as a modulating factor that can influence skin absorption has been discussed in great detail, particularly from a standpoint of increasing absorption in the delivery dermatopharmaceutics, and there is much interest in solvents such as dimethylsulfoxide and azone as vehicles because they act as penetrant enhancers. Postapplication loss of volatile components in the vehicles can also alter the permeation characteristics of the applied chemicals. For example, if a highly volatile vehicle is used this may result in the compound under investigation being deposited as a thin film of solid onto the surface of the skin. On the other hand, a nonvolatile vehicle, such as an ointment, may be occlusive and change the diffusional properties of the stratum corneum. Both of these scenarios can greatly influence the extent of percutaneous absorption. Therefore, the rationale used to justify the selection of an appropriate vehicle for dose application will have important bearing on the significance and validity of the *in vivo* observations.

The extent of skin absorption is greatly dependent on the concentration of the applied dose and the surface area of exposure. Increasing the concentration of the applied dose has been shown to result in a decrease in the percentage of the applied dose being absorbed, but total absorption is increased. This effect may be compound specific and may depend on the dose range under investigation. Moreover, increasing the surface area of exposure will also result in increases in the extent of absorption. In defining the dose applied, therefore, one must consider not only the amount of chemical applied per unit area but also the total surface area of application and the total dose applied. The frequency of application and the duration of exposure have also been shown to influence the extent of skin absorption. In the few times that it has been investigated, the results have shown that washing of the application site to remove the applied dose may enhance, reduce, or have no effect on absorption. Studies on the interrelationship and influence of the various parameters pertaining to dose application in skin absorption are in their infancy. How these parameters may influence the extent of skin absorption is being explored, and it is clear that the current knowledge in this area is far from complete.

Many of the variabilities associated with the lack of standardization of dosing techniques (dosing concentration, volume, applied surface area, application time, etc.) can be somewhat compensated for by

calculating a dermal penetration coefficient K_p from the data wherever possible. The advantage of estimating K_p (as opposed to, say, the percent absorbed) is that values from different experiments (and for different compounds) can be directly compared, since this is a normalized measure of dermal penetration capacity. (It is in fact the flux per unit applied concentration and per unit exposed skin area.) In addition, the extent of dermal absorption can also be readily extrapolated to other exposure conditions (different concentrations, doses, surface areas, application times, etc.). A disadvantage is that K_p is essentially a steady-state parameter, and care must be taken to ensure that a steady state with constant influx rate of the material at a constant applied concentration is in fact achieved (e.g., under 'infinite dose' conditions – see *In Vitro* Techniques below), or is at least compensated for by taking into account dose depletion.

In Vitro Techniques From a cursory review of the literature on percutaneous absorption it is evident that much of our current understanding of the mechanism of percutaneous absorption was derived from *in vitro* investigations. *In vitro* experiments generally afford the investigator the ability to manipulate and control the experimental conditions, and the approach provides the unique opportunity to monitor the rate and extent of percutaneous absorption in skin tissues removed from the confounding influences of the rest of the body. *In vitro* methods, primarily those involving excised skin mounted in diffusion chambers, are the most frequently employed techniques used in the assessment of skin absorption.

Generically, these diffusion chambers consist of a donor and a receptor compartment. Skin absorption is then determined based on the assumption that recovery of the compound of interest in the receptor compartment, following application to the skin surface in the donor compartment, will provide an accurate measure of penetration and permeation. The success and popularity of the *in vitro* approach stem from the fact that the techniques are relatively simple. In these experiments the investigator is provided with the ability to monitor the rate and extent of absorption through skin removed from the influence of other bodily organs. Experimental conditions can be readily manipulated and controlled and, compared to *in vivo* studies, *in vitro* results can be obtained relatively quickly. Furthermore, it is recognized that *in vitro* methodology has contributed significantly in defining the important physiochemical parameters underlying percutaneous penetration and is responsible for much of our current understanding on the mechanisms involved.

Typically, an appropriate fluid (ideally to mimic the properties of blood) is placed into the receptor compartment and an appropriate formulation of the compound under investigation (often radiolabeled) is placed in contact with the skin surface in the donor compartment. The recovery of radioactivity over time in the receptor fluid then provides an estimate of skin absorption. The justification of this methodology centers upon the generally accepted assumption that the passive barrier properties of the skin limit skin absorption. Since the outermost layer of the skin is composed essentially of nonliving tissues and is considered the principal barrier for most compounds, it is reasoned that biochemical processes are unlikely to influence the diffusional characteristics of the rate-limiting membrane and, hence, *in vitro* diffusion studies will accurately measure skin penetration and absorption. The preparation of the skin sample itself has some bearing here. Typically, skin samples are either 'full thickness' in which the skin, stripped merely of its underlying fat deposits, is mounted in the chamber, or 'split thickness', in which the skin is first dermatomed, or sliced parallel to its surface to a specific thickness (typically a few hundred micrometers). The advantage of full thickness skin is that it is less likely to be damaged, but it suffers from the great disadvantage that more lipophilic materials in particular are forced to penetrate through the primarily aqueous layers of the viable epidermis and the whole dermis, which provides a much more formidable barrier to these compounds than they would encounter on their way to the capillary bed *in vivo*. In the case of split thickness skin, a compromise is attempted between trying to simulate a thickness representative of the depth of skin capillaries and the tendency for the thinner preparations to suffer skin barrier damage due to the dermatoming process itself.

In vitro diffusion chamber experiments are based on measuring the compound under investigation in the receptor fluid and much of the research activity has naturally focused in this area. Therefore, recovery of material in the skin itself has received only limited attention. Cutaneous distribution, metabolism, and binding of the topically applied agent are integral parts of the percutaneous absorption process, however, implying that assessing the disposition of the applied chemical in the skin tissue should be an important measurement in the evaluations of skin absorption. Indeed, the amount of a chemical that passes through the stratum corneum into the viable epidermis and dermis is an important parameter for assessing local bioavailability, and it also contributes to the overall estimate of *in vitro* percutaneous absorption. Furthermore, analysis of the skin following

permeation studies would assist in determining mass balance and dose accountability. Such measurements are often not reported or conducted even though they are important in establishing the validity and the interpretation of *in vitro* observations. Because of obvious advantages, radiolabeled chemicals are routinely used in skin absorption studies, and frequently liquid scintillation spectrometry is the sole method used for detecting the penetrating substances in the receptor fluid. However, skin absorption may be accompanied by cutaneous metabolism; therefore, the radioactivity recovered in the receptor medium reflects not only the permeation of the parent substance but also its metabolites.

Experimental designs of *in vitro* studies utilize one of two main strategies. In the traditional steady state or 'infinite dose' technique, a well-stirred donor solution of the compound of interest, at a defined and constant concentration, is used to deliver the compound across the skin preparation. The absorbed compound is subsequently delivered into a well-stirred receptor compartment. The most important design feature of these studies is that the quantity of compound that penetrates the membrane must be kept small relative to the total amount available – there must be no appreciable reduction in the concentration of the compound in the donor compartment. This is so that steady-state flux conditions are not significantly violated and the studies are performed with rigorous compliance to the laws of diffusion. The conventional approach to presenting data from this type of study is to plot the cumulative amount of drug reaching the receptor as a function of time. From the linear portion of this plot, we obtain the most important piece of information, that is, steady-state flux of the compound across the skin membrane (slope $= J_s$). This value is generally normalized with respect to the area of the skin membrane and is usually expressed as amount of drug per unit area per unit time. The intercept on the x-axis, obtained by extrapolating the linear part of the curve, gives a measure of the time required to establish a linear concentration gradient across the skin membrane and is referred to as the lag time, or τ. From these two parameters it is relatively simple, using the diffusion equations, to calculate the permeability coefficient ($K_p = \text{slope}/\Delta c_s$) and derive the other mass transfer parameters such as the diffusion coefficient of the drug across the skin, the partition coefficient of drug between the skin and the receptor fluid, and the diffusional thickness of the membrane. Additional useful parameters can be determined from the earlier nonlinear portion of the curve, but this requires a detailed and often complex model of the kinetics of the absorption process.

While the infinite dose technique has been invaluable in the determination of important skin permeability parameters such as dermal penetration coefficients and in the development of transdermal drug delivery concepts, to mimic *in vivo* conditions, the so-called 'finite dose' technique was developed. This is essentially a modification of the traditional steady-state method. The important difference is that the skin preparation is supported over the receptor so that the epidermal surface is exposed in a manner that mimics the real-life exposure scenario, and the compound of interest is applied to the surface of the skin in a manner also similar to exposure *in vivo*. Although the results of such studies may give valuable information about the absorption of materials under specific exposure conditions, they are generally not amenable to extrapolation to other exposures since no invariant skin properties such as penetration coefficients can be readily calculated.

The techniques described for maintaining viability of the excised skin used in these studies are relatively simple. Basically, the skin preparations are maintained under appropriate conditions as short-term organ culture. They are supported over the culture medium so that their epidermal surfaces are not covered. Material of interest can be applied topically in a manner similar to exposure *in vivo*. This material reaches the epidermal cells by diffusion where it may be metabolized, and recovery of both metabolites and parent compounds in the culture fluid then provides a measure of skin permeation and the extent of cutaneous first-pass metabolism. Two systems have been described. In the 'static' system discs of freshly excised skin are maintained, epidermal side up, on filter paper on a stainless-steel ring support within individual culture dishes containing a suitable culture fluid. In the flowthrough system the skin discs, supported on a stainless grid, form the upper seal of tissue wells of a compact, water-jacketed, multisample skin penetration chamber. Fresh, oxygenated culture medium is continuously perfused through the tissue wells and the well effluents may be collected at timed intervals. The skin absorption and metabolism studies described previously utilizing this methodology demonstrated that, by maintaining the metabolic viability of the excised skin under appropriate culture conditions, the *in vitro* approach provided the means whereby the potential influence of skin metabolism may be evaluated in conjunction with the diffusional aspect of percutaneous absorption. This methodology, therefore, offers a possible approach with which an estimate for the contribution by skin to the percutaneous fate of topically applied chemicals may be determined. It has also been suggested that the metabolites found in the

perfusion medium result from metabolism of the parent compound after its permeation into the receptor fluid, and enzymes that have leaked into the receptor fluid from the cultured tissue mediate this biotransformation. Currently, there is no evidence to support this hypothesis.

The underlying assumptions inherent in the utility of using excised skin for diffusion experiments are (1) skin condition, particularly that of the stratum corneum, is comparable to that found *in situ* (i.e., a key variable is the barrier function of the skin sample once it is removed from the animal or man); (2) recovery of the applied substances in the receptor fluid provides a true reflection of the rates and extent of percutaneous absorption (i.e., tissue binding and partitioning into the receptor fluid are not confounding factors); (3) living processes have little or no effect on percutaneous absorption mechanism or kinetics (although some investigators have maintained the metabolic viability of the skin by using flow-through systems with a receptor solution rich in oxygen – see above); and (4) penetration through dermis is not rate limiting. Some of these premises are becoming increasingly difficult to justify in light of what we now know about active metabolism of some drugs within the skin and the influence of cutaneous blood flow on the clearance of drugs and their metabolites from the skin. For some other chemicals, on the other hand, *in vitro* data sets are becoming invaluable tools to predict their dermal penetration, particularly from aqueous vehicle. **Table 4** lists a number of important design considerations when assessing percutaneous absorption using in *in vitro* diffusion chamber experiments. As can be seen, many of these potential sources of error *in vitro* experiments could be predicted from the list of factors affecting skin absorption *in vivo* (**Table 3**). Others, such as skin thickness, barrier integrity/viability, and receptor fluid content, are true artifacts of the *in vitro* system.

Table 4 Experimental design considerations with *in vitro* assessments of percutaneous absorption

Factor	*Contributing factors*
Skin source	Animal species differences, fresh human vs. cadaver skin
Membrane thickness	Full-thickness vs. split-thickness (no dermis) vs. stratum corneum alone
Barrier integrity	Preparation and storage conditions, follicles and other holes, viability (metabolic capacity)
Receptor fluid	Solubility, maintenance of skin viability/barrier integrity
Dosing method	Finite vs. infinite, dose formulation
Environment	Ambient temperature and humidity, hydration state

In Vivo–In Vitro Correlation In the skin absorption literature there are only a few instances in which studies were designed specifically to correlate *in vivo* and *in vitro* observations. This is probably because such comparative experiments involve many variables that need to be controlled or monitored and are difficult to perform well. Meaningful comparisons can only be made when experimental parameters of the *in vitro* studies closely resemble those of the *in vivo* study or vice versa. Moreover, ethical and safety concerns often limit the extent to which *in vivo* experiments may be conducted in humans. Thus, *in vivo–in vitro* correlations using human skin are practically nonexistent. In addition, in humans the site of application is frequently the ventral forearm, whereas in animals the back is often used and potentially damaging pretreatments of the animal skin such as shaving, clipping, or chemical depilation (hair removal creams) are frequently necessary before skin absorption experiments can be conducted. Since these are treatment variables that can influence percutaneous absorption, the reported species differences and similarities in skin absorption may reflect the net result of many competing variables, and understanding the significance of these variables would provide additional insights into the mechanism of percutaneous absorption.

A general consensus among investigators in percutaneous absorption is that human skin is preferred and should be used for *in vitro* assessments. On the other hand, it is also recognized that a major liability of human skin as a research tissue *in vitro* is its notoriously high variability in barrier properties. The source of human skin is frequently from cadavers, and since the investigator often has little or no control over the source and characteristics of the donor skin, the high variability observed with human skin preparations is to be expected. Characteristics such as treatment of the cadaver, elapsed time from death to harvest of tissue, skin site, age, health, sex, race, and skin care habits are examples of variables which may bias the *in vitro* penetration studies. Also, when skin samples are derived from elective surgery, the preoperative procedures such as scrubbing with antimicrobial disinfectants, the surgical manipulations, and the manner in which the membrane is prepared from the excised tissue are important details of concern. Again, these variables may influence the *in vitro* penetration observations. It has been recommended that where possible, in an *in vitro* study with human skin, such information should be routinely collected and carefully documented.

Although human skin is the tissue of choice, its limited accessibility to many investigators and the variability experienced with human skin have led many researchers to explore skin from various animals as models for skin absorption. However, species differ considerably in the structure and function of their skin and it is unlikely that animal skin will have barrier properties that are identical to those of human skin. Nevertheless, animal skin is routinely used for evaluating dermal toxicity and percutaneous absorption. Histological evidence and physiochemical studies have concluded that animal skin can provide reasonable percutaneous absorption models that approximate human skin; however, the debate concerning the appropriate animal model continues. Numerous comparative studies, both *in vivo* and *in vitro*, have been conducted to identify the ideal animal model. From the results obtained thus far, it would appear that the choice of animal model will depend on the preference of the investigators and the compound under investigation. The pig, the monkey, hairless mice, hairless guinea pigs, and, recently, the fuzzy or hairless rat have been described as species with the potentials to be good candidates as predictive models of skin absorption in humans.

Because passive physical diffusion is assumed to be the principal determinant in skin absorption, and since the selection of an appropriate animal model remains controversial, artificial barrier systems have been explored as potential models for evaluating absorption in human skin. These systems offer some advantages over biological models in that they are reproducible, easily prepared, and the composition of the membrane can be readily manipulated. Such membranes offer a defined matrix with which basic physical concepts regulating permeation may be examined. Various materials have been used in the construction of artificial membranes, and they include chemicals such as silastic (silicone rubber), cellulose acetate, isopropyl myristate, mineral oil, and dimethyl polysiloxane. Materials such as collagen and egg shell membrane which are of biological origin have also been used. In general, the construction of these artificial membranes attempts to mimic the stratum corneum barrier, and their use in diffusion studies has provided some useful information on the underlying mechanisms governing the physiochemical properties of chemicals and the relative abilities of the chemicals to diffuse through lipid membranes. Artificial membranes have been used as models for evaluating potential drug formulations during the development of topical preparations and transdermal delivery systems, and they have proven particularly useful for exploring the bioavailability of chemicals from different vehicles. In cases in which the partitioning of the chemical from the vehicle into the skin and into the artificial membrane is rate limiting, the effect of changing the vehicle on dermal penetration can be predicted from artificial membrane studies. Indeed, the experimental procedure can be even further simplified, since it is only necessary in these cases to determine the relative partition coefficients *into* the artificial material from different vehicles (and not necessarily the relative steady-state fluxes), and simple probes or 'dip sticks' fashioned from the appropriate material will suffice for such purposes.

While there is no doubt that *in vitro* diffusion chamber experiments have made substantial contributions to the current knowledge concerning the diffusional aspects of skin absorption, there are limitations to their usefulness, particularly for developing predictive pharmacokinetic models of skin absorption. Standardized versions of these diffusion chambers are readily available commercially, and these systems are easy to use and are well designed, having incorporated many of the desirable features of diffusion chambers described by investigators with many years of research experience in the field of skin absorption.

Advanced Models: Isolated Organ Perfusion Methods A major limitation of all diffusion chamber experiments is the lack of normal vascular uptake mechanisms. One consequence of this inadequacy has been a large effort aimed at better diffusion chamber designs, particularly through the use of flowthrough devices mentioned previously. It should be noted that the recovery of material in the receptor fluid, which provides an overall measure of *in vitro* permeation (i.e., the net penetration through the various layers of the skin into the receptor), does not necessarily provide an accurate measure of percutaneous absorption. Material that permeates the skin and remains in the tissue is absorbed material that would not be included when receptor fluid only is assessed (although this does not matter if a steady-state dermal penetration coefficient for systemic absorption is being determined). Furthermore, various tissue slicing techniques or apparatus design considerations have failed to totally resolve the possibility that the thick dermis may represent an artificial and selective barrier limiting the permeation of lipophilic penetrants *in vitro*. There is no consensus on what constitutes an ideal receptor fluid. The selection of the optimum receptor fluid for a particular compound of interest is frequently empirical and often reflects the biases of the investigator. Therefore, caution should be exercised and *in vitro* observations should not always be considered to be true and

accurate representations of the *in vivo* situation with respect to cutaneous absorption and metabolism.

Given the obvious physiological limitations of the previously discussed organ culture and diffusion cell approaches, perfused skin preparations, with an intact and functional cutaneous microcirculation, appear to represent an ideal experimental methodology for investigating the pharmacokinetics and mechanisms of percutaneous absorption and metabolism. Following the development of the perfused rabbit ear model in the 1930s and the subsequent demonstration of its potential as a tool for studying skin absorption, surprisingly little progress was made over the next half century. Reports of perfused feline and canine skin flaps, both *in situ* (meaning 'on site,' or still attached to the animal) and *in vitro*, appeared sporadically in the literature. These models have provided useful information in studies of skin physiology and the basic pathways of cutaneous respiration and energy production. However, they have not been widely used to study skin absorption. In addition, although early attempts to develop human skin perfusion models have been documented, progress has been slow.

Recently, techniques for creating and maintaining isolated arterial sandwich skin flaps *in situ* in rats have been described. This rat skin flap is created on athymic nude rats by surgically raising a small area of skin, perfused by the superficial epigastric artery, and grafting a split-thickness skin sample from syngenetic rats (i.e., from the same breeding stock) onto the underside. Although athymic rats reject foreign skin grafts at a relatively high rate (up to 90%), some success has been achieved in creating and maintaining a hybrid rat–human sandwich flap (RHSF) on this animal by repeated low-dose cyclosporine therapy. It has been proposed that the RHSF might be useful for studying percutaneous absorption in human skin if it can be shown that the absorption mechanisms are unaffected by the surgical manipulations and cyclosporine treatments. The experimental advantages afforded by such human-grafted skin flaps, in addition to the fact that they are reusable, are intuitive. Unfortunately, the complicated surgical procedures, costly animal and housing requirements, and expensive cyclosporine therapy and its confounding effects on skin absorption, coupled with the apparently high variability in xenobiotic flux through the xenografts, place severe limitations on their utility as experimental models for studying cutaneous metabolism and skin absorption.

Since it has long been known that there is a high degree of anatomical and physiological similarity between skin obtained from certain pale-skinned porcine species and that of man, it is not surprising that various pig skin flaps have been pursued. The biochemistry and utility of pig buttock flaps, created surgically in several different patterns, for dermatological purposes have been extensively investigated. Proposed advantages for using pig skin flaps include the availability of large surface areas, similar vasculature and anatomic structure, and the ease and similarity in the types of clinical observations which can be made. Perhaps the most promising perfused skin preparation is the isolated perfused porcine skin flap (IPPSF), which has been developed recently and provides a novel *in vitro* approach for examining percutaneous absorption processes in intact, living skin. The biochemistry and morphology of the IPPSF, maintained using an isolated organ perfusion technique for skin which is essentially analogous to methods developed for other organs such as liver, lung, and kidneys, have been examined in great detail and appear to be consistent with that found in porcine integument *in vivo*. The absorption of a wide variety of topically applied xenobiotics has already been demonstrated using the IPPSF, including such diverse chemicals as organic acids and bases, organophosphate insecticides, and steroid hormones and organochlorines. In addition, the effects of applied surface concentration and coadministration of vasoactive drugs (tolazoline and norepinephrine) on lidocaine iontophoresis (electrically driven drug transport across biological membranes), as well as the iontophoretic transport of small peptides and proteins (insulin), have been examined using the IPPSF, demonstrating its potential for testing novel transdermal drug delivery systems. Cutaneous biotransformation of xenobiotics during percutaneous absorption has been demonstrated using the IPPSF with the chlorinated hydrocarbon, chlorbenzilate, and with the organophosphate, parathion. Among the limitations of this method are persistent issues with the choice of appropriate perfusion/receptor fluids (whole blood, plasma, artificial blood cocktails, etc.) and perfusion rates, and the often staggering complexity of the mathematical models needed to interpret some of the time-dependent absorption results.

Preliminary studies using the IPPSF have shown that compounds such as the cancer chemotherapeutic agents cisplatin and carboplatin and the antibiotics tetracycline and doxycycline readily distribute into the skin following intravascular administration. Also, compounds such as parathion, 1-aminobenzotriazole (ABT), and 25-hydroxyvitamin D are bioactivated in the skin following intravascular administration in the IPPSF. This demonstrates a role for the IPPSF as an ideal experimental model for studying the disposition of xenobiotics that are

distributed to skin from the systemic circulation. Interest in the so-called outward transdermal migration or reverse penetration concept, namely, that skin may function as a clearance organ following delivery of systemically administered substances via the cutaneous vasculature, has been stimulated by the development of noninvasive techniques for measuring and analyzing the pharmacokinetics of the distribution of substances to skin *in vivo*. The absence of confounding, extracutaneous metabolizing organs, such as the liver, lungs, and kidneys, is a distinct advantage in IPPSF investigations of this reverse penetration phenomenon.

In conclusion, there are many fundamental questions concerning skin absorption and metabolism that remain to be addressed. The potential role of the dermal vasculature, the contribution of skin appendages such as hair follicles and sebaceous glands, the influence of skin condition, age, disease state, and anatomic sites are just a few examples of questions that need to be resolved. When topical exposure results in local effects, pathological changes in the skin may be expected to affect its barrier functions. These changes may involve alteration of the physical barrier as well as the biochemical properties, such as the metabolic status of the skin. Such local changes may have important implications on the outcome of percutaneous absorption and fate of topically applied xenobiotics. The experimental techniques necessary to address these questions are available, and productive research in these areas will provide means whereby species differences in skin absorption and metabolism may be investigated. These studies should provide not only a better understanding of the mechanisms important in the percutaneous fate of topically applied chemicals but also a rational basis for cross-species extrapolation and, therefore, more predictive estimates for skin absorption and metabolism in man.

Etiology of Skin Toxicity

General Concepts

Because the skin is in direct contact with the external environment, it is constantly being exposed to drugs, chemicals, electromagnetic radiation, and physical materials capable of producing toxic responses in this organ. In addition, many drugs are delivered into the skin via the systemic circulation, which also may result in cutaneous toxicity. It is the purpose of this section to review and categorize the extensive list of agents that exert toxic effects within the skin. Without discussing specific mechanisms, which are described in detail later in this entry, it is necessary here to make a distinction between those agents that produce a direct irritant response and those that act via a systemic, immune-mediated pathway. The former is called irritant contact dermatitis (ICD), while the latter is called allergic contact dermatitis (ACD). Both ICD and ACD involve the participation of many immune cell types found in the skin and are often histologically and biochemically indistinguishable from each other. Moreover, a third category of skin reactions has emerged, called contact uticaria. Unfortunately, the mechanistic distinction (discussed later) between this syndrome and ICD is even more blurred than the ACD versus ICD comparison. Because the list of urticariants (**Table 5**) appears to be a subset of the contact irritants, representing materials from every chemical class, these agents will not be described separately in the categories developed for this section.

Table 5 Contact urticariants

Category	*Examples*
Natural agents	Birch bark, butter, cabbage, capsaicin, chicken, cinnamon, cobalt chloride, copper, cotton oils, eggs, fish, fruits (kiwi, strawberry), hawthorn, honey, horse saliva, laboratory animals, mahogany, milk, nickel, papain, prawn crust, seminal fluid, sorbic acid, spices, spider mites
Industrial sources	Alcohols, benzoates, BHT, carbamates, carbonless copying paper, chloramine, chlorhexidine, diethyl fumarate, DEETS, DMSO, formaldehydes, *p*-phenylenediamine, phosphorus sesquisulfide, plastics, rouge, rubber, sorbitan monolaureate
Pharmaceuticals	Aminophenazone, benzocaine, benzoyl peroxide, penicillins

Direct cutaneous irritation, or ICD, is one of the most common maladies in industrialized society. The symptoms of ICD are the classical inflammatory response markers: redness, swelling, pain, and loss of function. Although ICD is not often fatal, this disease does involve significant morbidity and takes a heavy economic toll due to its sheer prevalence. The incidence of ICD in the general populations of the United States and Western Europe has been variously estimated at between 1% and 10%; however, counting undiagnosed cases the true incidence may lie closer to 25%. It is well documented that ICD is the single most common occupational disease seen in the United States, with over 5000 man-made and natural chemicals known to be capable of irritating the skin. A simplistic classification of these irritants includes such agents as desiccants, abrasive materials, organic solvents, acids and alkalis, concentrated metallic salt

solutions, oxidizing/reducing agents, enzymes, plant extracts, and surfactants. The latter group of agents represents the various soaps and detergents used in the form of complex mixtures and marketed extensively as cleansers in personal, fabric, and hard surface care products. As such, surfactants are primarily responsible for ICD of household origin and are considered second only to organic solvents in producing occupational dermatitis.

Equally important from a dermatological viewpoint, although not nearly as prevalent, are the immune-mediated skin reactions, which can be broadly categorized as ACD. Whereas ICD is commonly thought to account for 60–80% of clinically recognized human contact dermatitis, ACD accounts for most of the remainder (20–30%). As will be discussed later in this entry, ACD is clinically and histologically indistinguishable from ICD in most cases. However, the presence of two etiologic factors renders this condition perhaps even more dangerous than ICD. First, once an individual has become sensitized to contact allergens, quite low amounts of the offending agent can subsequently elicit massive skin responses. Second, once induced, this hypersensitivity may persist for a long and varied period of time, possibly even for the rest of one's life.

Like ICD, ACD may also occur from a very large number of chemicals, but not from electromagnetic radiation or physical stimuli alone. Most substances are rarely allergenic and there is a great range in allergenic potency, with a small number of known strong sensitizers having been identified experimentally in man. These strong allergens are often aromatic substances with molecular weights less than 500, highly lipid soluble, and quite reactive with proteins (a mechanistic requirement, as will be detailed later). The simplistic classification of the principal ACD agents includes metallic salts, plant polyunsaturated alcohols and ketones, acrylates, plasticizers, antibiotics, aliphatic amines and phenols, and formaldehyde. The possibilities for human exposure to both contact allergens and contact irritants can be divided among four broad categories: consumer products, occupational or industrial chemicals, environmental agents, and pharmaceuticals.

Root Causes of ICD and ACD

Consumer Products As mentioned earlier, the soaps and detergents in cleaning products and cosmetics comprise the bulk of the household materials that are irritating to human skin. The molecules responsible for this type of ICD are called surface-active agents, or surfactants. There are four main classes of surfactants, which are listed in decreasing order of their irritancy: anionics (used as industrial-strength cleaners and fat-based soaps), cationics (mostly disinfectant cleaners), and nonionics and amphoterics (fabric cleaners, cosmetics, shampoos, and mild cleansers). The irritancy of surfactants is roughly correlated to their cleaning power and their ability to foam when mixed with water and air. Other consumer products likely to cause ICD are wool and fiberglass due to mechanical action of the fibers on the skin surface; formaldehyde residues found in newspaper inks, building materials, and clothing; and dry cleaning fluid residues of polychloroethylene. Diaper dermatitis is also a form of consumer product-induced ICD caused by the combination of enzymes in urine/feces and disinfectant cleansers used on the skin.

The largest group of agents capable of causing ACD in the household are perfumes and dyes used in cosmetics, toiletries, and clothing (**Table 6**). Metal salts, such as nickel salts, chromium salts, and cobalt;

Table 6 Chemical agents associated with ACD

Category	*Specific examples*
Plants	Barley dust, lichens (D-usnic acid), hops (colophony), hetzil, sawdust, sesquiterpene lactones (*compositae*, *frullania* spp.), tulips (tulipalin A), poison ivy (urushiol)
Plastics	Cyanoacrylate, epoxy resins, polyacrylates, phenolformaldehyde resins, polyurethane, rubber additives (thiuram, carbamates)
Metals	Nickel, cobalt, mercury, silver, chromates (welding fumes and cement), beryllium
Industrial chemicals	Bis-(4-chlorophenyl)-methylchloride, 3-bromo-3(4-chlorobenzoyl)-propionic acid, 4-bromomethyl-(6,8)-dimethyl-2(1 *H*) quinolone, bromomethyl-4-nitrobenzene, bromophthalide, 2-chlror-6-fluorobenzaldehyde-chlorooxime, hydrogen sulfide, *n*-hydroxyphthalimide, trimethyl hexamethylene diisocyanate, Solvents—formaldehyde, turpentine, persulfate, phosphorus sesquisulfide, thioureas, allylphenoxyacetate, dimethoxane, chloracetamides (paints, wood shavings)
Pharmaceuticals	Chloroquine sulfate, benzocaine, chlorpromazine, cytosine arabinoside, 4,7-dichloroquinoline, 2,6-dichloropurine, streptomycin, neomycin, vincamine tartarate, 2[4(5) methyl-5(4) imidazolyl-methyl-thio] C_{13} pyritinol hydrochloride
Pesticides	Calcium lignosulfate, captafol, captan, carbamates, dithianone, ethoxyquin, naled, pyrethrum, spiramycin, tetrachloroisophthalonitrile, thiuram, tylosine, virginiamycin
Cosmetics	Perfumes, deodorants, hair sprays, sunscreens, skin lotions/creams, nail polish, dyes, shampoos

organomercurials; and formalin are all sometimes used as preservatives in household products and cosmetics and can also become allergenic. In fact, reactions to nickel (jewelry) and nickel salts are typically the most prevalent response in diagnostic patch test studies involving a wide variety of known allergens. Certain pesticides (e.g., isothiazolone-containing biocides like Kathon) and sunscreens also produce ACD, although the more potent sensitizers, such as *p*-aminobenzoic acid, are no longer in general use as sunscreens. Finally, the component monomers from certain rubber and plastic materials may also leach out and cause ACD, although humans are more likely to be exposed to these molecules in the workplace.

Industrial Chemicals With the possible exception of consumer products, this category represents the largest and most widely studied group of irritants and sensitizers. Certainly, it consists of the widest range of chemical classes to which humans are routinely exposed. It has been estimated that occupational skin disease accounts for 40–60% of all lost work days and ~95% of the cost, with ICD being more prevalent than ACD. Moreover, 25% or more of the general population is considered to be atopic or predisposed to skin eruptions despite the lack of visual, or even histologic, evidence that the skin is compromised. Chronic exposure to damaging consumer products or environmental agents no doubt contributes to occupationally induced skin disease.

Table 7 lists high-risk occupations for developing ICD and ACD. A common factor in these occupations is the presence of water ('wet work') and exposures to organic solvents and surfactants. While water itself is not considered an irritant, continual wetting and drying of the skin usually produces many of the hallmark symptoms of ICD. Organic solvents are by far the chemical class most responsible for occupationally induced ICD. These chemicals are used as degreasing agents and lubricants in many processes in the electronics, manufacturing, and construction industries. They include, in decreasing order of their irritancy, chlorinated aliphatics (e.g., trichloroethylene and polychlorinated biphenyls), aromatics (benzene/toluene), aliphatics (*n*-hexanes), ketones (acetone), and alcohols. Surfactants, discussed earlier in the context of consumer products, represent the second most important class of industrial irritants.

Miscellaneous industrial irritants include alkalis, such as caustic soda, NaOH, cement, and lime used in mining, dying, tanning, and construction, as well as strong acids (sulfuric, chromic, nitric, hydrochloric, and hydrofluoric) used in ironworks, glass etching, and masonry. Hydrogen peroxide and organic peroxides in plastic manufacture and reducing agents, such as phenols, hydrazines, aldehydes, and thioglycollates, may also produce ICD in the workplace. Moreover, enzymes released from meats and fish have been known to cause ICD in processing/packing plants. Besides the rubber and plastics industries (monomers), the primary source of occupationally induced ACD is the manufacture of consumer products, or the raw materials thereof, containing perfumes, dyes, preservatives, biocides, and other specialty chemicals.

Table 7 High-risk occupations for ICD

Occupation	*Specific exposures of interest*
Baker	Soaps and detergents, fruit juices, spices, enzymes
Construction worker	Cement, chalk, acids, wood preservatives, glues, detergents, industrial solvents
Canner, food service industry	Soaps and detergents, brine, syrup, fruit and vegetable juices, fish, meat, poultry
Dental technicians	Soaps and detergents, soldering fluxes, adhesives, acrylics, solvents, mercury
Electricians	Soldering fluxes, metal cleaners (solvents), epoxy resins, PCBs and PBBs
Hairdressers	Soaps and detergents, shampoos, permanent wave liquids, bleaches, and dyes
Horticulture	Manure, fertilizers, pesticides, irritating plants
Mechanics	Detergents, degreasers (solvents), lubricants, petroleum products, battery acids, soldering fluxes, cooling system fluids (PEG), metal shavings
Nurses	Soaps and detergents, alcohols, disinfectants, hand creams
Printer	Solvents, acrylates, formaldehyde, phthalate esters (inks)
Agriculture	Pesticides, fertilizers, disinfectants, detergents, petroleum products, irritating plants, animal secretions

Environmental Agents Many plants contain rough hairs or large calcium oxalate crystals (*Dieffenbachia*, *Caladium*, and *Philodendron* spp.), both of which are capable of producing mechanical damage to the skin. In addition, enzymes like bromelin (pineapples) or mucanain (cowhage) and chemicals like capsaicin (nightshade) or polycyclic diterpene alcohols (spurges) are also somewhat irritating. Nettles produce a contact urticaria by direct injection of the inflammatory mediators acetylcholine, histamine, and 5-hydroxytryptamine (serotonin). Anthralin, a synthetic drug, but originally isolated from the araroba tree, is also an important environmental contact irritant. The primary

plant allergens are catechols present in the *Toxicodendron* genus which are responsible for the most common form of plant-induced ACD: poison ivy (urushiol)/oak/sumac. ACD is also caused by butryo- and sesquiterpene-lactones found in the *Primula obconica* and *Compositae* (ragweed and Australian bush) plant families. Finally, atmospheric changes can also cause or predispose certain individuals to have ICD since it has long been known that low ambient humidity (more common in winter) can impair the barrier function of skin.

Ultraviolet (UV) light, a principal toxic component of solar radiation, interacts with skin in a variety of different ways which deserve special mention here. Visible light, having wavelengths of 400–760 nm, is relatively harmless, but shorter wavelengths can produce devastating effects alone or in combination with 'photoreactive' drugs and chemicals (described below). The three important divisions of UV light are UVA (320–400 nm), UVB (280–320 nm), and UVC (220–280 nm). UVC is of little natural concern because these shorter wavelengths are almost entirely absorbed, or blocked, by the stratospheric ozone layer. UVB is the part of the solar spectrum responsible for the most damaging effects on the skin, although UVA is now felt to play a more prominent role in certain types of skin disorders. The more serious effects of UV exposure are pigmentation defects, actinic elastosis (premature skin aging), selective defects in immune function, actinic keratosis, squamous/basal cell cancers, and malignant melanomas. UVB alone produces a characteristic, ICD-like inflammatory response (sunburn) or can react with chemical agents in and on the skin to produce photoirritation, or photo-ICD. A list of common phototoxic chemicals is shown in **Table 8**. Most of the recognized photoirritants are drugs delivered into the skin from systemic, not topical, administration, although plant-derived phototoxins are also known. For example, a pigment from St. John's wort is delivered to the skin upon ingestion, reacts with sunlight, and causes a massive vascular leakage that may progress to sloughing of large patches of dead skin.

Photo-induced ACD, or photosensitization, is also a consequence of combined exposure to sunlight and certain chemicals. The vast majority of these reactions appear to result from UVA wavelengths acting on topical agents, although isolated and incompletely documented reports of photosensitization resulting from systemic administration have appeared in the literature. A list of selected photoallergens is shown in **Table 9**. All are substances that absorb UV light and most have a resonating structure, that is, aromatic ring(s). An important complication of photo-ACD is the development of persistent light reaction, seen with phenothiazines, wherein a marked sensitivity to light persists long after exposure to the photoallergenic chemical has ended.

Table 8 List of common photoirritants

Class	*Chemical agents*
Coumarins	8-Methoxypsoralen, 5-methoxypsoralen, trimethoxypsoralen
Polycyclic aromatic hydrocarbons	Anthracene, fluoranthene, acridine, phenanthrene
Pharmaceuticals	Tetracycline, sulfonamides, chlorpromazine, nalidixic acid, NSAIDs[a] (benoxaprofen)
Dyes	Eosin, acridine orange
Miscellaneous	Porphyrins, amyl-*O*-dimethylaminobenzoate

[a] Nonsteroidal antiinflammatory drugs.

Table 9 List of common photoallergens

Class	*Chemical agents*
Halogenated salicylanides	Tetrachlorosalicylanide, bithional, dibromosalicylanide, tribromosalicylanide, 4-chloro-2-hydroxybenzoic acid, *N*-butylamide (JADIT)
Coumarins	6-Methylcoumarin, 4-methyl-7-ethoxycoumarin, 7-methylcoumarin
Plants	*Compositae* family (ragweed, Australian bush)
Sunscreens	*p*-Aminobenzoate (PABA), glyceryl-PABA
Miscellaneous	Sulfonamides, phenothiazides, 4,6-dichlorophenylphenol, quinoxaline-1,4-di-*N*-oxide, musk ambrette

Pharmaceuticals Adverse drug reactions account for 3–5% of hospital admissions and occur in as many as 5% of patients who are already hospitalized. Cutaneous involvement is particularly common in these circumstances, especially in children, in part because these so-called skin rashes are easily identified. Although many of these conditions are relatively harmless, cutaneous adverse drug reactions (CADRs) may be only one symptom of a much larger, and potentially life-threatening, immune response to a drug, or CADRs may be severe in and of themselves. Moreover, except for the occasional irritancies produced by topical ointments or transdermal drug delivery devices (patches), which are often not drug related but are due to other chemicals/materials present in the formulation/device, CADRs are almost always a form of ACD.

The most common CADRs are the less severe exanthem-like (characterized by a small papular rash

which can cover large surface areas) and urticarial reactions, together accounting for over two-thirds of drug-induced skin rashes. **Table 10** presents a list of drugs that are often associated with CADRs. Antibiotics are the drug class most likely to produce skin reactions, particularly in children, in which they account for more than 50% of all prescriptions. In addition, this drug class is mostly responsible for the non-life-threatening skin rashes. However, as can be seen from **Table 10**, the situation is complex in that most of the drug-induced skin diseases have multiple causes, and many of the drugs are capable of causing more than one type of skin lesion. The probability that an individual drug will cause a particular CADR is under the control of many 'host' factors, such as genetics, age, sex, the presence of other drugs (interactions), and concurrent diseases (liver or kidney failure, for example). Thus, generalizations are not very useful in the case of CADRs.

Other types of skin reactions to drugs (**Table 11**) are less frequent, but some are much more severe and deserve special mention. These CADRs fall into three major classes: severe and life-threatening dermatoses, skin malignancies, and other skin reactions. The severe dermatoses are the erythrodermas, erythema multiforme, toxic epidermal necrolysis, and bullous or blistering diseases. The dangerous symptom common to most of these CADRs is the sloughing off of large areas of epidermis, leaving the underlying dermis unprotected from bacterial infection. Although exceedingly rare, the mortality of toxic epidermal necrolysis has been estimated at 34%. The other dermatoses generally respond better to withdrawal of therapy. Phototoxicity and photoallergic reactions to common drugs were described previously. Drug-induced skin tumors have provided increasing evidence for the role of the immune system in the inhibition of malignancy due to the observed higher frequency of skin tumors of patients receiving immunosuppressants. It is also possible that certain drug-induced dermatoses result in greater propensity toward skin malignancies. Although ideopathic lichen planus does not appear to result in greater incidence of skin tumors, lichenoid eruptions due to quinicrine appear to have predisposed some individuals to a subsequent squamous cell epithelioma. Since the latency period for skin cancers can be many years to decades, more examples of drug rashes leading to skin malignancies may be forthcoming. Finally, although not generally life-threatening, but often severely and socially debilitating, are pigmentation, hair, and nail changes, acne, and vascular inflammation, all of which are listed in **Table 11** under 'other lesions.'

Table 10 List of drugs often associated with CADRs

Class	*Specific drugs involved*
Antibiotics	Penicillins, cephalosporins, sulfatrimethoprim, sulfonamides, nitrofurantoin, isoniazid, rifampin
Anticonvulsants	Phenytoin, carbamazepine, barbiturates
Corticosteroids, gold, NSAIDs	Antiinflammatories
Others	Antineoplastics, allopurinol, diuretics (sulfa derivatives)

Skin's Response to Toxic Insult

General Considerations It is axiomatic that the body's reaction to injury is limited and it is often impossible to identify the causal agent based solely

Table 11 Types of CADRs

Category	*Subclass*	*Examples of drugs involved*
Exanthem-like erythemas (46%)[a]		Antibiotics, anticonvulsants
Urticarias (23%)		Antibiotics, antiinflammatories, opiate analgesics
Other erythemas (<20%)	SLE[b]	Antibiotics, anticonvulsants, oral contraceptives
	Erythrodermas	Sulfonamides, gold, isoniazid, streptomycin
	Lichenoid photosensitivity	quinicrine antimalarials (see **Table 5**)
Blistering diseases (<10%)	Erythema multiforme	Sulfonamides, penicillins, diclofenac, oxyphenbutazone, piroxicam, phenytoin, carbamazepine
	Ten[c] pemphigus	(Same as for erythema multiforme) penicillamine, captopril, piroxicam, penicillins, rifampicin
	Bullous pemphigoid	Frusemide, penicillamine, penicillin, PUVA therapy
Skin cancer (<1%)		Immunosuppressants, mexiletine, thioridazine, penicillamine, moduretic®, atenolol, quinacrine

[a] Where percentages are noted, this is the approximate frequency among all patients experiencing CADRs.
[b] Systemic lupus erythematosis.
[c] Toxic epidermal necrolysis.

on the observed responses. Toxic insults on the skin can result in a combination of functional, biochemical, and morphological changes. These alterations induced by toxicants do not differ, in general terms, from changes caused by physical or biological agents, but the magnitude of the changes that are observed at any point in time depends on the nature, rate, extent, depth, and duration of the insult. From a mechanistic viewpoint, toxic insults to the skin can be classified into two main categories, namely, direct injury (i.e., ICD or contact urticaria) and immune injury (i.e., ACD). However, as mentioned earlier, the basic pathological lesions and clinical features that are encountered in all inflammatory skin responses are essentially indistinguishable. Thus, irrespective of the mechanism, the manifestations of toxic responses of the skin to an insult are basically the same and are similar to those following any other cause of cell injury in other organs and tissues: degeneration, proliferation and repair, or any combination of these basic dynamic responses.

Degenerations are regressive changes within a cell or cell population in response to injury. They range from reversible changes such as atrophy, which may be considered an adaptative homeostatic response to an adverse environment, to irreversible changes such as necrosis or cell death, while still forming part of the living organ. In between are a range of cellular alterations, including hydropic changes, fatty changes, and other inclusions, resulting from cytoplasmic accumulation of water, lipids, and granular materials, respectively, all of which are derived from breakdown of intracellular components.

Proliferation, in contrast to degeneration, involves increased growth in response to an injurious stress. The hypertrophy and hyperplasia experienced may range from adaptative homeostatic responses to irreversible proliferation of a cell population, leading to cancer. Inflammation and repair are extracellular responses that often accompany degeneration and proliferation. They represent tissue responses that attempt to contain or remove the injurious agent and revitalize the damage tissue. The extent and nature of the inflammatory response varies according to the nature, extent, and duration of the injury and include vascular, neurological, humoral, and cellular responses at the site of injury. Acute inflammation is typically an immediate and early response to an injurious agent. The vascular and connective tissues adjacent to the injured cells are usually involved and may include local vasodilation with transient increased blood flow and increased vascular permeability, with egress of white blood cells into the injured tissue. These processes are coordinated and integrated by numerous inflammatory mediators (e.g., histamine and bradykinin) that are produced or released at the site of injury. Where injury persists, chronic inflammation ensues and is characterized by the accumulation or proliferation of macrophages, lymphocytes vascular endothelium, and fibroblasts at the damage site.

The goal of the inflammatory process is to rapidly effect the elimination of the causal agent and removal of debris from damaged cells by dilution and phagocytosis, as well as to initiate the repair process. Repair of the damaged tissue may be achieved by a process of regeneration, which involves the replacement of damaged cells with viable cells of the same type through proliferation of adjacent healthy cells. Where the intrinsic regenerative capacity of cells of the damaged tissue is limited or tissue damage is severe, repair will involve fibrosis, a process in which fibroblasts from adjacent connective tissue mediate the replacement of damaged cells, with a characteristic scar tissue formation as the inevitable consequence.

Nonneoplastic Lesions

Epidermal Lesions Since the skin is composed of various structures, the extent and degree of involvement of each component will depend on the agent itself and on the severity of the exposure. However, because of its location, the epidermis is always first exposed to externally applied toxicants. Consequently, many skin responses to adverse reactions are epidermal in nature and usually involve inflammation. In the mildest form of superficial skin injury, where damage is restricted solely to the epidermis and there is some degree of epidermal destruction, hyperplasia is generally the dominant response. The epidermal destruction ranges from focal keratinocyte swelling (e.g., spongiosis) to hydropic degeneration of the basal layers and focal cellular necrosis. Under these conditions, the basal cells typically respond by increasing cell division and the epidermis quickly regenerates to normal. However, when the insult is sustained the proliferative response continues and ultimately results in a thickening of the epidermis. A good example of such proliferative response is that observed in the thickened skin on the palms of manual workers and it is the result of a continued low-level abrasive injury. Depending on the particular cell layers of the epidermis that are affected, these hyperplasias are described as hyperkeratosis, hypergranulosis, and acanthosis for thickening of the stratum corneum, stratum granulosum, and stratum spinosum, respectively.

In severe injuries (e.g., corrosions), extensive epidermal necrosis, with accompanying damage to the

cells of the basement membrane as well the superficial dermis, is frequently encountered. In this case, the extensive epidermal necrosis may lead to various degrees of ulcerations and be seen as devitalized epithelial layers with pyknotic nuclei that loosely line the dermis. Alternatively, the epidermis itself has sloughed off leaving a denuded dermal surface exposed to the external environment. These ulcerations are frequently accompanied by inflammatory changes, with migration of inflammatory cells such as polymorphonuclear leukocytes to the site of ulceration at the junction of the necrotic and viable tissues. This is followed by regenerative and proliferative changes involving the surrounding viable epithelial and connective tissue elements in an attempt to repair the damage. The undamaged adnexal components (e.g., hair follicles) are generally the source of precursor cells involved in the regeneration of the epithelial layers, and fibroblasts from the surrounding dermis are responsible for repair by fibrosis. As the damaged epidermis is repaired, the dead layers are sloughed, eventually leaving a scar.

These scenarios represent the two extremes, with most forms of dermatitis falling somewhere in between. Mild to moderate injuries usually produce clinical conditions described as eczema and they represent a wide range of responses essentially involving various combinations of degeneration, proliferation, and inflammation. Inflammatory responses often dominate during the early stages and are characterized by erythema, exudation, and leukocyte migration. These responses are sometimes accompanied by bullae, or blisters, and abscesses, or pustule formation, resulting from epidermal accumulation of fluids and cellular debris, respectively. With chronic or protracted exposure to mild irritants, proliferation of the epithelium increases. The skin becomes thickened, fissures may develop, and the proliferating keratinocytes begin to differentiate abnormally in a process known as parakeratosis, where the nuclei are retained in the stratum corneum. Although proliferation, involving hyperplasia and/or hypertrophy, is the usual pattern of epidermal response to toxicant exposure, on rare occasions epidermal atrophy is observed wherein the epidermis responds with decreases in cell size or decreases in number of epidermal layers.

Dermal Lesions As alluded to previously, dermal responses to toxic insults can be elicited by direct penetration of the toxicant through the epidermis to the dermis and this may occur with or without the destruction of the epidermis. Furthermore, reactive processes, initiated in the epidermis as a consequence of epidermal exposure, while not injuring the dermis directly can also elicit dermal responses. In addition, dermal exposure to toxicants of systemic origin via diffusion through dermal capillaries may produce toxic responses in the dermis in the absence of associated injuries to the epidermis. As previously described, the extent and nature of the toxic response will depend largely on the severity of the insult and will likely involve a combination of mechanisms. Mild acute injuries can produce focal necrosis that may be accompanied by localized inflammatory infiltrations and possibly abscesses. On the other hand, severe injuries resulting from exposure to corrosive substances can produce dermal and eventual subcutaneous coagulative necrosis that may be very painful. Edema and congestion in both the dermis and the epidermis, with eventual formation of vesicles, often accompany allergic reactions in the dermis that result from either systemic or local exposure to toxicants. Prolonged dermal exposure to mild toxicants can result in chronic dermatitis and this is often associated with extensive subepidermal mononuclear infiltrates or with perivascular infiltrates. The presence of secondary infections often complicates the overall picture of the toxic response. Finally, proliferation of dermal fibroblasts accompanied by angioblastic activity completes the repair process and this frequently culminates in fibrosis, or dermal scarring.

Adnexal Lesions In response to toxicologic insults, the cutaneous adnexa (appendages) will also undergo the dynamic changes of degeneration, proliferation, inflammation, and repair in a manner similar to that described. Thus, during toxicant exposure, typical destructive and involutional changes (e.g., focal necrosis, edema, hypertrophy, and hyperplasia) are evident. However, severe acute or chronic injuries can result in the partial or, in certain instances, complete loss of skin appendages from the exposed area. This is due to the fact that although the epidermis can regenerate completely by cell migration from unaffected sites, the newly formed epidermis is unable to reconstitute the adnexal elements. When hair is the target of the toxic insult, alopecia (hair loss) is the main consequence. Hair is susceptible to damage by both external agents and agents reaching the hair matrix through the dermis. Two major types of injury are experienced, namely, matrix cell damage and keratolytic damage. Keratolysis, the dissolution of hair keratin, is generally associated with local or surface contact of the toxic agent with hair. The resulting hair loss, due to the increased fragility of the hair shaft, may involve local patches or extensive areas, depending on the extent of the exposure. Regrowth of hair generally occurs following removal

of the toxic agent as the hair matrix cells are not damaged.

Agents that damage the hair matrix cells may affect hair follicles at a specific phase of hair cycle, that is, during anagen or telogen. The effect of anagen toxicity is typically hair loss (anagen effluvium). The mechanism of toxicity involves interference of the rapid mitotic activity of the follicular cells, leading to either a cessation of growth and the loss of the hair or the later loss of excessively brittle hair at the site of a weak, constricted area in the hair shaft. Anagen effluvium can occur within one or two weeks of exposure to the toxic agent and a number of common cancer chemotherapeutic agents are known to be anagen toxicants. Hair loss is also a consequence of telogen toxicity. The onset of telogen toxicity is slower and occurs over months of exposure and may involve a variety of mechanisms. Anagen and telogen toxicity can occur simultaneously and typical early histological signs of toxicity may include the vacuolization, disappearance of mitosis, pyknosis of the nuclei in the follicular matrix, or the presence of nuclear and other debris in the hair shaft. When damage to the hair follicles is severe, there is the potential for complete and irreversible loss of hair follicles resulting in permanent alopecia. As indicated previously, although the epidermis has full regenerative capacity, the newly formed epithelium usually cannot regenerate the skin adnexa.

Another class of lesions of adnexal origin that is frequently seen as a result to exposure to a variety of agents, including grease, oils, coal tar, and cosmetic preparations, is acne. These acneiform lesions originate from the sebaceous glands and typically start with comedones and inflammatory folliculitis on the skin surface that is in direct contact with the causal agents. The resultant proliferation of the sebaceous gland follicular epithelium leads to the formation of lipid-filled keratin cysts, similar to those observed in acne vulgaris. Chloracne is a somewhat specific type of acneiform eruption which occurs after exposure to a group of halogenated aromatic hydrocarbons (e.g., polyhalogenated dibenzofurans, polychlorinated dioxins, polychlorinated naphthalenes, and polychlorinated biphenyls). Chloracne is characterized by small, straw-colored cysts, comedones, and, in severe cases, inflammatory pustules or abscesses may be seen. Histologically the changes that are seen during the development of chloracne begin with keratinization of the sebaceous gland epithelial duct and the outer root sheath of the hair follicle. The sebaceous gland is eventually replaced by a keratinous cyst and the typical fully developed lesion consists of a dilation of the upper third of the hair follicle, which is usually bottle shaped. No differentiation can be seen between the epithelia of the infundibulum and the sebaceous glands. Edema and mononuclear perivascular infiltrates are sometimes seen in the papillary dermis and late manifestations of chloracne often include mild fibrosis of the dermis, hypotrichosis, and hyperpigmentation. The affected areas are usually those located in the malar crescent of the face and behind the ears. The external genitalia, axillae, shoulders, chest, back, abdomen, and buttocks are sometimes involved, but lesions are rarely seen in the extremities. Chloracne often continues to appear even after exposure to the chemical agent responsible has ceased, possibly as a consequence of release from tissue depots since most chloracnegens tend to be highly lipophilic. Experimental chloracne has been produced in rabbits, monkeys, and hairless mice. This latter species is thought to be the most useful animal model for the disease, but the occasional presence of degenerative cystic hair follicles in normal hairless mice is a confounding factor with this model.

Selective local damage to other skin adnexa, such as the sweat glands, can occur with exposure to a number of cytostatic agents, such as cytarabine and bleomycin, which are used in human cancer therapy. The condition is characterized by necrosis of the epithelium lining the eccrine sweat duct, accompanied by acute inflammation and squamous metaplasia of the remaining cells of the eccrine apparatus. The mechanism for the selective toxicity is unknown, although high concentrations of these compounds in sweat may provide an explanation. Other chemicals that are toxic to the sweat gland include formaldehyde, arsenic, lead, fluorine, and thallium, all of which produce generalized anhidrosis (loss of the sweating mechanism) due to partial or total destruction of the eccrine system.

Neoplastic Lesions Cellular proliferation is one of the ways in which cells and tissues respond to an injurious insult, and the result is neoplasia, or cancerous growth when these proliferations show partial or complete loss of responsiveness to normal growth controls. Neoplastic lesions induced in the skin of experimental animals have played an important role in understanding the multistage process of chemical carcinogenesis. Tumors produced in this multistage process are initially benign exophytic lesions (e.g., papillomas), some of which may regress while others gradually convert into fully invasive, malignant, endophytic tumors (i.e., carcinomas). The mechanisms by which chemicals may lead to uncontrolled cell proliferation are outside the scope of this entry, but suffice it to say that chemical carcinogens may be divided into two categories based on their proposed

mechanisms of action: (1) genototoxic, or those acting intracellularly, usually directly damaging to DNA, and (2) nongenotoxic, or those which act via regulatory factors in the extracellular environment.

Papillomas are the most common neoplastic lesions occurring in rodent skin after exposure to chemical carcinogens. They generally arise from the infundibular region of metaplastic or hyperplastic hair follicles. They are composed of a series of folds, united by common stalks to the underlying skin, and have a cauliflower-like structure and appearance. The folds of a papilloma consist of a central connective tissue core covered by a thick layer of epidermis-like epithelium. The germinative layers of the epithelium contain numerous mitoses and there are distinct spinous and granular layers as well as a thick, fully keratinized stratum corneum. Papillomas may regress or continue their progression toward carcinomas, and confluency into larger malignant tumors can also occur.

Keratocanthomas are benign neoplastic skin lesions often found after exposure to UV radiation or complete carcinogens in various species, including humans. They originate in the hair follicles as an intradermal growth of epithelial prolongations. They have a cup-shaped architecture with a central horny crater that has a papillomatous exophytic component and an endophytic component of deeply penetrating epithelial cords, which appear not to invade the subcutaneous tissues. In mice, keratocanthomas generally progress to squamous carcinomas and regression is uncommon. In humans, however, they are generally considered to be abortive neoplasias that usually regress. Preneoplastic, intraepithelial lesions are commonly found in humans as the result of exposure to sunlight or arsenicals, but such lesions are not frequently inducible in animal models of chemical carcinogenesis. These preneoplastic lesions have the potential to progress to carcinoma.

Carcinomas of various types, for example, squamous cell and basal cell carcinomas, have been induced in many different laboratory species using UV light, other forms of ionizing radiation, and chemical carcinogens. Generally these tumors arise from existing papillomas, keratocanthomas, or intraepidermal preneoplastic lesions (in humans), as well as from otherwise normal or hyperplastic epidermis. In humans, cutaneous squamous cell and basal cell carcinomas are extremely common clinical problems and the major etiologic agent is generally considered to be chronic sun exposure. Fortunately, these tumors rarely metastasize and thus have low mortality, but they are locally destructive and can be associated with considerable morbidity. Melanomas, arising from the pigment-producing melanocytes in the epidermis, have been produced using chemical carcinogens in experimental animals. These melanotic tumors, which include both benign and malignant types, have generated considerable concern, particularly in relation to skin cancer in man. Melanomas, which metastasize widely, are responsible for more deaths than any other type of skin cancer. Chronic sun exposure is believed to be a major risk factor and the implication that UV radiation is a major causative agent in the pathogenesis of melanoma remains controversial. In experimental species, chemically induced melanotic tumors are less aggressive than the human malignant melanomas, thus they tend not to metastasize readily.

Other Responses Urticarias, or 'wheal and flare' reactions, are common skin responses produced by topical exposure to a variety of topical agents (**Table 5**), especially biogenic polymers released from plants and insects. The response generally occurs within one hour of exposure and involves the local release of vasoactive substances including histamine. Frequently, urticaria is associated with immunologic responses and is often an integral part of immediate hypersenstivity reactions to ingested agents (e.g., drugs involved in CADRs). Undesirable color or pigmentary changes are also encountered as adverse cutaneous responses to topical agents. Chemicals which show structural similarities to tyrosine, the major building block of melanin, are known to cause local loss of pigmentation, whereas increased pigmentation may result as a secondary consequence to a phototoxic response. Color changes in the skin may also occur as the result of cutaneous accumulation of endogenous (e.g., carotenemia from eating too many carrots) as well as exogenous (argyria from contacting silver) pigments. Subjective reactions such as itching, burning, or stinging sensations are often encountered by sensitive individuals following exposure to a variety of topical agents, primarily cosmetics and detergents. These reactions are entirely subjective and do not have any obvious manifestations that can be perceived by the outside observer. Nevertheless, they are considered by the affected individuals to be completely undesirable.

Mechanisms and Methods for Assessing Skin Toxicity

General Considerations

The classic signs of the inflammatory response in skin were recognized long ago in ancient Rome by the physician Celsus, who coined the Latin phrase, *Rubor et tumor, cum calore et dolor*, roughly

meaning redness and swelling, resulting in heat and pain. The underlying mechanisms whereby these processes take place in ICD, ACD, and contact urticaria were, of course, unknown at the time. Much work has been done to help clarify this mystery, and inflammation is now best described within the paradigm of two major phases: the vascular phase and the cellular phase. Although a third, more immediate, 'neurologic' phase has been identified recently, it is such a transient and poorly understood component of the inflammatory response that it bears little mention here.

The vascular phase represents the most acute response of the skin to the presence of an irritating chemical or to a potential allergen, taking place within minutes and generally lasting only a few hours. This phase is induced by several systems, first and foremost of which is the nonspecific release of inflammatory mediators by epidermal keratinocytes and dermal fibroblasts. Such vasoactive materials as IL-1β, other cytokines, and the arachidonic acid metabolites prostaglandin E_2, leukotriene D_4, and prostacyclin initiate a cascade of events resulting in vasodilation, increased vascular permeability, and the influx of blood cell constituents. A good analogy would be the situation presented by an overturned fuel tanker on a major highway. The roads would become swelled with traffic and the influx of police, fire trucks, ambulances, and onlookers would spill over into the surrounding countryside. Other systems involved in this early phase are the complement pathways, primarily C3a and C5a; the coagulation system (fibrin split-products, Factor XIIa, and thrombin); plasma bradykinin; and an immunological reaction mediated by mast cells, which are abundant in the dermis (7000–10 000 cells mm^{-3}). This latter component is the principal mechanism in contact urticaria (mentioned earlier) and the release of histamine, serotonin, heparin, and chemotactic factors from these mast cells is also important in initiating the cellular phase of the inflammatory response.

The cellular phase takes place over a period of several days and begins with leukocyte margination (contact with vascular walls) and the release of chemotactic factors causing the migration of neutrophils into the injured tissue. Neutrophils contain granules which provide microbicidal enzymes (myeloperoxidase and lysozyme), neutral serine proteinases (e.g., elastase and cathepsin G), β-glucuronidase, α-mannosidase, vitamin B_{12}-binding proteins, and collagenase. The net effect of these mediators is increased tissue oxygen consumption and the generation of reactive oxygen species, or free radicals (e.g., superoxide anions, peroxide radicals, and halide acids), all of which are lethal to invading pathogens and are somewhat responsible for the heat and pain which accompany the inflammatory response.

Table 12 Lymphocyte products acting on other cell types

Cell type affected	*Lymphocyte products involved*
Macrophage	Migration inhibitory factor (MAF), macrophage activating factor, macrophage aggregating factor, chemotactic factor, AG-dependent MIF
Nuetrophil	Chemotactic factor, leukocyte inhibitory factor (LIF)
Lymphocyte	Interleukins (IL-) 2, 3, 4, and 5; chemotactic factors
Eosinophil	AG-AB-dependent chemotactic factor, IL-5, migration stimulation factor
Basophil	Histamine releasing factor, IL-3
Other cells	Lymphotoxin, growth inhibitory factors, osteoclast activating factor (OAF)

Basophils and eosinophils may also be involved, especially in ACD. Basophils are similar to mast cells and play a role in delayed-type hypersensitivity, whereas eosinophilic migration is dependent on complement and chemotactic factors released early upon exposure to a contact allergen. Langerhan's cells and other macrophagic monocytes release IL-1 and other cytokines early and are important in antigen presentation to lymphocytes. The latter white blood cell type then proceeds to influence a number of other cellular responses (**Table 12**). Overall, there are three types of allergic reactions in skin: type I (anaphylaxis), typified by the 'wheal and flare' produced by IgA- and IgE-responsive mast cells; type III (immune complex), which is an anitgen–antibody response involving complement; and type IV (delayed-type hypersensitivity). The latter is by far the most prominent type of chemically induced ACD and begins with Langerhan's cell and lymphocyte presentation of antigen to regional lymph nodes, followed by a vascular phase 24–48 h later. As mentioned earlier, it is prerequisite for a molecule to produce ACD that it reacts chemically with proteins in the antigen presenting cells.

Experimental Models

***In Vivo* Techniques** Determination of eye and skin irritation potential is mandated for proper labeling of all consumer products, and is needed to meet various regulatory requirements (e.g., for chemicals or products to be transported across state lines in the United States, as required by the US Food and Drug Administration (FDA) and Department of Transportation (DOT), respectively). Animal testing for skin irritation (ICD) is almost exclusively restricted to modifications of the test first proposed by John

Draize at the FDA in 1944. The rabbit primary dermal irritation (PDI) bioassay, as recommended by the CPSC in 1981 (in the Federal Hazard Substances Act), provides the basis for the most modern version of this model. Slight modifications proposed by the Organisation for Economic Cooperation and Development (1981) and the US Environmental Protection Agency (1983) have recently been incorporated to reduce total animal use and eliminate the unnecessary discomfort of abraded test sites and overly long exposure periods. Briefly, 0.5 ml (liquids) or 0.5 g (solids) of each test substance are applied to unabraded sites only on three New Zealand White rabbits, under a 1×1 in. gauze pad, and the site is occluded with gauze and tape wrappings. Following a 4 h exposure period, the wrappings and patches are removed and the sites are gently swabbed free of residual test material. Scores ranging from 1 to 4 for both erythema and edema are based on visual observation of the test site immediately after patch removal and at 24 and 48 h postexposure. These scores are summed and divided by the total number of scores to calculate the PDI index, which serves as the *in vivo* response variable for each test substance. Occasionally, the guinea pig is used in place of rabbits in this assay or in full immersion studies and cumulative irritation (multiple doses) tests, while other species are used very infrequently. An exception to this rule may be the mouse ear swelling test (MEST), which has been undergoing extensive evaluation and validation in the past few years. Nevertheless, despite clear evidence that these animal models may not be relevant to the human condition, Draize-type testing is still a rather standard practice in the consumer products and cosmetics industries.

Human testing for ICD involves either single application patches or cumulative patching, usually fresh doses of the chemical every 48 h over a 21 day period, for most compounds. For soaps and detergents, specialized assays, called soap chamber tests and arm wash tests, are utilized. In the former, a modified Franz diffusion cell-type donor chamber is affixed to the forearm and the soap solution is left in contact with the skin surface for a few hours. Arm wash tests were devised to mimic actual use conditions. This test has been further modified to include multiple washes over a short time period, or an 'exaggerated' arm wash test, to help discriminate among milder irritants, which produce little or no response in the standard soap chamber, arm wash, or patch tests. The major limitation to all human tests is the large intersubject variability coupled with the heavy influence of environmental conditions on the skin's initial condition, which ultimately affects its ability to respond to irritant challenge. It is for this latter reason that most of these clinical assessments of ICD are performed in the summer months because cold, dry air alone can be very damaging to skin.

The situation with animal testing for ACD is somewhat more complicated than that for ICD tests, probably because the disease process and underlying mechanisms are more complex. The guinea pig is the standard animal model for ACD, based on the original intradermal injection studies of the nitro- and chlorobenzene classes of sensitizers in 1935. The following modifications of the original protocol are now in routine use: occluded patch test, ear-flank test, guinea pig maximization test, split-adjuvant test, guinea pig optimization or Freund's complete adjuvant test, and open epicutaneous test. The common feature to all these tests is that they are biphasic, employing an induction phase followed by a challenge phase. Their major limitations are the subjective nature of the visual scoring system and the fact that these are rather costly, time-consuming bioassays compared to the ICD counterparts. In addition, there are ethical concerns with the use of adjuvants, which are basically allergenic materials added to the assay to increase the response. Adjuvants alone, when injected intradermally, can cause considerable redness, swelling, and intense pain. Finally, there are two newer models under evaluation: a variation on the MEST and the local lymph node assay (LLNA). The LLNA is based on measurement of cellular proliferation and other parameters in white blood cells (lymphocytes) collected from the lymph node draining the site of exposure and has been accepted as an alternative to the guinea pig maximization test for assessing ACD by the US EPA, FDA and OSHA.

Human ACD assays are of two basic types: the so-called prophetic patch test or single-induction dose, which is insensitive and rarely used, and the repeat insult patch test (RIPT). The latter involves multiple applications (every other day for 2 or 3 weeks) of low concentrations of the test article during the induction phase, followed by a single 24 h exposure to a higher dose and visual scoring over a 3–7 day period during the challenge phase. A modification of the RIPT is Kligman's maximization procedure, which utilizes the irritating surfactant sodium lauryl sulfate to increase the skin's responsiveness to the test material. Besides the interfering factors cited previously for human ICD tests, a major limitation of the RIPT is the selection of nonirritating induction doses, vehicle effects, and the inability to properly evaluate the skin reactions. In addition, the results of RIPTs are the least quantitative of all the *in vivo* irritation and sensitization tests. This issue of quantitation is particularly important in human tests for both ICD and

Table 13 Instrumental methods for assessing cutaneous toxicity

Category	Instrument used (measured response)
Spectrophotometry	Dia-Stron erythema meter®, Minolta Chromameter®, Cortex Dermaspectrometer® (all measure a 'redness' index of erythema); laser Doppler Velocimeter (blood flow)
Evaporimetry	Servo-Med evaporimeter® (transepidermal or skin surface water loss)
Electrical properties	Skicon®, Corneometer®, Nova Dermal Phase Meter® (all measure conductance/capacitance to assess hydration state)
Calorimetry	Skin surface temperature, thermography
Mechanical properties	Dia-Stron Dermal Torque Meter®, rheometers, SEM 474 Cutometer®, gas-bearing electrodynamometer (all measure elasticity); Newcastle Friction Meter® (roughness); Cortex Dermascan®, and other ultrasound equipment (epidermal thickness)
Surface features	Anjinomoto Scopeman® or Microwatcher® image analyzers, ultrasound equipment, profilometers (roughness, flakiness, scaliness, etc.)
Miscellaneous	Differential scanning calorimetry, Fourier transform infrared spectrometry (changes in stratum corneum lipid structure/function)

Table 14 *In vitro* endpoints for predicting ICD

Class	*Specific examples of proposed markers*
Membrane integrity	Vital dyes (trypan blue, eosin); fluorescence (Hoechst, fluoresceins, rhodamine, ethidium bromide, propidium iodide); exogenous (^{51}Cr release); endogenous (LDH or alkaline phosphatase leakage, intracellular K^+, lipid peroxidation)
Subcellular function	Mitochondrial (MTT, XTT, Alamar blue, ATP); ribosomal (^{14}C-Leu or -URI incorporation); lysosomal (neutral red uptake/release); nuclear (^{3}H-Thy incorporation, DNA binding)
Cellular metabolism	Glucose utilization, O_2 consumption, growth inhibition, lactate/pyruvate ratios, glutathione/redox status
Inflammatory mediators	Arachidonic acid cascade (^{3}H-AA release, PGE_2 release, leukotrienes and HETEs); cytokine release (IL-1β, TNFα)
Morphology	Light and electron microscopic changes
Unknown mechanisms	Collagen swelling, Skintex®, Coumassie blue dye extraction from gelatin, quantitative structure activity relationships (QSAR computer models)

ACD, which normally depend entirely on subjective, visual assessments of erythema and edema. This need has led, in turn, to a large effort to develop instrumental methods for measuring the vast array of skin responses to toxic compounds (**Table 13**). Besides providing quantitative data for such diverse responses to cutaneous toxins as inflammation, altered hydration state (e.g., dryness and 'tight feel'), changes in elastic or mechanical properties, or altered surface morphology (e.g., roughness, scaliness, and flaking), these biophysical methods are much more sensitive than visual techniques. Moreover, some of these instruments have demonstrated utility in animal or *in vitro* studies of cutaneous toxicity.

***In Vitro* Techniques** During the past two decades, public pressure to reduce the use of animals in all areas of biomedical research has resulted in animal experimentation coming under close scrutiny and increased governmental regulation. One area in which alternatives to animal systems seem both feasible and justified is that of early screens in premarket safety evaluations. In fact, there are *in vitro* alternatives which have undergone large multiinstitutional validation studies and which are being extensively utilized for mutagenicity and ocular irritancy testing in the industrial setting. Furthermore, a number of alternative assays have been proposed as screens for cutaneous irritation, although the validation process has been much slower. Nevertheless, assays based on disruption of cell membrane integrity, metabolic activity, or growth; incorporation of radiolabeled nucleotides and amino acids; cellular release of inflammatory mediators; or induction of morphological alterations at the cellular level are all currently under evaluation (**Table 14**). These types of assays may be performed using human and nonhuman fibroblast and keratinocyte cell cultures or using the more complex, organotypic skin tissue and organ culture models. In addition, there are a number of techniques which do not involve tissue cultures, operate via unknown mechanisms or mechanisms that are unrelated to the ICD response *in vivo*, or which are known to be entirely correlative in nature. Many of the commonly used biochemical markers or endpoints associated with these alternative methods share significant limitations: (1) they often require high test substance concentrations, effectively killing a large fraction of the exposed cells, and there is no clear evidence that this degree of cytotoxicity is mechanistically relevant in ICD; (2) they produce extremely variable and unreliable results for diverse sets of test materials and are sometimes more costly than animal or human patch tests; and (3) they were primarily validated against *in vivo*

ocular irritation data. Since it is well documented that the potential for a chemical to produce eye irritancy is not well correlated with its irritability to the skin, the latter point is an important distinction to make in the validation of alternative models for predicting ICD.

The situation with *in vitro* models for predicting ACD, unlike its *in vivo* counterpart, is less complicated than that for ICD because there are very few *in vitro* systems which have even been proposed for ACD testing. This is also a consequence of the complexity of this disease since ACD involves the interaction of many organ systems, which cannot be properly simulated in any currently available cell or tissue culture model. Nevertheless, two assays that have shown some promise for predicting ACD with certain classes of allergens are the lymphocyte transformation test and the macrophage migratory inhibition test.

Conclusions

It is clear that skin is not just an inert, protective barrier that surrounds the body's internal organs, but rather is an active participant in the overall outcome of exposure to potentially injurious materials in the external environment. The significance of cutaneous reactions to topical agents, particularly the inflammatory response and carcinogenesis, is the subject of an increasing number of scientific investigations. From the perspective of the skin absorption process, this organ is at once a portal of entry for a variety of topically applied chemicals, a drug-metabolizing organ, and a target organ for local toxicity. Thus, knowledge of the mechanisms involved in translocating chemicals into and through the skin, coupled with its effect on the physiological disposition or availability of topically delivered chemicals to interact with skin and other body organs, is key to understanding cutaneous pharmacology and toxicology.

Local skin effects are not the only consideration for dermal toxicity. The role of the skin as a barrier preventing the free penetration of exogenous chemicals into the systemic circulation is equally important. Indeed, it is becoming apparent that the dermal route of exposure is in many cases comparable to inhalation and oral absorption as a potential source of potentially toxic chemicals in the body and forms an integral part of many multi-media multi-pathway risk assessments. In this context, for example, the (US) National Institute of Occupational Safety and Health is currently revising its current skin notations (which identify chemicals likely to present dermal hazards in the workplace) to take into account a compound's potential for dermal absorption, as well as its capacity to sensitize and damage the skin.

In this review, some of the theoretical models and experimental methodologies employed in dermatotoxicity studies, both *in vivo* and *in vitro*, have been described. It is suggested that a combination of these techniques may provide the basis for future experimental approaches toward increasing knowledge of the mechanisms of cutaneous toxicity. It should be emphasized that research in this area is evolving, such techniques are being developed, and the rationale by which *in vivo* or *in vitro* models are selected and utilized is under continual scrutiny. Further development in this area will necessitate improvements in bioanalytical techniques and a better understanding of the interplay between skin penetration, permeation, and metabolism, as well as the role of modulating factors that may influence the structure, function, and toxicology of the skin. As the underlying mechanisms are further elucidated, and experimental databases become more comprehensive, we will be seeing a greater role being played by quantitative structure–activity relationships and other physicochemical tools (including simple chemical measurements) in being able to more accurately predict the penetration and interaction of chemicals with the skin. Mathematical models that embody these mechanisms will allow predictions of dermal penetration for new compounds to be made from their physicochemical properties that will move from mere screening tools to increasingly powerful and useful predictors of skin penetration and potential local and systemic health effects.

See also: Acids; Alkalies; Carcinogen–DNA Adduct Formation and DNA Repair; Dioxins; Dyes; Fragrances and Perfumes; Hair; Hypersensitivity, Delayed Type; Nails (of the Fingers and Toes); Organophosphates; Photoallergens; Poisoning Emergencies in Humans; Safety Testing, Clinical Studies; Tissue Repair; Toxicity Testing, Alternatives; Toxicity Testing, Dermal; Toxicity Testing, Irritation.

Further Reading

Barile FA (1994) *Introduction to in Vitro Cytotoxicology. Mechanisms and Methods*. Boca Raton, FL: CRC Press.

Bronaugh RL and Maibach HI (eds.) (1989) *Percutaneous Absorption. Mechanisms–Methodology–Drug Delivery*, 2nd edn. New York: Dekker.

Drill VA and Lazar P (eds.) (1984) *Cutaneous Toxicity*. New York: Raven Press.

English JS, Dawe RS, and Ferguson J (2003) Environmental effects and skin disease. *British Medical Bulletin* 68: 129–142.

Goldsmith LA (ed.) (1983) *Biochemistry and Physiology of the Skin*, vols. I and II. London: Oxford University Press.
Hobson DW (1991) *Dermal and Ocular Toxicology: Fundamentals and Methods*. Boca Raton, FL: CRC Press.
Jackson EM and Goldner R (eds.) (1990) *Irritant Contact Dermatitis*. New York: Dekker.
Kemppainen BW and Reifenrath WG (eds.) (1990) *Methods for Skin Absorption*. Boca Raton, FL: CRC Press.
Maibach H (2000) *Toxicology of the Skin*. Boca Raton, FL: CRC Press.
Marzulli FN and Maibach HI (eds.) (1991) *Dermatotoxicology*, 4th edn. New York: Hemisphere.
Mukhtar H (ed.) (1992) *Pharmacology of the Skin*. Boca Raton, FL: CRC Press.
Roberts MS and Walters KA (1998) *Dermal Absorption and Toxicity Assessment*. New York: Marcel Dekker.
Smith Pease CK (2003) From xenobiotic chemistry and metabolism to better prediction and risk assessment of skin allergy. *Toxicology* 192(1): 1–22.

Relevant Website

http://www.epa.gov – US EPA. Dermal Exposure Assessment: Principles and Applications. EPA/600/8-91/011B. January 1992 Interim Report.

Smog *See* Great Smog of London.

Snake, Crotalinae

Gary W Everson

- SYNONYMS: Pit viper; Members of the Crotalinae subfamily in the United States include *Crotalus* species (rattlesnakes), *Agkistrodon* species (copperhead, cottonmouth), *Sistrurus* species (pigmy rattlesnake, massasauga)

Exposure Routes and Pathways

Most frequently, envenomation by North American pitvipers occurs subcutaneously. However, envenomation directly into an artery or vein has been documented and is associated with a rapid progression of life-threatening symptoms such as shock and cardiovascular collapse.

Toxicokinetics

Systemic absorption of venom is dependent on lymphatic transport following subcutaneous envenomation. The onset of local symptoms such as swelling and ecchymosis occurs within several hours. Cardiovascular, neurological, or hematological compromise varies in onset but may occur within 10–15 min following intravenous or intraarterial envenomation. The metabolism of venom components is not well understood. It is likely that venom components are inactivated by enzymes within tissues where the venom is ultimately distributed. The distribution of venom is variable and complex and possibly reaches different tissue sites unevenly. The biological half-life of Crotalinae venom has not been determined. Metabolized venom fractions are primarily eliminated by the kidney.

Mechanism of Toxicity

Snake venoms are complex mixtures of several different components or 'fractions' that can vary considerably within Crotalinae members. A complete review of venom components is beyond the scope of this review. Depending on the content of the venom, multiple organ systems may be affected. Historically, Crotalinae venom was classified as neurotoxic, hemotoxic, cardiotoxic, or myotoxic, depending on the species of snake involved in the envenomation. This oversimplifies the complex nature of Crotalinae venom. Clinically, a patient may develop such multisystem disorders as platelet destruction, internal bleeding, hypotension, paresthesias, and rhabdomyolysis.

Acute and Short-Term Toxicity (or Exposure)

Human

The severity of envenomation varies greatly and is dependent on various factors including the species involved, amount of venom injected, depth of envenomation (subcutaneous, venous, arterial), and the age of the victim. In general, Crotalinae venom initially produces local tissue changes that manifest

as swelling, ecchymosis, bruising, petechiae, pain, and erythema. Swelling may progress to involve the entire affected limb. These local symptoms commonly develop within minutes to several hours following envenomation. Because of poor tissue perfusion, local skin sloughing, and tissue necrosis may occur.

Crotalinae bites rarely penetrate the muscular fascial plane, consequently swelling of an envenomated extremity may be severe but rarely involves muscle compartments. In ~25% or more of Crotalinae bites, no venom is injected (dry bite). However, patients with dry bites may exhibit symptoms of erythema and slight swelling at the bite site due to trauma. Symptoms of dry bites are usually limited to the immediate area of the bite and require only wound management and follow-up care if necessary.

Systemic symptoms following envenomation may include paresthesias, coagulation disorders, thrombocytopenia, active bleeding, decreased hemoglobin, disseminated intravascular coagulation, hypotension, EKG changes, decreased level of consciousness, and rhabdomyolysis. In contrast to the venom of most rattlesnakes, the venom components of the Mohave Green rattlesnake (*Crotalus scutulatus scutulatus*) possess significant neurotoxic properties. Following Crotalinae envenomation, there is little local swelling and edema, which is normally used to measure extent of envenomation. Symptoms of envenomation from neurotoxic venom components may include paresthesias of the face and tongue and cranial nerve dysfunction resulting in ptosis and diplopia. The patient may experience confusion, disorientation, and coma. Generalized muscle weakness and shallow respirations may require intubation.

Clinical Management

Most first-aid measures that have been historically employed are of little value and some are dangerous and worsen medical outcome. The use of ice to prevent the spread of venom has been linked to an increased frequency of limb amputations and should never be employed. The incision of fang marks to relieve venom is ineffective and can result in nerve or artery damage. Tourniquet use may impede blood flow in the affected limb and contribute to local tissue damage. The application of electric shock at the bite site has shown to be ineffective in clinical trials and is also dangerous. However, the use of a properly applied constriction band as opposed to a tourniquet may possibly be effective in slowing the lymphatic distribution of venom. Anyone bitten by an unidentified snake requires evaluation in an emergency facility equipped to provide basic and advanced clinical life support. Aggressive supportive care is at least as important as the proper administration of antivenom in the outcome of a patient bitten by a venomous snake.

It is important to evaluate the clinical presentation of the patient as well as laboratory data to determine and guide the administration of antivenom. However, antivenom is not required in all patients who are envenomated and may not be necessary if there is no significant tissue swelling, systemic symptoms are absent, and laboratory parameters are normal.

When symptoms develop, a decision to start antivenom should be made. Patient response to the antivenom must be evaluated at frequent time intervals following administration of the initial dose to determine if further antivenom is required. CroFab™, a polyvalent sheep-derived (ovine) antivenom produced by Protherics, was approved by the Food and Drug Administration and is preferred over the older equine-based, Antivenin (Crotalidae) Polyvalent™ (Wyeth), because it is less antigenic and is now distributed much more widely than the Wyeth product. CroFab™ is marketed by Savage Laboratories. CroFab™ consists of highly purified ovine Fab fragments capable of neutralizing the toxic effects of most North American Crotalinae venoms. It contains a mixture of venom-specific Fab fragments and contains few of the proteins responsible for the allergic reactions associated with the Antivenin (Crotalinae) Polyvalent™ (Wyeth). Clinical trials to date have shown CroFab™ to be effective and safe in the treatment of Crotalinae envenomations. Acute reactions in the preliminary studies were minimal and serum sickness was virtually nonexistent. The initial dose of CroFab™ is four to six vials. Further doses may be given if symptoms continue to progress. Patients exhibiting life-threatening symptoms may require 30 or more vials of antivenom. The effectiveness of antivenom is directly related to its timely administration and the provision of adequate dosing. Clearly, the effectiveness of antivenom decreases as administration time is delayed.

See also: Animals, Poisonous and Venomous; Snake, Elapidae; Snakes.

Further Reading

Bond GR and Burkhart KK (1997) Thryombocytopenia following timber rattlesnake envenomation. *Annals of Emergency Medicine* 310: 40–44.

Clark RF, Williams SR, and Nordt SP (1997) Successful treatment of crotalid-induced neurotoxicity with a new polyspecific crotalid Fab antivenom. *Annals of Emergency Medicine* 310: 54–57.

Cruz NS and Alvarez RG (1994) Rattlesnake bite complications in 19 children. *Pediatric Emergency Care* 10: 30–33.

Snake, Elapidae

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- SYNONYMS: *Coral snake*; *Micruroides euryxanthus*; *Micrurus fulvius*; *Micruroides euryxanthus*

Exposure Routes and Pathways

Envenomation by North American species of Elapidae occurs subcutaneously. The bite differs from Crotalinae species in that coral snakes possess smaller fangs and tend to grasp and hold on rather than strike and release. Venom is discharged through hollow fangs and the chewing motion allows more venom to be injected into the bite site. Due to the relatively small fang size, envenomation into an artery or vein is not likely.

Toxicokinetics

Systemic absorption of Elapidae venom is dependent on lymphatic transport following subcutaneous envenomation. The onset of neurotoxic symptoms usually occurs within 4 h but can be delayed up to 10 h following a bite. The metabolism of venom components is not well understood. It is likely that venom components are inactivated by enzymes within tissues where the venom is ultimately distributed. The distribution of venom is variable and complex and possibly reaches different tissue sites unevenly. The biological half-life of Elapidae venom has not been determined. It is likely that metabolized venom fractions are eliminated primarily by the kidneys.

Mechanism of Toxicity

Elapidae venom is composed of different components that vary among species. The venom of North American species contains fractions that are primarily neurotoxic. The venom results in a bulbar-type cranial nerve paralysis. In contrast to Crotalinae species, venom from North American elapids lacks most of the enzymes and spreading factors that cause local tissue destruction. Elapids from countries other than the United States can contain venom components different than that of North American coral snakes.

Acute and Short-Term Toxicity (or Exposure)

Human

The severity of an envenomation varies greatly and is dependent on various factors including the species of snake, amount of venom injected, and the age of the victim. Envenomation may occur despite the absence of identifiable fang marks at the bite site. Coral snake venom causes very little local tissue changes. Mild swelling, pain, and redness at the immediate bite site are generally the limit of the local reaction. The degree of local tissue reaction does not correlate with the degree of systemic symptoms, which may appear much later. Typically, lightheadedness, dizziness, or drowsiness marks the onset of systemic toxicity. Generalized muscle weakness, fasciculation, and tremor may develop. Increased salivation, nausea, and vomiting are also common. Neurological symptoms may progress to include slurred speech, ptosis, dysphagia, visual disturbances, muscle paralysis, and respiratory depression. Neurological symptoms may be delayed for up to 12 h after envenomation. Seizures may occur, especially in children. Death results from respiratory depression, hypotension, and cardiovascular collapse. The bite of the Arizona coral snake is associated with less severe progression of symptoms than that of the Eastern or Texas coral snakes. Headaches, blurred vision, and ataxia may be the limit of neurological symptoms following envenomation by the Arizona coral snake. Venom from exotic Elapidae species (cobras, kraits, mambas, and allies) may contain toxins that target the heart, coagulation factors, and other sites in addition to the central nervous system. Although rare, bites from exotic species may occur within the United States (e.g., zoo employees and herpetoculturists). In these cases, contacting the local poison center is essential in determining the nearest location of specific antivenom.

Clinical Management

Basic and advanced clinical life support is essential in the successful management of any coral snake envenomation. A coral snake bite is a medical emergency and requires immediate transport to the emergency department. Aggressive respiratory and cardiovascular support can be life saving. Establishing intravenous fluid support should be started soon after the bite. Early administration of *Micrurus fulvius* (Equine)® antivenin is essential following envenomation by the Eastern and Texas coral snakes. The antivenom is not effective for bites of the Arizona coral snake. Since local symptoms do not correlate with the severity of the envenomation, antivenom should be administered as soon as possible following envenomation, despite the absence of neurological symptoms. Three to five vials of

antivenom should be diluted in 100–500 cc of 0.9% sodium chloride. This should be infused intravenously over 30 min. This antivenom is equine based; anaphylaxis and delayed hypersensitivity reactions are not uncommon. A small amount of diluted antivenom should be administered as a test dose to check for allergic response. This procedure is outlined in the package insert included with the antivenom. Patients who exhibit a negative allergic response following the test dose may still develop an anaphylactic reaction. Therefore, one should be prepared at all times to treat an allergic reaction to the antivenom. Epinephrine, intravenous antihistamines, and corticosteroids should be readily available. Close observation of the patient is required to determine the patient's response to the antivenom. Additional doses may be required should neurological symptoms progress. Most envenomations require from three to ten vials of antivenom and further doses may be required in those patients exhibiting life-threatening symptoms. As in any snakebite, infection is possible and a broad-spectrum antibiotic could be considered. In addition, tetanus prophylaxis should be provided. Serum sickness may occur following the use of the equine-based antivenom. Although serum sickness is usually mild, an outpatient course of corticosteroid may be required in some cases.

Most first-aid measures are of little value and some are dangerous. The use of ice to prevent the spread of the venom has been linked to an increased frequency of limb amputations and should never be employed. Field procedures such as fang mark incisions may result in vein or artery damage and improperly placed tourniquets may impede blood flow. Electric shock directed at the site of envenomation has not been proven effective and is a dangerous procedure.

See also: Animals, Poisonous and Venomous; Snake, Crotalinae; Snakes.

Further Reading

Coelho LK, Silva E, and Espositto C (1992) Clinical features and treatment of elapidae bites: Report of three cases. *Human & Experimental Toxicology* 11: 135–137.

Kitchens CS and Van Mierop LHS (1987) Envenomation by the Eastern coral snake (*Micrurus fulvius fulvius*) a study of 39 victims. *Journal of the American Medical Association* 258: 1615–1618.

Parrish HM and Khan MS (1967) Bites by coral snakes: Report of 11 representative cases. *American Journal of Medical Science* 253: 561–568.

Snakes

Randy Powell

Introduction

There are over 2950 species of snakes currently recognized. Although all are limbless ectotherms, snakes occupy a variety of ecosystems and environments and exhibit a wide assortment of morphological, physiological, behavioral, and ecological adaptations. These adaptations have enabled snakes to occupy terrestrial, fossorial, arboreal, aquatic, and marine niches in almost every ecosystem on every continent except for Antarctica. Size variation among snakes is considerable, ranging from the smallest, less than 150 mm blind snakes and shield-tail snakes to the largest species of pythons and boas, which can reach lengths of over 8 m. Because of several anatomical adaptations to the skull and head, snakes are able to swallow prey items much larger in relationship to their own size. All snakes are carnivores and they employ different methods to overpower and kill their prey including: seize and swallow, constriction, and the use of venom. Smaller snakes that eat invertebrates and small vertebrates (earthworms, insects, frogs, etc.) utilize the seize and swallow technique, which entails grabbing the prey item with their mouth and swallowing it whole, usually while it is alive. Snakes that constrict also grasp the prey item first with their mouths and then quickly wrap their body around it and contract their muscles. The prey item is incapacitated as blood flow and respiration is increasingly restricted until death occurs.

Approximately 30–40% of snake species produce some type of buccal toxins or venom. The major adaptive functions of venoms and buccal toxins in snakes have been associated with food acquisition, defense, and predigestion of prey items. Venomous species possess specialized structures (venom glands or Duvernoy's glands), which produce venom or toxins and enlarged teeth or fangs, which inject or introduce the venom into the prey item. Venom contains enzymes and proteins that break down cellular structures and interferes with critical functions such

as circulation, ventilation, and nerve impulses. When a prey item is envenomated, pronounced physiological responses occur within the animal resulting in immobilization and ultimately death. In the case of human envenomation, the effects are a very serious medical emergency that can result in permanent scarring, dysfunction, the loss of a limb, or death. While the majority of snakes are nonvenomous and harmless there are numerous species that are medically important and dangerous to humans. Venomous snakebite remains a public health problem in many areas throughout the world and the incidence of global mortality from snake envenomation is estimated at 125 000 cases per year (**Table 1**).

Venomous Species

All venomous snake species occur within the superfamily Colubroidea 'advanced snakes', a large and ecologically diverse group distributed worldwide. Within Colubroidea there are five families that have species (all or in part), which are considered venomous: Elapidae, Viperidae, Hydrophiidae, Atractaspididae, and Colubridae.

The Elapidae family (cobras, coral snakes, and mambas) are a group of fast moving alert snakes. They have fixed nonretractable fangs located anteriorly on each maxillary bone (proteroglyphs). The majority of elapids are terrestrial with a few arboreal, semifossorial, or aquatic species. Elapids are distributed throughout Australia, Africa, the Americas, and southern Asia. Most elapids are small to moderate in size (0.5–2 m) with a few species reaching 3 m or more (the king cobra, *Ophiophagus hannah*, reaches lengths of over 5 m). Other noteworthy examples include the black mamba, *Dendroaspis polylepis*, a large aggressive and extremely fast snake from central Africa, numerous species of brightly colored coral snakes found throughout the Americas, and the ringhal or ring-necked spitting cobra, *Hemachatus haemachatus*, one of several species that have the ability to project or spray their venom.

Table 1 Annual deaths from snakebite worldwide

Country	*Estimated number of human fatalities*
Africa	20 000
Asia	100 000
Australia	<10
Central America (including Mexico)	1000
Europe	<40
Middle East	100
North America	<20
Oceania	200
South America	4000
Total	125 000

The Viperidae family (vipers and pit vipers) members are robust bodied with a distinct neck and somewhat triangular or club-shaped heads. Their moveable fangs located anteriorly on the maxillary bone are retractable and are folded against the roof of the mouth when not in use (solenoglyphs). Most viperids are terrestrial with a few arboreal and semiaquatic species. They are distributed worldwide with the exception of Australia and oceanic islands. Viperids can range in size from less than 25 cm to the large bushmasters, *Lachesis* spp., which can reach 3 m in length. Examples of viperids include the sawscaled viper, *Echis carinatus*, a wildly distributed species responsible for thousands of human fatalities each year, the gaboon viper, *Bitis gabonica*, a large tropical African species whose fangs can measure up to 50 mm, and the rattlesnakes (genera *Crotalus* and *Sistrurus*) found throughout the Americas.

The family Hydrophiidae (sea snakes and allies) includes both aquatic and terrestrial species. The aquatic species have laterally compressed bodies and paddle-like tails with many terrestrial taxa resembling viperids in body and head shape. All hydrophiines are proteroglyphs with fixed fangs located anteriorly on each maxillary bone. Their distribution includes tropical Indian and Pacific oceans, Australia, and New Guinea. Their size ranges from 30 cm to over 2.5 m. Some of the most toxic venoms are found in hydrophiines (**Table 2**). Noteworthy examples include the inland taipan or fierce snake, *Oxyuranus microlepidotus*, from central Australia, which has some of the most toxic venom of any terrestrial snake, the death adders, *Acanthophis* spp., and the strikingly colored yellowbelly sea snake, *Pelamis platura*.

The Atractaspididae family (burrowing asps, mole vipers, and stiletto snakes) are a group of terrestrial and fossorial snakes with short heads, cylindrical bodies, blunt noses, and rather small eyes. They are distributed throughout sub-Sahara Africa and the Arabian peninsular coast. Atractaspidids can range in size from less than 25 cm up to 1 m in length. The movable fangs are located on the maxillary bone, either anteriorly or posteriorly. They are unique in that each fang can be rotated laterally allowing it to be extended out from the side of the snake's closed mouth. Most atractaspidids are not considered dangerous to humans. However, some of the larger *Atractaspis* spp. should be regarded as dangerous and deaths have resulted from bites.

The family Colubridae (rat snakes, water snakes, racers, and allies) represents by far the most speciose

Table 2 Comparative toxicities of snake venoms

Species	*Family*	LD_{50}
Inland taipan, *Oxyuranus microlepidotus*	Hydrophiidae	0.025
Eastern brown snake, *Pseudonaja textiles*	Hydrophiidae	0.037
Reef shallows sea snake, *Aipysurus duboisii*	Hydrophiidae	0.044
Yellow bellied sea snake, *Pelamis platurus*	Hydrophiidae	0.067
Spiny-headed sea snake, *Acalyptophis peronii*	Hydrophiidae	0.079
Northern taipan, *Oxyuranus scutellatus*	Hydrophiidae	0.106
Black mamba, *Dendroaspis polylepis*	Elapidae	0.32
Eastern coral snake, *Micrurus fulvius*	Elapidae	1.30
King cobra, *Ophiophagus hannah*	Elapidae	1.8
Sidewinder, *Crotalus cerastes*	Viperidae	4.00
Puff adder, *Bitis arietans*	Viperidae	7.75
Boomslang, *Dispholidus typus*	Colubridae	10.00
Gaboon viper, *Bitis gabonica*	Viperidae	12.5
Copperhead, *Agkistrodon contortrix*	Viperidae	25.6

LD_{50} values equal milligram per kilogram administered subcutaneously in mice. LD_{50} values can be useful in establishing relative toxicity but do not necessarily extrapolate to other species. Venom toxicities within the same species can vary considerably across geographic ranges.

family of snakes. They are a diverse group both in body form and ecology with species inhabiting terrestrial, arboreal, fossorial, and aquatic habitats. Colubrids are distributed worldwide with the exception of oceanic islands and range in size from 20 cm to 3.5 m or more. Most of the colubrids have teeth without any groove or canal (aglyphic) and are considered harmless. However, many colubrids possess enlarged posterior maxillary teeth (opisthoglyphs) and specialized oral glands (Duvernoy's gland). Buccal toxins or 'venom' are secreted from Duvernoy's gland under low pressure from around the base of the enlarged teeth. The venom is not directly injected but is introduced more indirectly into the bite wound. Most of the 'venomous' colubrids are not considered dangerous to humans. However, there are several larger species that are 'mildly toxic' and bites can result in localized pain, edema, and ecchymosis and a few species are regarded as quite dangerous. Noteworthy examples in which human fatalities have been attributed to include the boomslang, *Dipholidus typus*, the tiger keelback, *Rhabdophis tigrinus*, and the vine snake, *Thelotornis capensis*.

Snake Venom

Snake venoms are complex mixtures of enzymes and proteins of various sizes, amines, lipids, nucleosides, and carbohydrates. Venoms also contain various metal ions that are presumed to act as cofactors and include sodium, calcium, potassium, magnesium, and zinc. Snake venoms have been studied much more thoroughly in members of the families Elapidae, Hydrophiidae, and Viperidae, with considerably less knowledge regarding venoms from Atractaspididae and Colubridae. There is a large degree of variability in venom composition at all taxonomic levels. In addition, within the same species, venom components have been shown to vary considerably among populations and across geographical areas. Venoms act on a variety of cells and tissues with pronounced physiological responses. Some of the actions of venom components include the digestion of cells and cell membranes, disruption of procoagulant and anticoagulant activities of blood, production of oxidizing agents, breakdown of collagen and the intercellular matrix between cells, and the disruption of nerve tissue. Snake toxins with defined actions include neurotoxins, hemotoxins, cardiotoxins, cytotoxins, and myotoxins.

Snake venom components can be grouped by their molecular weight. Low-molecular weight components (<1500 Da) are usually considered the least physiologically active and includes peptides, lipids, nucleosides, carbohydrates, amines, and metal ions. Larger venom components (mol. wt. 4500–10 000 Da) include polypeptide toxins such as postsynaptically acting neurotoxins and myotoxins. The largest components, the enzymes (mol. wt. 13 000–150 000 Da), comprise a diverse group and produce marked physiological effects. The percentage of enzymes in snake venom can vary widely and can constitute as much as 90% or more in some of the viperid venoms and as little as 25% in some elapid venoms. There are over 30 enzymes that have been identified in snake venoms (**Table 3**) including some that are common to all venomous snake families.

Venom and Research

Venomous snakes and venom have always been of interest to biologists. Historically, snake venoms were viewed as a valuable aid and were frequently used in early medical therapies. Ancient Egyptian and Chinese physicians utilized snake venoms as treatment for a variety of ailments and diseases. For over a century, snake venom has been used to develop antivenoms to treat snakebite envenomation. Currently, there are over 30 facilities worldwide that produce $\sim$120 different commercially available antivenoms. These antivenoms include both monovalent forms (effective for a specific species) and polyvalent forms (generally effective for several species that occur

Table 3 Enzymes found in snake venom

Family	*Enzymes*
Common in all families: Atractaspididae, Elapidae, Viperidae, Hydrophiidae, Colubridae	Adenosine triphosphate, L-amino acid oxidase, amylase, catalase, deoxyribonuclease, hyaluronidase, NAD-nucleosidase, 5′-nucleotidase, peptidase, phosphodiesterase, phospholipase A_2, phosphomonoesterase, ribonuclease
Prominent in Elapidae and Hydrophiidae	Acetylcholinerase, dehydrogenase lactate, glycerophosphatase, phospholipase B
Prominent in Viperidae	Argine ester hydrolase, collagenase, endopeptidase, factor X activator, fibrinogenase, kininogenase, metalloproteinase, prothrombin activator, serine protease, thrombin-like enzyme
Found in some Viperidae and Elapidae	Alkaline phosphatase, acid phosphatase, heparinase, lysophospholipase

within a limited region or country). Currently, there is an intensified interest in venom, particularly venom components or fractions. Numerous bioactive components and enzymes are present in snake venom. The modes of action and interaction on cells and tissues produced by these bioactive components make snake venom a tremendous interest to researchers. Venom components are used in basic research in physiology and biochemistry to delay or increase biochemical and cellular processes. Snake venom components are being used in a variety of medications and diagnostic tests. Examples of snake venom derived medications include Captopril (an angiotensin-converting enzyme inhibitor used for the treatment of hypertension and other cardiovascular disorders), Ancrod (an anticoagulant used in stroke patients), Reptilase (used to measure blood plasma clotting time and to diagnose dysfibrinogenemia), Cobroxin (an analgesic drug used to block nerve transmission), Nyloxin (used for severe arthritis pain), and Integrelin (used to treat acute coronary syndrome). In addition, preliminary data suggests snake venom components may yield new drugs to treat a variety of conditions from strokes and cancer to hypertension, heart disease, and neuromusculoskeletal disorders.

See also: Animals, Poisonous and Venomous; Snake, Crotalidae; Snake, Elapidae.

Further Reading

Greene HW (1997) *Snakes: The Evolution and Mystery in Nature*. Los Angeles: University of California Press.
Harvey AL (ed.) (1991) *Snake Toxins International Encyclopedia of Pharmacology and Therapeutics,* section 134. New York: Pergamon.
Russell FE (1983) *Snake Venom Poisoning*. New York: Scholium International, Inc. New York.
Tu AT (ed.) (1991) *Handbook of Natural Toxins Reptile Venoms and Toxins*, vol. 5. New York: Dekker.
Uetz P, Etzold, T, and Chenna R (2002) The European Molecular Biology Laboratory Reptile Database.
Zug GR, Vitt LJ, and Caldwell JP (2001) *Herpetology: An Introductory Biology of Amphibians and Reptiles*. London: Academic Press.

Relevant Website

http://www.embl-heidelberg.dc – The European Molecular Biology Laboratory Reptile Database.

Sodium

Shayne C Gad

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- Representative Compounds: Sodium chloride (NaCl); Sodium azide (NaN_3)
- Chemical Abstracts Service Registry Number: CAS 7440-23-5
- Synonym: Natrium
- Chemical/Pharmaceutical/Other Class: Alkali metals
- Chemical Formula: Na^+

Uses

Numerous industries use sodium compounds. They are used in detergents, hair straighteners, glass, paper, textiles, and wood pulp. Sodium chloride (table salt) is used in ion exchangers to soften water, and sodium bicarbonate is used in beverages, baking soda, and antacid pills. Sodium azide is used in

insecticides, and was investigated as an antihypertensive. Sodium violently decomposes in contact with water, forming sodium hydroxide and hydrogen, which may ignite spontaneously.

Background Information

Sodium is 2.83% of the crust of the earth. It is extremely water reactive, forming explosive hydrogen gas and Lye (NaOH).

Exposure Routes and Pathways

Ingestion is the primary route of exposure to sodium. Many foods contain sodium chloride naturally (e.g., milk, cheese, shellfish, and, to a lesser extent, meat and poultry). Nonetheless, most people add extra table salt to their food to the extent of 2000–7000 mg day^{-1}. In addition, all water supplies tested and nearly all carbonated beverages contain sodium. Inhalation of sodium is a minor route of exposure except in some industrial environments. Sodium in the air comes from the oceans. Dermal absorption is not normally considered an important exposure pathway.

Toxicokinetics

Ingested sodium compounds are usually completely absorbed. Once absorbed, sodium is distributed throughout all tissues in the body. Most sodium is found in the plasma. Urine and perspiration are the major routes of excretion. Heat and hard physical labor can contribute to excessive loss of sodium.

Mechanism of Toxicity

Very little is known about sodium's mechanism of toxicity. There is practically no information on the effect of sodium on enzymes. No information is available on metabolic alterations of the sodium ion.

Acute and Short-Term Toxicity (or Exposure)

Human

The metal itself is very corrosive to eye or skin. Sodium is associated with hypertension. Excess sodium results in an increase of extracellular fluid volume. Under these conditions the plasma protein concentration decreases. Sodium is an emetic; intake of excess sodium leads to nausea and vomiting. The accidental substitution of table salt for sugar has resulted in sodium poisoning in infants. These infants experienced increased body temperature, muscle twitching, and convulsions; in some cases, their kidneys were damaged. Sodium compounds with high pH values in solution (e.g., sodium hydroxide) are extremely corrosive to the skin and mucous membranes.

Chronic Toxicity (or Exposure)

Animal

Laboratory animals given a high salt diet develop hypertension.

Clinical Management

For extremely high sodium intake, peritoneal dialysis is the treatment of choice to lower the plasma sodium concentration. For exposure to sodium hydroxide, clinical management of skin corrosion is indicated.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists short-term exposure ceiling limit is 2 mg m^{-3}.

See also: Iodine; Potassium.

Further Reading

Fozzard HA (2002) Cardiac sodium and calcium channels: A history of excitatory currents. *Cardiovascular Research* 55: 1–8.

Glynn IM (2002) A hundred years of sodium pumping. *Annual Review of Physiology* 64: 1–18.

Goyer RA, Klaassen CD, and Waalkes MP (1995) *Metal Toxicology*. San Diego, CA: Academic Press.

Relevant Website

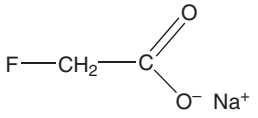
http://www.foodandhealth.com – Kenney JJ. Salt toxicity: Is it a major threat to public health? *Communicating Food for Health Newsletter*, April 1999–June 1999.

Sodium Fluoroacetate

David R Wallace

This article is a revision of the previous print edition article by Janice Reeves and Carey Pope, volume 3, pp. 186–187, © 1998, Elsevier Inc.

- Chemical Abstracts Services Registry Number: CAS 62-74-8
- Synonyms: 1080; SFA; Sodium monofluoro-acetate; FAA; Gifblaar poison; FAA
- Chemical/Pharmaceutical/Other Class: Rodenticide; Predacide
- Chemical Formula: $C_2H_2FNaO_2$
- Chemical Structure:



Uses

Fluoroacetate is primarily used as a rodenticide and is only available to licensed pesticide applicators. It is also used as a predacide against coyotes.

Exposure Routes and Pathways

Ingestion, inhalation, and dermal exposures are all possible, but ingestion is the major route of exposure. Sodium fluoroacetate is rapidly absorbed from the gastrointestinal tract and by the lungs.

Toxicokinetics

Fluoroacetate is rapidly absorbed by the gastrointestinal tract but not well absorbed dermally. Fluoroacetate is converted to the ultimate toxicant, fluorocitrate. Fluoroacetate is distributed to lipid-rich organs, such as the liver, brain, and kidneys. Fluoroacetate is primarily eliminated through urine. Up to 50% of the fluoroacetate is excreted unchanged in the urine by 72 h following administration. The kinetic half-life for sodium fluoroacetate is species dependent. Reported half-lives in rabbits, goats, and sheep are 1.1, 4–7, and 13.3 h, respectively.

Mechanism of Toxicity

Fluoroacetate produces its toxic action (after conversion to fluorocitrate) by inhibiting the Kreb's cycle. The compound is incorporated into fluoroacetyl coenzyme A, which condenses with oxaloacetate to form fluorocitrate. This inhibits the enzyme aconitase, which inhibits conversion of citrate to *cis*-aconitic acid/isocitrate. This inhibition will lead to a buildup of citric acid resulting in convulsions and death from cardiac failure or respiratory arrest. Mitochondrial uptake of acetate may also be affected. The heart and central nervous system (CNS) are the tissues most affected by this inhibition of oxidative energy metabolism. Oxygen consumption is markedly reduced. In addition to blockade of energy production, depletion of calcium may also be involved in the clinical manifestations associated with sodium fluoroacetate toxicity.

Acute and Short-Term Toxicity (or Exposure)

According to EPA RED facts about sodium fluoroacetate is characterized as a Toxicity Category I compound, the highest level of toxicity, with acute oral administration. It is a Toxicity Category II (moderate toxicity) for acute dermal exposure, Toxicity Category III (slightly toxic) as an eye irritant, and Toxicity Category IV (virtually nontoxic) for acute dermal exposure. Acute systemic toxicity resembles that of a metabolic poison with the target organs being the cardiovascular system, lungs, kidneys, and CNS. Generally, cold-blooded animals are more resistant to the effects of sodium fluoroacetate than are warm-blooded animals. There are also differences QJ;between herbivores and carnivores, with herbivores exhibiting more cardiovascular toxicity and carnivores more CNS effects. Omnivores show mixed effects.

Animal

Fluoroacetate is a compound of very high acute toxicity. Oral LD_{50} values in laboratory rodents range from 0.2 to 2 mg kg^{-1}. In mid- to high doses, testicular atrophy and renal tubule degeneration were observed in subchronic studies. There is a large variation in toxicity of fluoroacetate which is not due to differences in size of animal, type of digestive system, or basal metabolic rate. The variation may be due to the rate of elimination or rate of condensation of the poison with oxaloacetate. The oral LD_{50} in mammals is 110 μg kg^{-1}. In a 13-week oral gavage study in rats the no observed adverse effect level and the lowest observed adverse effect level were determined to be 0.05 and 0.20 mg kg^{-1} day^{-1}, respectively. The signs associated with this study were increased heart rate in both males and females,

decreased testis weight and altered spermatogenesis in males.

Human

Acute fluoroacetate poisoning can result in nausea, vomiting, cardiac arrhythmia, cyanosis, generalized convulsions, hypotension, and death from ventricular fibrillation or respiratory failure. Residual effects are uncommon if the patient survives the acute toxicity.

The LD_{Lo} (oral) for humans is $714\,\mu g\,kg^{-1}$. The American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value – time-weighted average (TLV – TWA) is $0.05\,mg\,m^{-3}$. The probable lethal dose in humans is less than $5\,mg\,kg^{-1}$.

Chronic Toxicity (or Exposure)

Chronic exposure can lead to profound effects, both in the central and peripheral nervous systems.

Animal

Exposure to sodium fluoroacetate at a concentration of 26 ppm resulted in reversible growth retardation in rats with damage to the testes and sperm in males. In sheep, inhalation of $0.11\,mg\,kg^{-1}\,day^{-1}$ resulted in myocardial damage. Myocardial damage in animals is usually fatal, but may be asymptomatic while the animal is at rest.

Human

Chronic inhalation in humans can result in neurological dysfunction, liver dysfunction, and renal degeneration in humans exposed up to 10 years. The initial symptoms evident are nausea and mental apprehension. Soon following initial symptoms, convulsions, generalized CNS depression, and coma will result. Cardiovascular effects following chronic exposure are usually fatal and are characterized by ventricular arrhythmias.

In Vitro Toxicity Data

Differential outcomes are evident depending on the preparation used to study the *in vitro* effects of sodium fluoroacetate. Mitochondria-free preparations exhibit a K_i value of 22–45 μM for inhibition of aconitase by fluoroacetate. Aconitase bound to mitochondria appears to be much more sensitive to the effects of fluoroacetate: fluoroacetate inhibits aconitase in these preparations in the picomolar concentration range. Inhibition of the tricarboxylic acid cycle in actrocytes results in a depletion of ATP and a concomitant reduction in glutamine synthetase activity, resulting in elevations in glutamate concentration. Reuptake of glutamate is also inhibited due to reductions in ATP-dependent Na^+/K^+ pumps.

Clinical Management

The patient should be moved to fresh air. Decontamination of eyes and skin should be immediate. Treatment for skin and eye contamination should consist of rinsing with copious amounts of water for 10–15 min. For oral exposure, gastric lavage is preferable to emesis and should be prompt. Emesis should be avoided due to the potential for arrhythmias and convulsions. Charcoal should be administered as a slurry to block absorption of sodium fluoroacetate. Although there are no antidotes available, acetamide has been used with some success in a 10% solution in 5% glucose. Solution of calcium gluconate and sodium succinate ($130{:}240\,mg\,kg^{-1}$) has also exhibited some therapeutic benefit. Treatment is largely symptomatic. Respiratory and cardiovascular support is often necessary with significant exposures. Anticonvulsants (barbiturate) and antiarrhythmic (procainamide) agents are useful. Competition with acetate (in the form of acetamide or monoacetin) is recommended. Ethanol appears to be beneficial. Mephentermine is more efficacious than norepinephrine in raising blood pressure. Evidence of fluoroacetate poisoning can be difficult, but may be determined by fluorocitrate concentration in blood or by measuring increasing levels of citrate.

Environmental Fate

There is evidence that leaching and metabolism are the major routes of dissipation. Sodium fluoroacetate that has not undergone degradation is considered mobile by the Environmental Protection Agency (EPA) and has a high risk for movement into the soil and the ground water. Once adsorbed in soil, sodium fluoroacetate can be degraded by halidohydrolase in many microbial and fungal species. The 'half-life' of sodium fluoroacetate in soil is dependent on temperature, weather, initial amount of chemical, and decomposition of the host animal. There have been no reports that sodium fluoroacetate can leach into water and reach levels exceeding that which would be deemed toxic.

Ecotoxicology

Acute oral exposure of sodium fluoroacetate has been shown to be highly toxic to mallard ducks, chukar, ring-necked pheasants, widgeons, golden

eagles, and black vultures. Due to its use as 'toxic collar' predacide, sodium fluoroacetate has been shown to be extremely toxic to coyotes and other small wild rodents. Other nontarget animals which may be affected by sodium fluoroacetate toxicity include birds and other small animals that may feed on the neck of deceased animals which had a toxic collar. Reptiles and amphibians are relatively resistant.

Exposure Standards and Guidelines

The exposure limits (permissible exposure limits) set by the Occupational Health an Safety Administration is 0.05 mg m^{-3} for skin in both general industry and construction industry. This is the same limit (0.05 mg m^{-3}) established by ACGIH (TLV) and the National Institute for Occupational Safety and Health (recommended exposure limit).

See also: Gastrointestinal System.

Further Reading

Gribble GW (1973) Fluoroacetate toxicity. *Journal of Chemical Education* 50: 460–462.

Smith FA, Gardner DE, Yuile C, de Lopez OH, and Hall LL (1977) Defluorination of fluoroacetate in the rat. *Life Sciences* 20: 1131–1138.

United States Environmental Protection Agency (US EPA) (1995) *R.E.D. FACTS: Sodium Fluoroacetate*. EPA-738-F-95-022.

Relevant Websites

http://www.osha.gov – Occupational Safety and Health Administration. Chemical Sampling Information: Sodium Fluoroacetate.

http://www.epa.gov – United States Environmental Protection Agency Integrated Risk Information System (IRIS), Sodium Fluoroacetate (CASRN 62-74-8).

Sodium Sulfite

Stephen R Clough

- Chemical Abstracts Service Registry Number: CAS 7757-83-7
- Synonyms: Anhydrous sodium sulfite; Disodium sulfite; Exsiccated sodium sulfite; Sulftech; Natriumsulfit (German); Sodium sulfite anhydrous; Sodium sulphite; Sulfurous acid; Disodium salt; Sodium salt (1:2)
- Chemical/Pharmaceutical/Other Class: Inorganic salt
- Chemical Formula: Na_2SO_3

Uses

Sodium sulfite is an odorless, solid white powder with a salty sulfurous taste that is soluble in water. It is a reducing agent that is used as a food preservative and antioxidant. Its use is prohibited in meats and other sources of vitamin B_1. Sodium sulfite is also used in the treatment of semichemical pulp in the paper industry, in the treatment of water, as a photographic developer, and in textile bleaching (antichlor). It has also found historical use in the water treatment field as a dechlorinating agent.

Exposure Routes and Pathways

Sodium sulfite is not an 'environmental' pollutant *per se*, but its wide use as a food additive may lead to widespread exposure of the general population to trace amounts through ingestion. This may pose a problem for a small percentage of people who are hypersensitive to this chemical. Exposure to elevated concentrations (i.e., those that might cause abject toxicity) of this compound would only be expected to occur in the workplace, primarily involving sources of production or bulk use as mentioned previously. Because this compound is packaged as a powder, exposure would be expected to occur from airborne dust. Potential exposure routes would thus include skin, inhalation, and possibly involvement of the eye, nose, and throat.

Mechanism of Toxicity

The exact mechanism of toxicity has not been elucidated, although there is a lot of information on how sulfur-based compounds are detoxified by the liver. Sodium sulfite is a mild reducing agent that would most likely cause burning or irritation at the site of exposure or application by altering oxidation–reduction potential and pH.

Sulfites are used widely as antioxidants to keep foods from prematurely spoiling and to keep them looking 'fresh' by preventing oxidation and subsequent 'browning'. Many people, however, are 'sulfite sensitive'. After ingestion of food or beverages containing sulfite, these people may have allergic-type reactions such as asthmatic wheezing, hypotension,

tingling sensations, and flushing of the skin. The mechanism is unclear but probably has to do with an individual-specific chemical stimulation of the immune system, which in turn releases small amounts of vasoactive substances.

Acute and Short-Term Toxicity (or Exposure)

Animal

The median lethal dose (LD_{50}) measured for a mouse was $820\,mg\,kg^{-1}$. The LD_{50} for a rabbit ($2825\,mg\,kg^{-1}$) indicated that sodium sulfite was more than 3 times less toxic to rabbits than to mice. The lowest lethal dose for a cat or dog, administered subcutaneously, was $1300\,mg\,kg^{-1}$, whereas only half that dose was required to have the same effect on a guinea pig or rabbit. The LD_{50} for a mouse, administered intraperitoneally, was similar to the oral route ($950\,mg\,kg^{-1}$).

Human

Concentrated forms (e.g., powders, mixtures) of sodium sulfite may be harmful following exposure by inhalation, ingestion, and skin contact. It is an eye, skin, and respiratory irritant. At the concentrations used as a food additive, sodium sulfite is not toxic *per se* in humans; however, as mentioned previously, it will pose a problem for individuals who are sensitive to this chemical following ingestion. Allergic-type responses include asthmatic wheezing, a feeling of increased warmth and flushing of the skin, hypotension, and tingling sensations. Because some food manufacturers may use sulfites sporadically, it may be difficult for sensitive persons to avoid these additives altogether.

Clinical Management

Persons who are sensitive to sulfites should avoid foods containing this additive (e.g., wines), and those exhibiting severe allergic-type reactions (e.g., difficulty breathing) following a meal or beverage should seek immediate medical attention.

Persons exposed to large quantities of the dust in air should vacate the high-exposure area and seek conventional medical treatment if adverse symptoms are seen or if discomfort persists. As with exposure to any potentially irritating dust, eyes should be irrigated with water immediately following exposure and skin should be thoroughly washed with warm soapy water.

Ecotoxicology

There is very little environmental effects data on sodium sulfite. Water fleas (*Daphnia* sp.) exposed to sodium sulfite for 24–48 h have an LC_{50} between 200 and $300\,mg\,l^{-1}$ (US Environmental Protection Agency ECOTOX database). The pH of the solution, however, can strongly affect how much of the compound is ionized and thus greatly affect the toxicity.

See also: Food Additives.

Further Reading

Nair B, Elmore AR; Cosmetic Ingredients Review Expert Panel (2003) Final report on the safety assessment of sodium sulfite, potassium sulfite, ammonium sulfite, sodium bisulfite, ammonium bisulfite, sodium metabisulfite and potassium metabisulfite. *International Journal of Toxicology* 22(Suppl 2): 63–88.

Relevant Website

http://toxnet.nlm.nih.gov – Specialized Information Systems, National Library of Medicine, Search for Sodium Sulfite.

Soil Pollution *See* Pollution, Soil.

Solanum Genus

Christopher P Holstege

This article is a revision of the previous print edition article by Regina M Rogowski, volume 3, pp. 188–189, © 1998, Elsevier Inc.

- Synonyms: *S. aculeatissimum* (devil's apple, bull nettle); *S. americanum* (black nightshade); *S. carolinense* (horse nettle); *S. dulcamara* (European bittersweet, blue nightshade, woody nightshade, climbing nightshade); *S. eleagnifolium* (white horse nettle); *S. gracile* (bull nettle, wild tomato); *S. melongena* (eggplant); *S. nigrum* (common nightshade); *S. pseudocapsicum* (Jerusalem cherry, natal cherry); *S. rostratum* (Buffalo burr, sandbur, Colorado burr, Texas thistle); *S. seaforthianum* (blue flowered 'potato vine'); *S. sodomeum* (apple of Sodom); *S. tuberosum* (Irish or common potatoes); *S. triflorum* (three-flowered nightshade); *S. villosum* (harry nightshade). Related plants to *Solanum* genus include: *Lycopersicum esculentum* (tomato); *Physalis heterophylla* (ground cherry); *Physalis longifolia* (husk tomato)
- Chemical/Pharmaceutical/Other Class: Solanine is a glycoalkaloid. Solanine is found throughout the plant with highest concentrations in areas of high metabolic activity such as the sprouts, green skin, and stems. Solanine is in highest concentration in the unripe fruit and its presence decreases as the fruit ripens.

Exposure Routes and Pathways

Reports of toxicity are secondary to ingestion of berries or herbage.

Toxicokinetics

The time to peak serum levels of solanaceous alkaloids is variable and depends on the species and amount ingested. Reports suggest peak levels are attained in 4–8 h. Solanine is converted to solanidine by hydrolysis. Solanine is rapidly excreted in urine and feces. The elimination half-life of solanidine is reported to be 11 h.

Mechanism of Toxicity

The mechanism of human toxicity has not been clearly delineated. In animal models, solanaceous alkaloids inhibit cholinesterase activity and demonstrate cardiac glycoside activity. Solanine inhibits hepatic microsomal enzymes and can cause hemolysis.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals may develop acute mental status changes including confusion, indifference to surroundings, prostration, and stupor. Ulcerative stomatitis, conjunctivitis, eczema, and diarrhea have been reported. Treatment focus is on symptomatic and supportive care.

Human

Gastrointestinal and neurological effects predominate in toxicity form in the solanaceous alkaloids. Reported clinical effects include nausea, vomiting, diarrhea, hyperthermia, tachycardia, bradycardia, hypotension, dehydration, blurred vision, mydriasis, salivation, flushing, diaphoresis, muscular cramps, headache, drowsiness, confusion, weakness, hallucinations, delirium, paresthesias, coma, and death.

Chronic Toxicity (or Exposure)

Human

Chronic exposure may produce more pronounced neurological effects and gastrointestinal effects, especially in times of starvation involving malnourished individuals.

Clinical Management

General supportive care is the focus of therapy. There is no antidote. Administration of activated charcoal may decrease absorption of the plant if given within an hour of the ingestion. Intravenous fluid and electrolyte replacement should be administrated as needed. Symptomatic patients should have continuous cardiac monitoring. Symptomatic bradycardia may be treated with atropine. For patients whose blood pressure does not respond to fluid replacement, vasopressors may be needed. Recovery can occur within hours to days.

See also: Plants, Poisonous.

Further Reading

Ceha LJ, Presperin C, and Young E (1997) Anticholinergic toxicity from nightshade berry poisoning responsive to physostigmine. *Journal of Emergency Medicine* 15: 65–69.

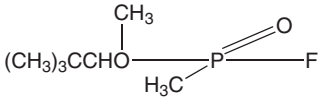
Dalvi RR and Bowie WC (1983) Toxicology of solanine: An overview. *Veterinary and Human Toxicology* 25: 13–15.

Soman

Harry Salem and Frederick R Sidell*

Published by Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 96-64-0
- Synonyms: GD; Phosphonoflouridic acid; Zoman; PFMP; G-agent; Methyl-1,2,2-trimethylpropyl ester; Pinacolyl methylphosphonoflouridate; Methylpinacolyloxyfluorophosphine oxide; Pinacolyloxymethylphosphonyl fluoride; Pinacolyl methanefluorophosphonate; Methylflouropinacolylphosphonate; Fluoromethylpenacolyloxyphonphine oxide; Methylpinacolyloxyphosphonyl fluoride; Pinacolyl methylfluorophosphinate; 1,2,2-Trimethylpropoxyfluoromethylphosphine; Nerve gas; Nerve agent
- Chemical/Pharmacetical/Other Class: Soman is a human-made nonpersistent anticholinesterase compound or organophosphate (OP) nerve agent, irreversible cholinesterase inhibitor, and chemical warfare agent. It is a light liquid with a camphor-like odor.
- Chemical Formula: $C_7H_{16}FO_2P$
- Chemical Structure:



Uses

Soman is a nerve agent used in chemical warfare.

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. Soman 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 soman for ~30 min, which could lead to exposure of other people. Soman vapor is heavier than air, and can sink to low-lying areas.

*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.

Toxicokinetics

Soman is absorbed both through the skin and via respiration. The half-life of soman in water at 30°C and pH 7.5 was reported to be 577 min compared to sarin at 30°C and pH 7.6, which was 5 min. Soman consists of a mixture of four stereoisomers:

1. C (−) P (−)
2. C (−) P (+) C (−) – soman
3. C (+) P (−)
4. C (+) P (+) C (+) – soman

The enzyme OP hydrolase hydrolyzes soman, tabun, sarin, and diisopropyl fluorophosphates at approximately the same rate.

Mechanism of Toxicity

Soman and the other nerve agents are organophosphorus cholinesterase inhibitors. They inhibit the enzymes butyrylcholinesterase in the plasma, the 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, and nasal discharge), respiratory effects (bronchoconstriction and increased bronchial secretion), gastro-intestinal effects (anorexia, vomiting, abdominal cramps, and diarrhea), sweating, salivation, and cardiovascular (bradycardia and hypotension) effects. The nicotinic effects include muscular fasciculation and paralysis. The effects on the CNS can include ataxia, confusion, loss of reflexes, slurred speech, coma, and paralysis.

Following inhalation of soman, the median lethal dosage (LCt_{50}) in humans has been estimated to be 70 mg min m^{-3} at a respiratory minute volume of 15 l min^{-1} for ten minutes. For percutaneous liquid the LD_{50} has been estimated to be 350 mg per 70 kg human. The permissible airborne exposure concentration of soman for an 8-h workday or a 40 h work week is an 8 h time-weighted average of 0.00003 mg m^{-3}.

Doses that are potentially life threatening may be only slightly larger than those producing minimal effects. Vapor exposure to the eyes and nose causes miosis and runny nose at ECt_{50} dosages of less than 2 mg min m^{-3}. The median incapacitation dosage (ICt_{50}) of vapor inhalation has been estimated as 35 mg min m^{-3}, while the LCt_{50} is 70 mg min m^{-3}. These vapor exposure durations are from 2 to 10 min. Individuals intoxicated with soman exhibit miosis, visual disturbances, headache and pressure sensation, runny nose, nasal congestion, salivation, tightness in the chest, nausea, vomiting, giddiness, anxiety, difficulty in thinking, difficulty in sleeping, nightmares, muscle twitching, tremors, weakness, abdominal cramps, diarrhea, and involuntary urination and defecation. These effects may progress to convulsions and respiratory failure. Depending on dose, the onset of signs and symptoms may occur within minutes or hours.

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 two 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 two or three 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. It is available in 30 mg tablets and the tablets should be administered every 8 h. When used prior to exposure, it should be followed by atropine and pralidoxime chloride after exposure.

Supportive therapy may include ventilation via an endotracheal airway if possible and suctioning of excess secretions from the airways.

Animal Toxicity

The stereoisomers of soman have different median lethal doses. The C(+)P(+) soman and the C(−) P(+) soman are the least toxic and subcutaneous LD_{50} values $\geqslant 5000$ and $\geqslant 2000\,\mu g\,kg^{-1}$, respectively. The more toxic stereoisomers, C(−) P(−) soman and C(+)P(−) soman, have subcutaneous LD_{50} values of 38 and $99\,\mu g\,kg^{-1}$, respectively. The racemic mixture of soman has a subcutaneous LD_{50} of $156\,\mu g\,kg^{-1}$ in mice.

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, 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.

An LCt_{50} of $30\,mg\,min\,m^{-3}$ was reported in rats following a 30-min inhalation exposure to soman. The acute toxicities by other routes of exposure in various animal species are presented in **Table 1**.

Table 1 Acute toxicities of soman in various species by various routes of exposure

Route of exposure/species	*LD_{50} ($\mu g\,kg^{-1}$)*
Percutaneous	
Rat	7800
Subcutaneous	
Chicken	50
Dog	12
Guinea pig	24
Monkey	13
Rabbit	20
Mouse	10
Rat	71
Intramuscular	
Monkey	9.5
Mouse	89
Rat	62
Intraperitoneal	
Chicken	71
Frog	251
Mouse	393
Rat	98
Intravenous	
Cat	15
Rat	44.5
Mouse	35

See also: Nerve Agents; Sarin; 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.

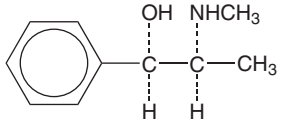
http://sis.nlm.nih.gov – US National Library of Medicine, Specialized Information Services, Chemical Warfare Agents.

Speed

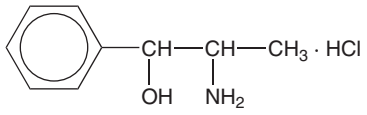
Henry A Spiller

This article is a revision of the previous print edition article by Carole Wezorek, volume 3, pp. 192–193, © 1998, Elsevier Inc.

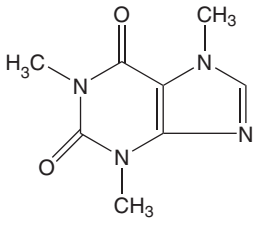
- Representative Chemicals: Ephedrine; Caffeine
- Chemical Abstracts Service Registry Numbers: CAS 299-42-3 (Ephedrine); CAS 58-08-2 (Caffeine)
- Synonyms: Street speed; 'Look alike' drugs; White crosses; Pink hearts; Black beauties; 357s; 357 magnums; Dexies; Robin eggs; Minithins; Stacker 2
- Chemical/Pharmaceutical/Other Class: Speed generally contains one or more agents belonging to the drug class of sympathomimetics
- Chemical Formulas: $C_{10}H_{15}NO$ (Ephedrine); $C_9H_{13}NO$ (Synephrine); $C_8H_{10}N_4O_2$ (Caffeine)
- Chemical Structures:



Ephedrine



Phenylpropanolamine hydrochloride



Caffeine

Uses

Ephedrine is labeled for sale as a bronchodilator. Caffeine is marketed as a minor stimulant to produce greater alertness or reduce drowsiness. Synephrine is available from plant sources as an 'herbal' weight loss supplement.

Background Information

Speed is the nomenclature used for a number of preparations that resemble and are often misrepresented as prescription amphetamines. They are used as substitutes for amphetamines. Speed is commonly composed of ephedrine, caffeine, synephrine, or a combination of these agents. Ephedrine is probably the most frequently encountered component of street speed. Herbal weight loss products containing ephedra, which is made up a number of sympathomimetic alkaloids, were removed from the US market in 2003. Phenypropanolamine, previously marketed as a weight loss supplement and oral decongestant and abused as a 'look alike', was removed from the US market in 2002.

Exposure Routes and Pathways

Oral speed preparations may be in tablet or capsule form. Ingestion is the most common route of intentional or accidental exposure.

Toxicokinetics

Ephedrine, synephrine, and caffeine are all well absorbed from the gastrointestinal tract. Following ingestion of an oral dose, clinical effects are seen within 60 min and persist from 2 to 6 h. Ephedrine is metabolized in the liver by oxidative deamination, demethylation, aromatic hydroxylation, and conjugation. Metabolites of ephedrine include norepinephrine, benzoic acid, and hippuric acid. Ephedrine is resistant to metabolism by monoamine oxidase. Ephedrine, synephrine, and caffeine have wide distribution throughout the body following oral administration. Ephedrine is presumed to cross the placenta and distribute into breast milk.

Ephedrine, synephrine, and caffeine are excreted in the urine. The rate of urinary excretion of ephedrine is dependent on urinary pH. The elimination half-life of ephedrine is 3 h when the urine is acidified to a pH of 5.0 and 6 h when the urinary pH is 6.3.

Mechanism of Toxicity

Sympathomimetic agents frequently found in speed stimulate α-adrenergic and β-adrenergic receptors and also stimulate the release of neuronal norepinephrine. Sympathomimetic drugs stimulate the sympathetic division of the autonomic nervous system. Stimulation of β-adrenergic receptors in the heart initially produces a positive inotropic effect on the myocardium. However, large or frequent doses produce a negative inotropic effect. With prolonged use, ephedrine, in particular, may deplete norepinephrine stores in sympathetic nerve endings, and tachyphylaxis to the cardiac and pressor effects may develop.

Acute and Short-Term Toxicity (or Exposure)

Animal

The clinical effects after overdose include anorexia, diarrhea, dehydration, hyperexcitment, tachycardia, tremor, weakness, seizures, and death.

Human

The clinical effects following overdose of sympathomimetic agents depend on the particular receptor selectivity and consist of α-adrenergic and/or β-adrenergic stimulation. Hypertension is usually the predominating symptom and may be accompanied by tachycardia or bradycardia, depending on the drug involved. The bradycardia is primarily a reflex bradycardia in response to hypertension. Cardiac arrhythmias, hypertensive crisis, and myocardial ischemia are possible effects of excessive exposure to sympathomimetic agents. Anxiety, muscle tremor, central nervous system (CNS) stimulation, seizures, and cerebral hemorrhage may occur. Hypokalemia is a possible transient serum electrolyte finding. Vomiting commonly is seen with caffeine overdose.

Chronic Toxicity (or Exposure)

Animal

No evidence of carcinogenicity has been seen in studies on mice and rats with doses up to 250 ppm ephedrine in diet over 2 years.

Human

Long-term use of large doses of ephedrine (350–2500 mg day^{-1} for 3–20 years) may produce psychotic episodes characterized by paranoia, hallucinations, depression, and bizarre mentation. Following withdrawal of the drug, aberrant mental effects will resolve but reinstitution of ephedrine use may result in a return of the psychotic symptoms. A tolerance may develop with chronic use, allowing larger doses. Tolerance is lost after 4–6 weeks of removal from the drug.

In Vitro Toxicity Data

Mutagenicity studies using the Ames *Salmonella*, Chinese hamster ovary cells, and Syrian hamster liver assays have been negative.

Clinical Management

Basic and advanced life-support measures should be instituted as indicated. Gastric decontamination may be performed depending on the specific drug involved, the patient's symptomatology, and the history of the ingestion. Activated charcoal may be used to adsorb ephedrine, synephrine, and/or caffeine. Careful monitoring of the heart and hemodynamic status should be performed. Hypertension and symptoms of CNS stimulation usually resolve spontaneously with only supportive measures. Antiarrhythmic and antihypertensive agents may be necessary in severe exposures. If treatment of hypertension is necessary, a direct vasodilator such as nitroprusside or nifedipine should be utilized. Treat agitation and seizures as necessary with benzodiazepines. Management of concurrently ingested drugs should be appropriate to the agent involved.

Environmental Fate

Limited data indicate that caffeine has the potential to biodegrade in soil. If released into water, caffeine will not volatilize from water to the atmosphere. It will not bioconcentrate in fish nor will it adsorb to sediment. Limited data indicate that caffeine has the potential to biodegrade in water.

No information is currently available on breakdown in soil groundwater or surface water for ephedrine or synephrine.

See also: Caffeine.

Further Reading

Pentel P (1984) Toxicity of over-the-counter stimulants. *Journal of the American Medical Association* 2: 1898–1903.

Spider, Black Widow

Gary W Everson

- Synonyms: *Latrodectus mactans*; 'Hour glass' spider

Exposure Routes and Pathways

Envenomation occurs subcutaneously due to the small biting apparatus of the spider. Bites occur most frequently on the extremities.

Toxicokinetics

Following a bite, the specific disposition of *Latrodectus* venom is not well understood. The distribution of venom to the central and peripheral nervous system occurs following absorption through the lymphatic system. The onset of muscle cramping and pain ranges from 30 min to several hours. Resolution of symptoms is usually complete within 24–72 h. However, occasionally a longer clinical course is experienced.

Mechanism of Toxicity

Black widow spider venom contains several different protein fractions. However, the high-molecular-weight neurotoxin is the only fraction that is of clinical significance. This neurotoxin acts at the neuromuscular synapse damaging nerve terminals and causing the release and ultimately the depletion

of such neurotransmitters as alpha-aminobutyric acid, norepinephrine, and acetylcholine. Neurotransmitter release is most likely responsible for hypertension, muscle fasciculation, and muscle spasms experienced by victims of a bite. Generalized muscle weakness and labored breathing may develop in severe cases. While the venom of the black widow spider has been characterized as being more potent than that of many poisonous snakes, the small amount of venom injected limits the extent of toxicity.

Acute and Short-Term Toxicity (or Exposure)

Human

Several species of *Latrodectus* exist and all produce a similar clinical course. The severity of an envenomation is dependent on patient age and the presence of any preexisting cardiovascular disease. Fatalities are extremely rare. Infants and the elderly are at greater risk for developing severe symptoms. Initially, the bite is associated with mild local pain and redness limited to the immediate bite site. As the venom causes no tissue damage, little or no swelling occurs. Most frequently, patients present with painful muscle cramping, spasms, and rigidity within a few hours of the bite. In general, the location of the bite determines which muscle groups will be affected. Bites occurring to the upper body commonly affect muscles of the back, shoulders, and chest. Lower extremity bites are associated with abdominal spasms and rigidity. In some cases, the presentation resembles an acute abdomen. Most problematic is the severe pain that commonly accompanies the muscle spasms. Nausea, vomiting, headache, dizziness, diaphoresis, and mild hypertension are other commonly encountered symptoms. Severe clinical manifestations are rare but can include clinically significant hypertension, respiratory insufficiency, and seizures.

Clinical Management

Although life-threatening envenomations are extremely rare, basic and advanced clinical life support should be employed when necessary. Healthy adult patients bitten by black widow spiders often do not develop symptoms significant enough to require medical evaluation. However, the primary complaint of most patients evaluated in the emergency department is moderate to severe pain due to muscle spasms. Therapy is directed toward making the patient as comfortable as possible while monitoring for the development of severe symptoms such as hypertension and labored breathing. Agents commonly employed to treat muscle spasms and pain includes muscle relaxants, narcotic analgesics, and occasionally intravenous calcium. The most effective treatment in reducing muscle spasm and pain appears to be the combined use of intravenous diazepam and a narcotic analgesic, such as morphine sulfate. Titrating to an effective dose is necessary while minimizing adverse effects. Readministration of this combination is usually required until symptoms abate. Intravenous calcium salts, as either calcium chloride or calcium gluconate, have been attempted. However, treatment failure with this treatment is common and is generally not recommended. Occasionally, intravenous calcium is added to the regimen of muscle relaxants and narcotic analgesics. Calcium gluconate, a less concentrated form of calcium than calcium chloride, is often preferred since it is less irritating to blood vessels during administration. The use of *L. mactans* Antivenin or Antivenin (*Latrodectus mactans*) (Black Widow spider antivenin) Equine Origin®, in general, should be limited only to those patients experiencing severe, potentially life-threatening, symptoms. Most studies indicate that the routine use of antivenin is unnecessary and therefore should be discouraged. Since life-threatening symptoms following a black widow spider bite are rare, the benefits of giving the antivenin rarely outweigh the risk of potentially life-threatening anaphylaxis from this horse serum derived antivenin. Therefore, the vast majority of patients will recover fully with only supportive care and the use of muscle relaxants and narcotic analgesics to manage pain. 'High-risk' patients, such as infants, the elderly, or those with significant cardiovascular disease, who exhibit significant toxicity, are potential candidates for receiving antivenin. *Latrodectus* antivenin is derived from horse serum. Both anaphylactic and delayed hypersensitivity reactions have occurred. If antivenin use is indicated, the intravenous administration is the preferred route. One vial of *Latrodectus* antivenin is reconstituted and commonly diluted further in 50 cc of normal saline and administered intravenously over 30 min. A test dose of not more than 0.02 ml, of test material (1:10 dilution of normal horse serum in physiologic saline) should be administered intradermally prior to intravenous infusion of the antivenin to check for hypersensitivity reactions. Wheal formation at the test site indicates the possibility that an allergic reaction may occur and antivenin use should be reconsidered. A negative reaction to the test dose does not necessarily rule out the possibility of an allergic reaction to the antivenin. One should always be prepared for the possibility of anaphylaxis whenever antivenin derived from horse serum is given. The

benefits of giving antivenin in a particular patient should be weighed against the potential risks. Elevation in blood pressure is frequent following black widow spider envenomation but rarely requires treatment with an antihypertensive agent. Tetanus prophylaxis should be provided as necessary.

See also: Spider, Brown Recluse; Spiders.

Further Reading

Clark RF, Wethern-Kestner S, and Vance MV (1992) Clinical presentation and treatment of black widow spider envenomation: A review of 163 cases. *Annals of Emergency Medicine* 21: 782–787.

Gueron M, Ilia R, and Margulis G (2000) Arthropod poisons and the cardiovascular system. *American Journal of Emergency Medicine* 18: 708–714.

Spider, Brown Recluse

Gary W Everson

- SYNONYMS: *Loxosceles reclusa*; Fiddle-back spider; Violin spider

Exposure Routes and Pathways

Envenomation occurs subcutaneously due to the small biting apparatus of the spider. The Brown Recluse (*Loxosceles reclusa*) spider, as its name implies, is found in secluded areas. Bites occur most frequently to the hands and arms while reaching into woodpiles or other well-protected areas.

Toxicokinetics

In humans, the specific disposition of venom is not well understood. The local distribution of venom is enhanced by the presence of hyaluronidase and other spreading factors found in the venom. Systemic absorption of venom components is likely dependent on lymphatic transport. The onset of local symptoms such as redness and pain may develop within a few hours of the bite.

Mechanism of Toxicity

Brown Recluse spider venom contains many diverse protein fractions including spreading factors and enzymes such as hyaluronidase, collagenase, protease, phospholipase, and others. These venom components cause coagulation of blood and, ultimately, the occlusion of small blood vessels at the bite site. This leads to local skin and tissue necrosis due to ischemia. Hemolysis of red blood cells may also occur. The normal inflammatory processes that follow, such as edema and hemorrhage, contribute to the tissue damage caused by the venom. Occasionally, the local tissue necrosis expands as the tissue ischemia spreads from the initial bite site.

Acute and Short-Term Toxicity (or Exposure)

Human

The Brown Recluse spider is one species of the genus *Loxosceles* which is found in the central Midwest: Nebraska south to Texas and eastward to southernmost Ohio and north-central Georgia.

The Brown Recluse spider bite produces symptoms that range from mild local tissue inflammation to wide spread systemic toxicity. The extent of toxicity is dependent on the amount of venom injected and the age and general health of the patient. Although life-threatening symptoms are possible, a localized skin and tissue reaction is much more common. It is important to note that Brown Recluse spider bites often do not progress to a significant necrotic lesion. However, when this lesion is present, the tissue necrosis is usually self-limiting and often responds to general wound management.

There are some brown spiders that somewhat resemble the Brown Recluse to the layperson. Without proper identification by an entomologist, it is often not possible to immediately diagnose a Brown Recluse spider bite. Complicating the diagnosis further is the fact that there are several other species of spider (e.g., *Chiracantheum* species, *Argiope* species, and *Phidippus* species) that can cause a necrotic skin lesion, without systemic complications.

The bite of the Brown Recluse is usually painless and often goes unnoticed initially. The spider is seldom seen. Therefore, most patients do not seek treatment until a necrotic lesion develops. Within several hours of envenomation, local symptoms of redness and pain occur. Within 24 h, a reddish to violet colored blister becomes surrounded by a

blanched, ischemic ring that is bordered by a reddish ring. This represents the often described 'bull's eye lesion'. Over the next several days, the blistered, ischemic area may turn darker and sink below the level of skin due to subcutaneous tissue necrosis. This necrotic reaction may stop or continue to expand, producing a lesion as large as 5–30 cm in diameter. In 7–14 days, the top layer of the blister sloughs off leaving an ulcerated lesion. Depending on the size of the lesion, healing may require several months. The necrosis tends to be more extensive following bites in fatty areas such as the thighs and buttocks. Rarely, systemic involvement may occur. Systemic effects can include fever, chills, weakness, vomiting, muscle pain, generalized rash, seizures, disseminated intravascular coagulation, thrombocytopenia, and hemolytic anemia. Renal failure and death may occur due to widespread hemolysis.

These potentially severe reactions are extremely rare and the vast majority of Brown Recluse spider bites have a relatively benign progression of local symptoms and resolve with supportive care. It should be pointed out that symptoms of localized infection following a bite of any insect or spider may produce localized lesions that might be mistaken for an expanding necrotic spider bite. Cellulitis should be included on the differential diagnosis and ruled out before considering a patient with a necrotic spider bite.

Clinical Management

Since home identification of a Brown Recluse spider is rarely possible, a 'brown spider bite' should be treated as any other bite of an unknown insect or spider. Management should include the application of a topical disinfectant such as 3% hydrogen peroxide or isopropyl alcohol and brief application of a cold compress for pain. Should the bite site develop increased redness and swelling or a local ulcer develop over several days, a physician should evaluate the wound to rule out infection.

Many controversial techniques have been employed in the management of true Brown Recluse spider bites. Unfortunately, no scientific evidence exists which supports an ideal method or methods of management. However, case reports advocate a variety of therapies as potentially useful. Most agree, however, that good local management of the cutaneous lesion is the most important aspect of care. Tetanus prophylaxis should always be included. In general, antibiotics should be withheld unless there is evidence of infection. Local and systemic injections of steroids have also been employed, but research has shown that neither the extent nor the duration of tissue necrosis is affected. Dapsone has been shown to be effective in research done in the animal model. In addition, several case reports have described some success with dapsone in decreasing local pain and preventing further induration and tissue necrosis. Doses have ranged from 50 to 200 mg day^{-1}. This drug appears to decrease the extent of tissue necrosis by inhibiting polymorphonuclear leukocytes, the mediators of the inflammatory response to the bite. However, side effects of dapsone are potentially severe. Hemolytic anemia and liver toxicity have been described.

There appears to be no benefit to early surgical excision of the bite site. Some time is required before a clear boundary is established marking the end of the spread of venom. Excising the necrotic area too soon may leave some venom at the boundary that can produce further tissue necrosis. In cases where the necrotic lesion expands and is unresponsive to local treatment, 'delayed' surgical excision of the wound after 2 or 3 weeks may be indicated.

Although several case reports describe some success in patients following the use of hyperbaric oxygen, no scientific studies have been completed yet to determine the effectiveness of this approach. Management of systemic toxicity is primarily supportive and includes good wound management with emphasis on pain management, wound debridement, and observation for local infection. Also, adequate hydration is important to maintain good urine output. Clotting abnormalities and anemia should be managed with appropriate blood products. The Brown Recluse spider antivenin is still experimental and not commercially available.

See also: Animals, Poisonous and Venomous; Spiders.

Further Reading

Anderson EJ (1987) Recognizing the recluse spider bite in children: Necrotic arachnidism. *The Journal of the Louisiana State Medical Society* 139: 33–36.

Gendron BP (1990) *Loxosceles reclusa* envenomation. *The American Journal of Emergency Medicine* 8: 51–54.

Wright SW, Wrenn KD, and Murray L (1997) Clinical presentation and outcome of brown recluse spider bite. *Annals of Emergency Medicine* 30: 28–32.

Spiders

Julie Weber

- Representative Genera: *Latrodectus*; *Loxosceles*; *Argiope*; *Chiracanthium*; *Lycosa*; *Phidippus*; *Tegenaria*
- Synonyms: *Latrodectus* species: *L. mactans* (Black Widow); *L. variolus* (Northern Black Widow); *L. hesperus* (Western Black Widow); *L. bishopi* (Red Widow); *L. geometricus* (Brown Widow); *L. hasselti* (Red Back); *L. mactans tredecimguttatus* (European Black Widow). *Loxosceles* species: *L. deserta*; *L. arizonica*; *L. laeta*; *L. rufescens*; *L. unicolor*; *L. recluse*. Necrotizing spiders: *Argiope* (Orb Weaver); *Chiracanthium* (Running or sac spider); *Lycosa* (Wolf spider); *Phidippus* (Jumping spider), *Tegenaria agrestis* (Hobo spider or Northwestern brown spider)

Exposure Routes and Pathways

Envenomation occurs subcutaneously due to the small biting apparatus of the spider. Bites occur most frequently on the extremities. Typically, the victim is bitten while dressing in clothing that has been undisturbed, when rolling over in bed onto a spider, moving stored boxes in an attic or basement or while reaching into woodpiles or other well-protected areas.

Toxicokinetics

In humans, the specific disposition of *Latrodectus* and *Loxosceles* venom is not well understood. Distribution of *Latrodectus* venom to the central and peripheral nervous system occurs following absorption through the lymphatic system. Whereas the local distribution of *Loxosceles* venom is enhanced by the presence of hyaluronidase and other spreading factors found in the venom. Systemic absorption of venom components is likely dependent on lymphatic transport, possibly similar to the *Latrodectus* venom. In *Latrodectus* envenomation, the onset of muscle cramping and pain ranges from 30 min to several hours. Resolution of symptoms is usually complete within 24–36 h. Occasionally, a longer clinical course is experienced with symptoms persisting for several days. In comparison, the onset of local symptoms with *Loxosceles* envenomation may develop within a few hours of the bite with redness and pain, full progression of symptoms and healing may not occur for 1 month or longer.

Mechanism of Toxicity

Latrodectus

Black Widow spider venom contains several different protein fractions. The most significant component of the venom is the neurotoxin, α-latrotoxin. This neurotoxin acts at the presynaptic membrane of the neuronal and the neuromuscular junctions. The binding of the α-latrotoxin results in the opening of nonspecific cation channels, a massive influx of calcium, release of acetylcholine and norepinephrine and decreased uptake of the neurotransmitter. The neurotransmitter release is most likely responsible for hypertension, muscle fasciculations, and spasms frequently experienced by victims of a bite. Later, generalized muscle weakness and labored breathing may develop in severe cases. While the venom of the black widow spider has been characterized as being more potent than that of many poisonous snakes, the small amount of venom injected limits the degree of toxicity.

Loxosceles

The *Loxosceles* venom is complex and contains multiple enzymes including alkaline phosphatase, hyaluronidase, 5-ribonucleotide phosphohydrolase, esterase, and sphingomyelinase D. The venom components cause coagulation of blood and ultimately, the occlusion of small blood vessels at the bite site. This leads to local skin and tissue necrosis due to ischemia. A release of inflammatory mediators resulting in polymorphonuclear leukocyte infiltration is also associated with the local reaction. Hemolysis of red blood cells can occur. Sphingomyelinase D appears to be the major dermonecrotic factor. When calcium and serum amyloid protein are present sphingomyelinase D reacts with sphingomyelin to release choline and *N*-acylsphingosine phosphate stimulating platelet aggregation and release of serotonin. Occasionally, the local tissue necrosis expands as the tissue ischemia spreads from the initial bite site.

Acute and Short-Term Toxicity (or Exposure)

Human

Latrodectus Several species of *Latrodectus* exist and all produce a similar clinical course. The severity

of an envenomation is dependent on patient age and the presence of any preexisting cardiovascular disease. Fatalities are extremely rare. Infants and the elderly are at greater risk for developing severe symptoms. Initially, the bite is associated with mild local pain and redness limited to the immediate bite site. The venom causes no tissue damage, so little or no swelling occurs. Patients most frequently present with painful muscle cramping, spasms, and rigidity that commonly occurs within 15 min to 3 h of the bite. The location of the bite determines which muscle groups will be affected. Bites occurring to the upper body commonly affect muscles of the back, shoulders, and chest. Lower extremity bites are associated with abdominal spasms and rigidity. In some cases, the presentation resembles an acute abdomen. Most problematic is the severe pain which commonly accompanies the muscle spasms. Nausea, vomiting, headache, dizziness, diaphoresis, and mild hypertension are other commonly encountered symptoms. Severe clinical manifestations are rare but can include clinically significant hypertension, respiratory insufficiency, and seizures. Other less common effects that have been reported include ptosis, pulmonary edema, facial edema, encephalopathy, generalized rash, itching, and toxic epidermal necrolysis.

Loxosceles The Brown Recluse spider bite produces symptoms that range from mild local tissue inflammation to widespread systemic toxicity. The extent of toxicity is dependent on the amount of venom injected, the location of the bite, and the age and general health of the patient. Although life-threatening symptoms are possible, a localized skin and tissue reaction is much more common. It is important to note that Brown Recluse spider bites often do not progress to a necrotic lesion. However, when present, the tissue necrosis is usually self-limiting and often responds to general wound management. The bite of the Brown Recluse is usually painless and initially often goes unnoticed. Since the spider is seldom seen, most patients do not seek treatment until a necrotic lesion develops. Local symptoms of pruritus, redness, and pain occur within several hours of the envenomation. Within 24 h, a reddish to violet colored blister becomes surrounded by a blanched, ischemic ring that is bordered by a reddish ring. This represents the often described 'bull's eye' or 'halo' lesion. Over the next several days, the blistered, ischemic area may turn darker and sink below the level of skin due to subcutaneous tissue necrosis. This necrotic reaction may stop or continue to expand, producing a lesion as large as 5–30 cm in diameter. In 7–14 days, the top layer of the blister sloughs off leaving an ulcerative lesion. Depending on the size of the lesion, healing may require several months. The necrosis tends to be more extensive following bites in fatty areas such as the thighs, buttocks, and abdomen. Neck and facial wounds can cause significant edema. Systemic reactions from *Loxosceles* envenomation are infrequent. A systemic reaction typically occurs within 24–96 h. Symptoms can include fever, chills, weakness, vomiting, muscle pain, generalized rash, seizures, disseminated intravascular coagulation, thrombocytopenia, and hemolytic anemia. Renal failure and death may occur due to widespread hemolysis.

Argiope, Chiracanthium, Lycosa, Phidippus, Tegenaria agrestis There are some brown spiders that somewhat resemble the Brown Recluse to the layperson. Without proper identification, it is often not possible to immediately diagnose a brown recluse spider bite. Complicating the diagnosis further is the fact that there are several other species of spiders that can cause a necrotic skin lesion, although not as severe.

Argiope The spider will bite if stressed or provoked. Initial symptoms include sharp pain and swelling. Induration with a surrounding erythema can occur. The bite may cause a necrotic lesion, but no systemic symptoms expected.

Chiracanthium The venom is similar to *Loxosceles*. The bite initially causes a sharp pain and red wheal formation. Several days later, a crust forms at the site with necrotic tissue underneath. Erythema, pruritus, and pain typically surround the bite site and may take one month to heal. Systemic symptoms are rare, but nausea, anxiety, headache, and abdominal cramps have been reported.

Lycosa The initial reaction often consists of erythema, pain, edema with a violaceous discoloration and tenderness. Necrosis is seldom, but may occur. Nausea and light-headedness have been reported.

Phidippus The jumping spider produces a sharp painful bite with redness, pain, edema, and pruritus. The swelling usually subsides within 48 h, but in one report symptoms persisted for 1 week. A small ulcer with eschar may form. No systemic toxicity is expected.

Tegenaria agrestis The initial bite of the hobo spider may be painless and unnoticed. It is the most similar to the brown recluse. Induration surrounded by erythema may be present within 30 min. A blister formation typically follows within 15–35 h. About

half of the reported envenomations may develop an eschar covering a necrotic ulcer. Systemic symptoms reported include headache, lethargy, weakness, nausea, vomiting, memory loss, and visual impairment.

Clinical Management

Latrodectus

Although life-threatening envenomations are extremely rare, basic and advanced clinical life support should be employed when necessary. Normally healthy adult patients bitten by Black Widow spiders often do not develop symptoms significant enough to require medical evaluation. However, the primary complaint of most patients evaluated in the emergency department is moderate to severe pain due to muscle spasms. Therapy is directed toward making the patient as comfortable as possible while monitoring for the development of severe symptoms such as hypertension and labored breathing. Agents commonly employed to treat muscle spasms and pain include benzodiazepines, muscle relaxants, intravenous calcium, and narcotic analgesics. The bite should be cleansed with soap and water. Measures to remove or decrease the spread of venom are ineffective due to the small amount of venom necessary to produce toxicity and its rapid spread to the circulation. Tetanus prophylaxis should be provided as necessary. Diazepam has been found to be effective for the relief of muscle spasms. Methocarbamol may also be effective. Parenteral opioids (codeine, morphine, or meperidine) or a combination of parenteral opioids and sedative-hypnotics such as diazepam or lorazepam are recommended for patients with severe envenomations who are not candidates for antivenin. Cautious use of opioids is recommended as they may be contraindicated by a patient's symptoms (i.e., respiratory difficulty, central nervous system (CNS) depression, seizures, etc.). The efficacy of intravenous calcium has not been firmly established in controlled trials. Calcium gluconate is often preferred and it is less irritating to blood vessels during administration than calcium chloride. It alone though often fails to provide adequate relief of symptoms and is not recommended for symptomatic relief. Use of both intravenous diazepam and a narcotic analgesic appears to be the most effective method in reducing muscle spasm and pain. The use of *L. mactans* antivenin, in general, should be limited only to those patients experiencing severe symptoms. Most studies indicate that the routine use of antivenin is unnecessary and therefore should be discouraged. The vast majority of patients will recover fully with only observation and the use of muscle relaxants and narcotic analgesics to manage pain. 'High risk' patients, such as infants, the elderly, or those with significant cardiovascular disease, are potential candidates for receiving antivenin. *Latrodectus* antivenin is derived from horse serum. Both anaphylactic and delayed hypersensitivity reactions have occurred. If antivenin use is indicated, intravenous administration is the preferred route. Prior to administration of the antivenin the patient should be tested for sensitivity. A small dose should be administered subcutaneously prior to intravenous infusion to check for hypersensitivity reactions. Wheal formation at the test site indicates the possibility of an allergic reaction. While a positive skin test warrants increased caution with antivenin administration, it should not necessarily preclude its use when indicated. A negative reaction to the test dose does not necessarily rule out the possibility of an allergic reaction to the antivenin. One should always be prepared for the possibility of anaphylaxis whenever antivenin derived from horse serum is given. The benefits of giving antivenin in a particular patient should be weighed against the potential risks. One vial (2.5 ml) of *Latrodectus* antivenin is reconstituted and commonly diluted further in 50–100 ml of D_5W or normal saline and administered intravenously over 30 min. Elevation in blood pressure is frequent following Black Widow spider envenomation but rarely requires treatment with an antihypertensive agent.

Loxosceles

Many controversial techniques have been employed in the management of Brown Recluse spider bites. Unfortunately, no scientific evidence exists which supports an ideal method or methods of management. However, case reports advocate a variety of therapies as potentially useful. Most agree, however, that good local management of the cutaneous lesion is the most important aspect of care. Tetanus prophylaxis should always be included. Immobilization, elevation and rest of a bitten extremity may be beneficial as increased activity and metabolic heat production may enhance enzyme activation. Local application of cool compresses has been reported to reduce inflammation and pain, and slow the evolution of lesions. It is postulated that the activity of sphingomyelinase D may be reduced by the cold. Analgesics can be administered for pain. Avoid analgesics and antiinflammatory drugs that affect platelet function. Antipruritics and antianxiety drugs may be administered as needed. In general, antibiotics should be withheld unless there is evidence of infection. Local and systemic injection of steroids has been employed, but research has shown that neither the extent nor the duration of tissue

necrosis is affected. Dapsone has been shown to be effective in research done in the animal model. In addition, several case reports have described some success with dapsone in decreasing local pain and preventing further induration and necrosis. Doses have ranged from 50 to 200 mg day^{-1} in adults. This drug appears to decrease the extent of tissue necrosis by inhibiting polymorphonuclear leukocytes, the mediators of the inflammatory response to the bite. However, side effects of dapsone are potentially severe. Hemolytic anemia and liver toxicity have been described. Dapsone should not be used routinely.

There appears to be no benefit to early surgical excision of the bite site. Some time is required before a clear boundary is established marking the end of the spread of venom. Excising the necrotic area too soon may leave some venom at the boundary that can produce further tissue necrosis. Corrective surgery should be delayed at least 6–8 weeks until the area of necrosis is clearly demarcated.

Although several case reports describe some success in patients following the use of hyperbaric oxygen, it is not considered standard therapy at this time. However, hyperbaric oxygen may provide wound healing benefits in certain patients with vascular insufficiencies. Management of systemic toxicity is primarily supportive and includes the use of steroids to prevent red blood cell hemolysis. Also, adequate hydration is important to maintain good urine output. Clotting abnormalities and anemia should be managed with appropriate blood products. If hemolysis occurs, maintain hydration and urine output to prevent renal failure. Alkalinization of the urine may enhance hemoglobin case excretion. Hemodialysis has no effect on elimination of the venom, but is indicated in the presence of renal failure. The Brown Recluse spider antivenin is still experimental and is not commercially available.

Argiope, Chiracanthium, Lycosa, Phidippus, Tegenaria agrestis

As with the treatment of the *Loxosceles* species, treatment remains controversial. Typically, good local management of the cutaneous lesion is the most important aspect of care. Tetanus prophylaxis should be updated. Immobilization, elevation and rest of a bitten extremity may be beneficial. Analgesics can be administered for pain. Antipruritics and antianxiety drugs may be administered as needed. In general, antibiotics should be withheld unless there is evidence of infection. The use of intralesional, intramuscular or oral corticosteroids is not of proved efficacy, although some case reports have shown a good outcome with the use of a corticosteroid.

Miscellaneous

There are five representative species of the widow spider in the United States. The species are the Black Widow (*L. mactans*, *L. hesperus*, *L. variolus*), Red Widow (*L. bishopi*), and the Brown Widow (*L. geometricus*). The five species of *Loxosceles* documented to produce necrotic bites are *L. arizonica*, *L. deserta*, *L. laeta*, *L. reclusa*, and *L. rufescens*. The *L. reclusa* is responsible for most cases of clinical significance of necrotic arachnidism.

There is no routine test for the diagnosis of trivial or cutaneous arachnidism. The use of a passive hemagglutination inhibition test has been used successfully to identify venom from Brown Recluse spider bites in animal studies. This test has not yet been used for diagnostic purposes in human trials and is not routinely available to clinicians.

See also: Animals, Poisonous and Venomous; Spider, Black Widow; Spider, Brown Recluse.

Further Reading

Barrett SM, Romine-Jenkins M, and Campbell JP (1989) Passive hemagglutination inhibition test for diagnosis of Brown Recluse spider bite envenomation. *Annals of Emergency Medicine* 18: 441.

Cacy J and Mold JW (1999) The clinical characteristics of Brown Recluse spider bites treated by family physicians. *Journal of Family Practice* 48(7): 536–542.

Clark RF, Wethern-Kestner S, Vance MV, *et al.* (1992) Clinical presentation and treatment of Black Widow spider envenomation: A review of 163 cases. *Annals of Emergency Medicine* 21(7): 782–787.

Rees RS, Altenbern DP, Lynch JB, *et al.* (1985) Brown Recluse spider bites: A comparison of early surgical excision versus dapsone and delayed surgical excision. *Annals of Surgery* 202: 659.

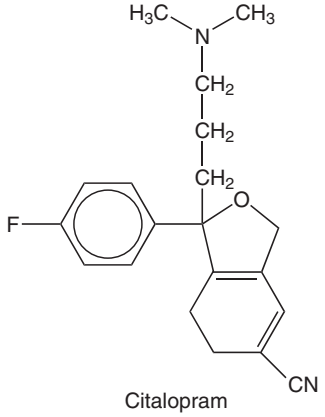
Sams HH, Hearth SB, Long LL, *et al.* (2001) Nineteen documented cases of *Loxosceles reclusa* envenomation. *Journal of the American Academy of Dermatology* 44(4): 603–608.

Yarbrough BE (1987) Current treatment of Brown Recluse spider bites. *Current Concepts in Wound Care* 10: 4–6.

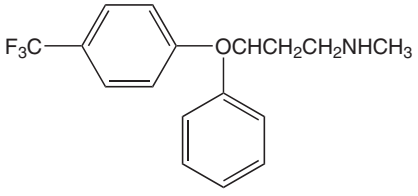
SSRIs (Selective Serotonin Reuptake Inhibitors)

Samantha E Gad

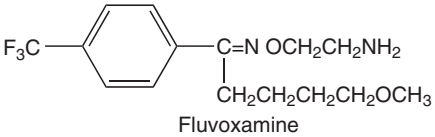
- Trade Names and Chemical Abstracts Service Registry Numbers:
  Citalopram hydrobromide (CAS 59729-32-7) Celexa® (Lundbeck)
  Flouxetine hydrochloride (CAS 59333-67-4) Prozac® (Lilly)
  Fluvoxamine maleate (CAS 61718-82-9) Luvox® (Solvay)
  Paroxetine hydrochloride (CAS78246-49-8) Paxil® (SmithKline-Beecham)
  Sertraline hydrochloride (CAS 79559-97-0) Zoloft® (Pfizer)
  Escitalopram oxalate (CAS 219861-08-2) Lexapro® (Forest)
  These are the six main selective serotonin reuptake inhibitors (SSRIs); others are under development and possibly in use in small populations or foreign countries.
- Therapeutic Class: Antidepressant
- Routes of Administration: Oral
- Chemical Structures: Escitalopram is the *S*-isomer, the active isomer of citalopram's racemic mixture



Citalopram



Fluoxetine



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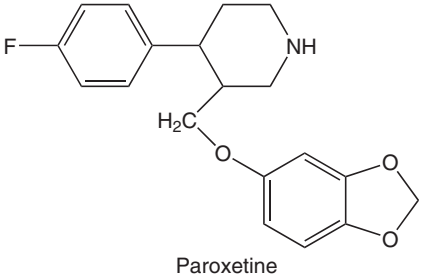
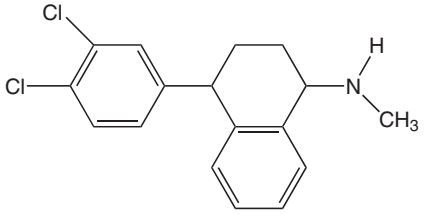


Paroxetine



Sertraline

Uses

Obsessive compulsive disorder, panic disorder, generalized anxiety disorder, bulimia nervosa, social anxiety disorders, post-traumatic stress disorder, dementia, dysthymia, premature ejaculation. Citalopram (investigational) is used for dementia, smoking cessation, ethanol abuse, OCD in children with diabetic neuropathy. Sertraline and Sarafem (contains fluoxetine) are also used to treat premenstrual dysphoric disorder.

Mechanism of Action

SSRIs selectively inhibit the reuptake of serotonin (5-hydroxytryptamine (5-HT)) into nerve endings in the central nervous system (CNS). This allows for an increasing concentration of serotonin at the synapse, enhancing serotonergic neuronal transmission. If depression represents some inadequacy in transmission between the nerves in the brain, as is believed, regulating transmission may go some way toward reversing this inadequacy. Dysfunction of serotonin in neurons is thought to play a major role in a wide variety of diseases, including major depression. It is believed that increasing the availability of serotonin will improve the clinical signs of depression. Serotonin is implicated in brain functions such as perception of pain, sleep, appetite, thermal regulation, reproductive function, balance, gut regulation, sensory interpretation, higher cognitive function, and motor function.

SSRIs have no direct effect on the reuptake of noradrenaline, dopamine, or GABA. Unlike tricyclic antidepressants (TCAs), they have no significant affinity for a1-adrenergic, H1-histamine, and muscarinic receptors. The selectivity of SSRIs may account for the lower incidence of some adverse effects such as sedation or thustatic hypotension, and anticholinergic effects. Seretonin is a neurotransmitter in the human brain; serotonin containing neurons are highly localized in specific clusters in the brainstem and spinal cord. From these sites, the cells send out axons that end in serotonin-containing terminals innervating the diverse areas throughout the brain.

SSRIs affect many postsynaptic serotonin receptors (e.g., 5-HT 1A, 5-HT 1D, 5-HT 2A, 5-HT 2C, and 5HT-3), therefore affecting many neural systems. With the exception of fluoxetine, none of the currently approved SSRIs have metabolites with clinically relevant effects on any of the neural sites. However, every SSRI that has been studied has metabolites with approximately the same activity as the parent drug for the inhibition of specific CYP enzymes and contributes to the effects mediated by this action.

SSRIs appear safer in overdose than most other classes of antidepressants. SSRIs are not associated with clinically significant anticholinergic side effects, or cardiotoxicity.

Pharmacokinetics

Citalopram is distributed in human breast milk. Escitalopram (*S*-citalopram) enters the breast milk. These should only be used during pregnancy if the potential benefit to the mother outweighs the possible risk to the fetus (**Table 1**).

Known Major Drug Interactions

All SSRIs have common 5-HT agonistic effects and because of this, SSRIs have common interactions and side effects. SSRIs are potent inhibitors of serotonin reuptake by CNS neurons and may interact with other drugs such as monoamine oxidase inhibitors (MAOIs) or circumstances which cause serotonin release. A minimum 2 weeks wash-out period should be observed between stopping a MAOI and starting an SSRI. Conversely, a MAOI should not be started for at least 1 week after an SSRI has been stopped, 5 weeks after fluoxetine, and 2 weeks for paroxetine and sertraline. Escitalopram and citalopram are hypersensitive to each other.

Combining SSRIs with the following drugs has caused a drug–drug interaction; the SSRI(s) involved are in italics:

- 3,4-Methylenedioxymethamphetamine (MDMA): The psychological effects of MDMA ('Ecstasy') are markedly reduced by the concurrent use of *citalopram*. It seems likely that other SSRIs will also reduce or block the effects of MDMA. An isolated report describes a neurotoxic reaction in a man on citalopram when he took unknown amounts of MDMA.
- 5-HT agonists (Triptans): The SSRIs normally appear not to interact with the triptans, but there are a few rare cases of dyskinesias and there is some evidence to suggest that the serotonin syndrome may occasionally develop.
- Amphetamines: SSRIs may increase the sensitivity to amphetamines; amphetamines may increase the risk of serotonin syndrome.
- Antiepileptics: These lower the convulsive threshold.
- Antipsychotics: Seven patients developed delirium when given *fluoxetine*, *paroxetine*, or *sertraline* with benztropine in the presence of perphenazine

Table 1 Pharmacokinetics of SSRIs

SSRI	*Initial dose (mg qAM)*	*Maintenance dose (mg day^{-1})*	*Half-life (h)*	*Metabolite half-life*	*Bioavailability (%)*	*Peak plasma level (h)*	*Plasma level*[a] (ng ml^{-1})	*In vitro potency IC_{50}*[b]	*5-HT uptake inhibition (%)*	*% Protein-bound*	*Metabolism*
Citalopram (Celexa™)	20	20–60	35	*S*-Desmethyl-citalopram: 59 h	80	4	≅85 (260 nM)	1.8 (14)	≅60	80	Extensively hepatic
Fluoxetine (Prozac™)	10–20	10–80	Initial: 24–72; Chronic: 96–144	Norfluoxetine: 4–16 days	72	6–8	≅200 (660 nM)	6.8 (3.8)	≅80	95	Hepatic to norfluoxetine
Fluvoxamine (Luvox™)	50	50–300	16	N/A	53	3	≅100 (300 nM)	3.8	≅70	80	Extensive to inactive metabolites
Paroxetine (Paxil®™)	10–20	20–50	21	N/A	>90	5	≅40 (130 nM)	0.29	≅80	95	Extensive by cytochrome P450 to catechol and other inactive metabolites
Sertraline (Zoloft®)	25–50	100–200	26	*N*-Desmethyl-sertraline: 62–104 h	88	5–8	≅25 (65 nM)	0.19 (NA)	≅80	98	Extensive to desmethyl-sertraline
Escitalopram (Lexapro®)	10	10–20	27–32	*S*-Desmethyl-citalopram: 59 h	80	5				56	Hepatic, metabolized to *S*-didesmethyl-citalopram

[a] Plasma level for fluoxetine represents total fluoxetine plus norfluoxetine given the comparable effect of each on 5-HT uptake pump. Plasma levels are a total of both enantiomers for citalopram and fluoxetine.

[b] Value for parent drug; value for the respective major metabolite is in parentheses; no data available for sertraline metabolite.

or haloperidol. Other patients remained symptom-free.

- Aspirin (and benorilate): *Citalopram* may increase the risk of bleeding.
- Artemether with lumefantrine: Avoid concomitant use of *citalopram*.
- Astemizole: The makers of astemizole contraindicate the concurrent use of SSRIs (because of possible increased astemizole serum levels) and those proarrhythmic drugs which might additively prolong the QT interval and thereby increase the risk of serious arrhythmias.
- β-Blockers (carvedilol and metoprolo): *Citalopram* and *fluvoxamine* may increase the levels of some β-blockers.
- Barbituates: They lower the convulsive threshold.
- Benzodiazepines: *Sertraline* and *fluvoxamine* may inhibit the metabolism of alprazolam and diazepam, resulting in elevated serum levels potentially causing sedation and psychomotor impairment.
- Benztropine: Seven patients developed delirium when given *fluoxetine*, *paroxetine*, or *sertraline* with benztropine in the presence of perphenazine or haloperidol. Other patients remained symptom-free.
- Buspirone: An isolated report describes the development of the serotonin syndrome with buspirone and *citalopram*; the same may happen with *fluvoxamine*. Busprone with *fluoxetine* can be effective, but some adverse reactions have been reported. *Fluvoxamine* may possibly reduce the effects of buspirone.
- Carbamazepine: Some, but not all, reports indicate that carbamazepine serum levels can be increased by *fluoxetine* and *fluvoxamine*. Toxicity may develop. *Sertraline* normally appears not to affect carbamazepine, but sertraline levels may be reduced by carbamazepine. Isolated cases of Parkinson-like and serotonin syndrome have occurred with *fluoxetine* and carbamazepine, while an isolated case of pancytopenia has been reported with *sertraline* and carbamazepine. The metabolism of citalopram may be increased.
- Carvedilol: Serum concentrations may be increased.
- Cilostazol: Inhibitors of cytochrome P450 isoenzyme CYP3A4 are predicted to increase the serum levels of cilostazol or its active metabolite (all SSRIs potentially affected).
- Cimetide: Cimetide causes a moderate rise in the serum levels of *sertraline*. It may inhibit the metabolism of citalopram. Cimetide may reduce the first-pass metabolism of *paroxetine*, resulting in elevated *paroxetine* serum concentrations.
- Cisapride (propulsid): Do not take *fluvoxamine*.
- Clozapine: *Fluoxetine*, *paroxetine*, *sertraline*, and possibly *citalopram* can raise serum clozapine levels. Particularly large increases can occur with *fluvoxamine*. Toxicity has been seen in some patients.
- Coumarins: Anticoagulant effect possibly enhanced by *citalopram*.
- CYP2D6 substrates (desipramine, nortriptyline, haloperidol, thioridazine, flecainide, codeine, propanolol, metprolol): SSRIs can inhibit *in vitro* and *in vivo* the hepatic isoenzymes 2D6 of the cytochrome P450 system (CYP2D6), which is involved in the oxidative metabolism of numerous drugs. SSRIs can cause a significant increase in the serum concentrations of these drugs.
- Cyclosporine: *Fluoxetine* may increase the serum levels of cyclosporine (and possibly tacrolimus)
- Cyproheptadine: Reports say that cyproheptadine can oppose the antidepressant effects of *fluoxetine*, *fluvoxamine*, and *paroxetine*.
- Dextromethorphan: Some SSRIs inhibit the metabolism of dextromethorphan; visual hallucinations have occurred; it may cause serotonin syndrome
- Digitalis glycoside: Neither *citalopram* nor *fluvoxamine* appear to interact with digoxin, but an isolated report describes increased serum digoxin levels attributed to the use of *fluoxetine*.
- Digoxin: *Fluoxetine* may increase serum levels of digoxin.
- Dihydroergotamine: Three isolated cases of the serotonin syndrome have been seen in patients on *paroxetine* with imipramine, amitriptyline, or *sertraline* when given dihydroergotamine.
- Disulfram (Antabuse): Do not take with *Zoloft oral concentrate* which contains alcohol and may cause a reaction.
- Erythromycin: An isolated report describes the development of what was thought to be serotonin syndrome in a 12-year-old child taking *sertraline* when erythromycin was added.
- Haloperidol: Serum may be increased slightly by *sertraline*. *Fluoxetine*, *paroxetine* and *fluvoxamine* may inhibit the metabolism of haloperidol and cause extrapyramidal symptoms.
- Human menopausal gonadotropin-CoA reductase inhibitors: *Sertraline*, *paroxetine*, *fluvoxamine*, and *fluoxetine* may inhibit the metabolism of lovastatin and simvastatin resulting in myosis and rhabdomyolysis; although its inhibition is weak, these combinations are best avoided.
- Isoniazid: No important interaction appears to occur between isoniazid and the SSRIs or nefazodone. However, adverse reactions have been seen

during concurrent use but they are thought unlikely to have been due to an interaction.

- Lamotrigine: Toxicity has been reported following the addition of *sertraline*.
- Linezolid: Hyperpyrexia, hypertension, tachardia, confusion, seizures, and deaths have been reported with agents that inhibit MAO (serotonin syndrome), *escitalopram*, and *citalopram*.
- Lithium: There is increased risk of CNS effects; lithium toxicity is reported.
- Loop diuretics: *Sertraline*, *paroxetine*, and *fluvoxamine* may cause hyponatremia; additive hyponatremic effects may be seen with combined use.
- Macrolide antibacterials: An isolated case report describes what appeared to be acute *fluoxetine* intoxication in a man brought about by the addition of clarithromycin.
- MAOI: This is contraindicated owing to potential risk of serotonin syndrome. Hyperpyrexia, hypertension, tachycardia, confusion, seizures, and deaths have been reported. A minimum 2 week's wash-out period should be observed between stopping a MAOI and starting an SSRI. Conversely, a MAOI should not be started for at least 1 week after an SSRI has been stopped (at least 5 weeks for *fluoxetine*; at least 2 weeks for *paroxetine* and *sertraline*).
- Meperidine: Combined use theoretically may increase the risk of serotonin syndrome.
- Methadone: Methadone serum levels may rise if *fluvoxamine* is added, possibly resulting in increased side effects. *Sertraline* may also increase methadone levels, but no interaction appears to occur with *fluoxetine*.
- Mesoridazine: *Fluoxetine* and *paroxetine* may inhibit the metabolism of mesoridazine, resulting in increased plasma levels and increasing the risk of QT_c interval prolongation. This may lead to serious ventricular arrhythmias, such as torsade de pointes-type arrhythmias, and sudden death. Wait at least 5 weeks after discontinuing these SSRIs prior to starting mesoridazine.
- Methylphenidate: Metabolism of *citalopram* may be inhibited.
- Metoprolol: *Escitalopram* and *citalopram* may increase plasma levels of metoprolol.
- Moclobemide: Concurrent use with *escitalopram* or *citalopram* make cause serotonin syndrome.
- Nefazodone: Concurrent may cause serotonin syndrome.
- NSAIDs: There is increased risk of bleeding when NSAIDs are used with escitalopram.
- Olanzapine: *Fluvoxamine* causes a rise in serum olanzapine levels.
- Perhexiline: Three case reports describe an increase in perhexiline serum levels with toxicity due to the concurrent use of *fluoxetine* or *paroxetine*.
- Phenothiazines: *Sertraline* may inhibit metabolism of thioridazine or mesoridazine, potentially leasing to malignant ventricular arrhythmias.
- Phenytoin: Phenytoin serum levels can be increased in some patients by *fluoxetine*. Toxicity may occur. There are also isolated reports of phenytoin toxicity with the concurrent use of *fluvoxamine* and *paroxetine*. Phenyltoin and *sertraline* do not normally interact; nevertheless, two patients have shown increased serum phenytoin levels.
- Pimozide (Orap): Do not take *fluvoxamine*.
- Primidone: When used with escitalopram, convulsive threshold is decreased.
- Propafenone: Serum concentrations and/or toxicity may be increased by *fluoxetine* and *fluvoxamine*.
- Quinidine: Serum concentrations may be increased with *fluvoxamine*.
- Risperidone: *Paroxetine* inhibits the metabolism of risperidone resulting in elevated risperidone levels; it may cause extrapyramidal symptoms.
- Ritonavir: Plasma concentrations of SSRI are possibly increased. It may cause serotonin syndrome in HIV patients when used with *citalopram*.
- Selegiline: Concurrent use has been reported to cause mania, hypertension, and in some cases serotonin syndrome. As a MAO type-B inhibitor, the risk of serotonin syndrome may be less than with nonselective MAO inhibitors.
- Sibutramine: May increase the risk of serotonin syndrome. (Manufacturers recommend avoiding concomitant use.)
- Sumatriptan (and other serotonin agonists): Concurrent use may result in toxicity; weakness, hyperreflexia, and incoordination. This combination may also increase the risk of serotonin syndrome; it also includes naratriptan, rizatriptan, and zolmitriptan.
- SRIs and SSRIs: Concurrent use with other reuptake inhibitors may increase the risk of serotonin syndrome.
- St. John's wort (*Hepericum perforatum*): Four patients on *sertraline* and one on nefazodine developed symptoms diagnosed as serotonin syndrome when St. John's wort was taken concomitantly. Another patient on St. John's wort developed severe sedation after taking a single dose of *paroxetine*.
- Sympathomimetics: Concurrent use may increase the risk of serotonin syndrome.

- Tacrine: *Fluvoxamine* inhibits the metabolism of tacrine; use alternative SSRI.
- Tacrolimus: *Fluvoxamine* may inhibit the metabolism of tacrolimus.
- TCAs: SSRIs inhibit the metabolism of TCAs resulting in elevated serum levels; if necessary, a low dose of TCA should be used.
- Theophylline: *Fluvoxamine* inhibits the metabolism of theophylline. *Paroxetine* may also inhibit the metabolism of theophylline.
- Thioridazine (Mellaril): Dangerous, even fatal, irregular heartbeats may occur when taken with *fluoxetine*, *fluvoxamine*, or *paroxetine*. These may inhibit the metabolism of thioridazine, resulting in increased plasma levels and increasing the risk of QT_c interval prolongation. This may lead to serious ventricular arrhythmias, such as torsade de pointes-type arrhythmias, and sudden death. Wait at least 5 weeks after discontinuing these prior to starting thioridazine.
- Tramadol: Five reports describe the development of the serotonin syndrome in patients on *fluoxetine*, *paroxetine*, or *sertraline* when tramadol was added. Another patient developed hallucinations with tramadol and *paroxetine*. Other reports suggest that the SSRI/tramadol combination is therapeutically valuable and normally safe. Also, carefully monitor concomitant use with *fluvoxamine*.
- Trazodone: Concurrent use may cause serotonin syndrome. SSRIs may inhibit the metabolism of trazadone resulting in increased toxicity.
- Tryptophan: It causes agitation and nausea.
- Valproic acid: *Fluoxetine* may increase serum levels of valproic acid.
- Venlafaxine: Combined use may increase the risk of serotonin syndrome.
- Warfarin: *Sertraline*, *fluoxetine*, *fluvoxamine*, and *paroxetine* may alter the hypoprothrombinemic response to warfarin.
- Zolpidem: A case of delirium has been reported when used in combination with *paroxetine*.
- *Herbal*: Avoid valerian, St. John's wort, SAMe, kava kava, and gotu kola; these may increase CNS depression.

Side Effects

Drowsiness, tremor in the upper extremities, lightheadedness, nausea, and vomiting are the most common symptoms. Other symptoms include tachycardia (occasionally bradycardia), hypo/hypertension, dilated pupils, agitation, dry mouth, and sweating. Citalopram also has shown effects of insomnia, somnolence, and erostomia.

Side effects are similar for all SSRIs, but have different degrees of severity. Data from the Committee on Safety of Medicines showed more reports of suspected reactions (including discontinuation reactions) to *paroxetine*, and of gastrointestinal reactions to *fluvoxamine* and *paroxetine* than to the other SSRIs during the first 2 years of marketing. Prescription–event monitoring revealed a higher incidence of adverse events related to *fluvoxamine* than its comparators. There were higher incidences of gastrointestinal symptoms, malaise, sedation, and tremor during treatment with fluvoxamine and of sedation, tremor, sweating, sexual dysfunction, and discontinuation reaction with paroxetine. It has been shown that *sertraline* caused significantly more sexual dysfunction and libido problems than did *fluvoxamine*. *Fluoxetine* caused more side effects than did *sertraline* in geriatrics. Escitalopram had low discontinuation rates due to its high tolerability.

Discontinuation of SSRIs can cause adverse events including dizziness, insomnia, nervousness, nausea, and agitation. See Discontinuation Syndrome under the section Human Toxicology (Acute) for more information.

SSRIs can inhibit hepatic isoenzymes 2D6 of the cytochrome P450 system (CYP2D6), which is involved in the oxidative metabolism of numerous drugs.

Serotonin Syndrome

Antidepressants are considered to have additive effects, therefore combined use is not recommended. Inhibitors of serotonin reuptake by CNS neurons may interact with other drugs or circumstances which cause serotonin release. The enhancement of the serotonergic effects may produce a life-threatening serotonin syndrome. Drugs which can increase the serotonin level when taken in combination with SSRIs include: TCAs, MAOIs, reversible inhibitors of monoamine oxidase, carbamazepine, lithium, or serotoneric substances. These drugs should not be coadministered with SSRIs and they may increase the risks of developing a serotonin syndrome.

Serotonin syndrome is characterized by the presence of at least three of the following symptoms: mental status changes, agitation, myoclonous, hyperreflexia, sweating, shivering, tremor, diarrhea, motor incoordination, muscle rigidity, and fever. Severe complications may occur, including severe hyperthermia, rhabdomyolysis, disseminated intravascular coagulation, convulsions, respiratory arrest, and death.

No prospective studies have been performed to evaluate the treatment of serotonin syndrome, and

treatment strategies are primarily based on case reports. Nonspecific serotonin receptor antagonists such as methyseride and cyproheptadine have been used successfully. Propanolol, which blocks serotonin 1A receptors, has also been used. Benzodiazepines can reduce the muscular rigidity. Dantrolene, a direct skeletal muscle relaxant, has also been found to be useful. The efficacy of these agents is yet to be evaluated.

Animal/Nonclinical Toxicology

Citalopram: Animal reproductive studies have revealed adverse effects on fetal and postnatal development at a dose higher than the human therapeutic dose.

Escitalopram: Teratogenic effects have been reported in animal studies.

Human Toxicology (Acute)

Ingestion: Nausea, vomiting, abdominal pain, diarrhea, tremor, confusion, agitation, drowsiness, insomnia, flulike syndromes, blurred vision, and in rare cases, seizures and coma. Significant cardiovascular toxicity is unusual (except with citalopram). Effects include mild hypo- or hypertension, tachycardia, and ventricular dysrhythmia. Escitalopram also has been shown to cause dermatologic, gastrointestinal, sexual, respiratory, and other miscellaneous adverse reactions.

More serious toxicity may be expected with high doses or coingestion, e.g., TCAs and MAOIs, which may result in a life-threatening serotonin syndrome.

Discontinuation Syndrome

SSRIs can cause adverse effects after withdrawal, either from a reduction in dosage or from the abrupt cessation of the drug. The most frequent symptoms include vertigo, dizziness, paresthesia (shocklike sensations, tingling and burning sensations); less frequently, symptoms include irritability, anxiety, headache, orthostatic hypotension, and sleep disturbances. The symptoms usually occur within 48 h after stopping the SSRI and they last $\sim$2 weeks.

Human Toxicology (Chronic)

Ingestion: A case of sertraline abuse has been reported with high doses, almost daily, for a period of 6 months. Effects include relaxation, euphoria, then intense excitement, marked tremor, and visual and auditory hallucinations.

Patients have survived large overdoses of each of the compounds, but there is concern over six fatalities following overdoses of citalopram.

Clinical Management of Overdose

Pure SSRI overdoses usually have a fairly benign course. However, a few deaths are reported in the literature, most involving a coingestion and/or very high doses.

Treatment is symptomatic and supportive, with diazepam for sedation if necessary. Cardiac monitoring is recommended in symptomatic cases. Coma and convulsions may occur in large overdose. If the patient presents within 2 h, a dose of activated charcoal should be given (50 g for adults; $1\,g\,kg^{-1}$ for children). Observation of vital signs and neurological status for 6 h is recommended.

Treatment of the serotonin syndrome in more severe intoxications or where there is a coingestion may require more aggressive measures such as establishment of an airway, ventilation, administration of intravenous fluids, and control of seizures and hyperthermia.

Intensive supportive care is rarely required. Measures that may be required based on the clinical presentation include endotracheal intubation and assisted ventilation if coma is present, intravenous fluid resuscitation if hypotension is present, pharmacological control of seizures, cooling if hyperthermia is present.

Gastrointestinal decontamination by administration of a single oral dose of active charcoal may be indicated. Gastric lavage followed by activated charcoal should be advocated in patients who have ingested large doses and/or when there has been a significant coingestion. There is no effective method known to enhance elimination.

See also: Food and Drug Administration, US; International Conference on Harmonisation.

Further Reading

DiPiro JT, Talbert RL, Yee GC, *et al.* (eds.) (2002) *Pharmacotherapy, A Pathophysiological Approach*, 5th edn. Chicago: McGraw-Hill.

Masand PS and Gupta S (1999) Selective serotonin-reuptake inhibitors: An update. *Harvey Reviews in Psychiatry* 7(2): 69–84.

Stockley I (ed.) (2003) *Drug Interactions*, 6th edn. Chicago: Pharmaceutical Press.

Vaswani M, Linda F, Ramesh S, *et al.* (2003) Role of selective serotonin reuptake inhibitors in psychiatric disorders: A comprehensive review. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 27(1): 85–102.

Relevant Websites

http://www.inchem.org – Selective Serotonin Reuptake Inhibitors (Poisons Information Monograph from the International Programme on Chemical Safety)

www.Preskorn.com – Clinical Pharmacology of SSRIs, Applied Clinical Psychopharmacology, Psychiatric Research Institute.

Staphylococcus aureus

Melanie J Karst

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Description

Staphylococcus aureus is a spherical coccoid which on microscopic examination appears in pairs, short chains, or bunched grape-like clusters. *S. aureus* forms a fairly large yellow colony on rich agar. The organisms are Gram-positive facultative anaerobes that grow by aerobic respiration or by fermentation yielding primarily lactic acid. The bacterium is capable of producing many highly heat-stable protein toxins.

Sources of Exposure

Staphylococci exist in air, dust, sewage, water, milk, and food or on food equipment, environmental surfaces, humans, and animals. Normally, this species lives in the human oropharynx, nose, large intestine, vagina, and on the skin without causing harm. However, if a breach in the skin or mucosal barrier occurs, *S. aureus* gains access to nearby tissues or the bloodstream where it can colonize and cause disease. The relationship between *S. aureus* and its human host, then, is dynamic in nature, capable of quickly shifting from mutualistic or commensualistic to parasitic.

Pathogenesis

S. aureus causes a variety of suppurative (pus-forming) infections and toxinoses in humans. It may cause superficial skin lesions (boils and styes); infections such as pneumonia, mastitis, phlebitis, meningitis, and urinary tract infections; and deep-seated infections such as osteomyelitis and endocarditis. *S. aureus* is associated with nosocomial infections of surgical wounds and infections with indwelling medical devices. *S. aureus* can cause toxic shock syndrome by releasing pyrogenic exotoxins into the bloodstream.

Food Poisoning

Human intoxication is caused by ingesting enterotoxins produced in food by some strains of *S. aureus*, usually because the food has not been kept hot enough (60°C, 140°F, or above) or cold enough (7.2°C, 45°F, or below). Foods that are frequently incriminated in staphylococcal food poisoning include meat and meat products; poultry and egg products; salads such as egg, tuna, chicken, potato, and macaroni; bakery products such as cream-filled pastries, cream pies, and chocolate éclairs; sandwich fillings; and milk and dairy products. Any foods that require considerable handling during preparation and that are kept at warmer temperatures after preparation are frequently involved in staphylococcal food poisoning.

Staphylococcal food poisoning is one of the most common types of food poisoning in the United States. The true incidence of staphylococcal food poisoning is unknown due to lack of information from victims, misdiagnosis of the illness, and inadequate collection of samples for laboratory analyses. A toxin dose of less than 1.0 μg in contaminated food will produce symptoms of staphylococcal intoxication. This toxin level represents a *S. aureus* population exceeding 100 000 organisms per gram.

Mechanism of Toxicity

S. aureus expresses many virulence factors: (1) surface proteins that promote colonization; (2) invasins that promote spread of bacteria in tissues; (3) surface characteristics that inhibit phagocytic engulfment (capsule, protein A); (4) biochemical properties that enhance their survival in phagocytes (carotenoids, catalase); (5) membrane-damaging toxins (hemolysins, leukotoxin, leukocidin); and (6) exotoxins that damage host tissue (toxic shock syndrome toxins, exfoliation toxin). The toxic shock toxin is a 'superantigen' that stimulates T-cells and the release of

cytokines causing symptoms mimicking endotoxic shock.

Diagnosis of Human Infection/Illness

Staphylococcal skin infections can usually be diagnosed by their appearance without laboratory testing. Serious staphylococcal infections require samples of blood or infected fluid for culture and diagnosis of which antibiotics should be used. Some strains are resistant to many antibiotics. Methicillin-resistant *S. aureus* is resistant to nearly all antibiotics and is increasingly common.

Proper interviews with the victims and analyzing epidemiological data are essential in the diagnosis of staphylococcal foodborne incidents. The most conclusive evidence is the linking of an illness with a specific food or detection of the toxin in a food sample.

Analysis of Foods

The staphylococcal toxin must be separated from food constituents and concentrated to detect trace amounts. The toxin is then identified by specific precipitation with antiserum as follows: (1) the selective adsorption of the enterotoxin from an extract of the food onto ion exchange resins and (2) the use of physical and chemical procedures for the selective removal of food constituents leaving the enterotoxin in solution. More recently rapid methods based on monoclonal antibodies (e.g., enzyme-linked immunosorbent assay, reverse passive latex agglutination) have been developed for detecting very low levels of enterotoxin in food.

Nature of the Disease

Staphylococcus bacteria are one of the most common causes of skin infection. There are many kinds of staphylococcal skin infections. The least serious is folliculitis, an infection of a hair root that produces a slightly painful pimple at the base of a hair. Most of these infections are minor (pimples and boils); however, *Staphylococcus* bacteria may also cause serious infections. Impetigo causes shallow, fluid-filled blisters and may itch or hurt. *Staphylococcus* skin abscesses are warm, painful, collections of pus below the skin surface and staphylococcus cellulitis is a spreading infection that develops under the skin producing pain and redness. More serious skin infections include toxic epidermal necrolysis and scalded skin syndrome in newborns.

The onset of symptoms of staphylococcal food poisoning is usually rapid and in many cases acute. The severity depends on the individual's susceptibility to the toxin, the amount of toxin ingested from contaminated food, and the general health of the person. The most common symptoms are nausea, vomiting, retching, abdominal cramping, and prostration. In more severe cases headache, muscle cramping, and transient changes in blood pressure and pulse rate may occur. All people are believed to be susceptible to this type of bacterial intoxication; however, the intensity and severity of symptoms may vary. Death from staphylococcal food poisoning is very rare but may occur among sensitive populations such as the elderly, infants, and immunocompromised individuals.

Clinical Management

Infections can usually be treated with penicillinase-resistant B-lactams. Infections acquired in the hospital are often antibiotic resistant strains and can only be treated with vanomycin. Vaccines are not currently available.

When contaminated food is ingested, the toxins, not the bacteria, produce the illness. Since this food poisoning is not an infectious disease antibiotics are of no value. Most cases do not require hospitalization but fluid replacement may be required. Prevention of staphylococcal food poisoning by cleanliness of food preparation areas, proper refrigeration, and good hand washing is the most effective control strategy.

See also: Food and Drug Administration, US; Food Safety and Toxicology; Gastrointestinal System; Skin.

Further Reading

Le Loir Y, Baron F, and Gautier M (2003) *Staphylococcus aureus* and food poisoning. *Genetics and Molecular Research* 2(1): 63–76.

Relevant Websites

http://vm.cfsan.fda.gov – US Food and Drug Administration. Center for Food Safety and Applied Nutrition. Foodborne Pathogenic Microorganisms and Natural Toxins Handbook. *Staphylococcus aureus*.

http://www.cdc.gov – CDC. MRSA – Methicillin Resistant *Staphylococcus aureus*. Fact Sheet.

Statistics

Shayne C Gad

Introduction

Statistics are an essential (and required by regulation) tool in all aspects of toxicology. They provide a means of objectively evaluating our tests of belief; for example, experiments. Increasing amounts and types of toxicological data are being generated each year. As a result, problems of data analysis have become more complex and toxicology has drawn more deeply from the well of available statistical techniques. Statistics has also changed and grown during the last 35 years, to some extent, at least, because of the parallel growth of toxicology. These simultaneous changes have led to an increasing complexity of data analysis. Before turning to the specific issues related to using statistics in interpreting toxicological data, it is important to discuss some of the basics of statistics.

Functions of Statistics

Statistical methods may serve to do any combination of three possible tasks. The most familiar is hypothesis testing, that is, determining if two (or more) groups of data differ from each other at a predetermined level of confidence. A second function is the construction and use of models which may be used to predict future outcomes of chemical–biological interactions. This is most commonly seen in linear regression or in the derivation of some form of correlation coefficient. Model fitting allows us to relate one variable (typically a treatment or 'independent' variable) to another. The third function, reduction of dimensionality, continues to be less commonly utilized than the first two. This final category includes methods for reducing the number of variables in a system while only minimally reducing the amount of information, therefore making a problem easier to visualize and understand. Examples of such techniques are factor analysis and cluster analysis. A subset of this last function, discussed later under 'descriptive statistics', is the reduction of raw data to single expressions of central tendency and variability (such as the mean and standard deviation (SD)).

There is also a special subset of statistical techniques that is part of both the second and third functions of statistics. This is data transformation, which includes such things as the conversion of numbers to log or probit values.

Data

Each measurement made, each individual piece of experimental information gathered, is called a datum. Generally, multiple pieces of information are gathered and analyzed at one time, and the resulting collection is called data.

Data are collected on the basis of their association with a treatment (intended or otherwise) or as an effect (a property) that is measured in the experimental subjects of a study, such as body weights. These identifiers (i.e., treatment and effect) are termed variables. The treatment variables (those that the researcher or nature control, and that can be directly controlled) are termed independent, while our effect variables (such as weight, lifespan, and number of neoplasms) are termed dependent variables; their outcome is believed to be dependent on the 'treatment' being studied.

All the possible measures of a given set of variables in all the possible subjects that exist are termed the population for those variables. Such a population of variables cannot be truly measured; for example, to achieve this it would be necessary to obtain, treat and measure the weights of all the animals of a particular type; for example, Fischer-344 rats, that were, are, or ever will be. Instead, studies are performed on a representative group, a sample. If the sample of data is appropriately collected and of sufficient size, it serves to provide good estimates of the characteristics of the parent population from which it was drawn.

Data can be classified into different types. **Table 1** shows one such classification.

The nature of the data collected is determined by three considerations. These are the source of the data

Table 1 Types of variables (data) and examples of each type

Classified by	*Type*	*Toxicological example*
Scale		
Continuous	Scalar	Body weight
	Ranked	Severity of a lesion
Discontinuous	Scalar	Weeks until the first observation of a tumor in a carcinogenicity study
	Ranked	Clinical observations in animals
	Attribute	Eye colors in fruit flies
	Quantal	Dead/alive or present/absent
Frequency distribution	Normal	Body weights
	Bimodal	Some clinical chemistry parameters
	Others	Measures of time to incapacitation

(the system being studied), the instrumentation and techniques being used to make measurements, and the design of the experiment. The researcher has some degree of control over each of these, the least over the system; for example, biological organism (he or she normally has a choice of only one of several models to study), and the most over the design of the experiment or study. Such choices, in fact, dictate the type of data generated by a study.

Statistical methods are based on specific assumptions. Parametric statistics, those most familiar to the majority of scientists, have more stringent underlying assumptions than do nonparametric statistics. Among the underlying assumptions for many parametric statistical methods (such as the analysis of variance) is that the data are continuous. The nature of the data associated with a variable (as described previously) imparts a 'value' to that data, the value being the power of the statistical tests which can be employed.

Continuous variables are those that can at least theoretically assume any of an infinite number of values between any two fixed points (such as measurements of body weight between 2.0 and 3.0 kg). Discontinuous variables, meanwhile, are those which can have only certain fixed values, with no possible intermediate values (such as counts of five and six dead animals, respectively).

Limitations on the ability to measure constrain the extent to which the real-world situation approaches the theoretical, but many of the variables studied in toxicology are in fact continuous. Examples of these are lengths, weights, concentrations, temperatures, periods of time, and percentages. For these continuous variables, the character of a sample may be described using measures of central tendency and dispersion that are most familiar: the mean, denoted by the symbol $\bar{X}$ and also called the arithmetic average, and the SD, denoted by the symbol σ and calculated as being equal to

$$\sigma = \sqrt{\frac{\sum X^2 - (\sum X)^2/N}{N-1}}$$

where X is the individual datum and N is the total number of data in the group.

Contrasted with these continuous data, however, are discontinuous (or discrete) data, which can only assume certain fixed numerical values. In these cases the choice of statistical tools or tests is, as will be seen, more limited.

Descriptive Statistics

Descriptive statistics are used to summarize the general nature of a data set. As such, the parameters describing any single group of data have two components. One of these describes the location of the data, while the other gives a measure of the dispersion of the data in and about this location. Often overlooked is the fact that the choice of which parameters are used to generate these pieces of information implies a particular type of distribution for the data.

Most commonly, location is described by giving the (arithmetic) mean and dispersion in terms of the SD or the SEM. The calculation of the first two of these has already been described. If the total number of data in a group is N, then the SEM would be calculated as

$$\text{SEM} = \frac{\text{SD}}{\sqrt{N}}$$

The use of the mean with either the SD or SEM implies, however, that there is reason to believe that the sample of data being summarized is from a population that is at least approximately normally distributed. If this is not the case, then it is more appropriate to use a set of statistical descriptions which do not require a normal distribution. These are the median, for location, and the semiquartile distance, for a measure of dispersion. These somewhat less familiar parameters are characterized as follows.

Median When all the numbers in a group are arranged in a ranked order (i.e., from smallest to largest), the median is the middle value. If there is an odd number of values in a group, then the middle value is obvious (in the case of 13 values, e.g., the seventh largest is the median). When the number of values in the sample is even, the median is calculated as the midpoint between the $(N/2)$th and the $((N/2)+1)$th number. For example, in the series of numbers 7, 12, 13, 19 the median value would be the midpoint between 12 and 13, which is 12.5.

The SD and the SEM are related to each other but yet are quite different.

The SEM is quite a bit smaller than the SD, making it very attractive to use in reporting data. This size difference is because the SEM actually is an estimate of the error (or variability) involved in measuring the mean values of samples, and not an estimate of the error (or variability) involved in measuring the data from which mean values are calculated. This is implied by the central limit theorem, which makes three major assumptions.

- The distribution of sample means will be approximately normal regardless of the distribution of

values in the original population from which the samples were drawn.
- The mean value of the collection equals the mean of all possible sample means, so the collection mean can be estimated from the sample means.
- The SD of the collection of all possible means of samples of a given size, called the SEM, depends on both the SD of the original population and the size of the sample.

The SEM should be used only when the uncertainty of the estimate of the mean is of concern, which is almost never the case in toxicology. Rather, toxicology is concerned with an estimate of the variability of the population for which the SD is appropriate.

Semiquartile Distance When all the data in a group are ranked, a quartile of the data contains one ordered quarter of the values. Typically, we are most interested in the borders of the middle two quartiles Q_1 and Q_3 which together represent the semiquartile distance and which contain the median as their center, are of most interest. Given that there are N values in an ordered group of data, the upper limit of the jth quartile (Q_j) may be computed as being equal to the $((jN-1)/4)$th value. After using this formula to calculate the upper limits of Q_1 and Q_3, it is possible to compute the semiquartile distance (which is also called the quartile deviation, and as such is abbreviated as QD) with the formula $\mathrm{QD} = (Q_3 - Q_1)/2$.

For example, for the 15-value data set 1, 2, 3, 4, 4, 5, 5, 5, 6, 6, 6, 7, 7, 8, 9, the upper limits of Q_1 and Q_3 can be calculated as

$$Q_1 = \frac{1(15+1)}{4} = \frac{16}{4} = 4$$

$$Q_3 = \frac{3(15+1)}{4} = \frac{48}{4} = 12$$

The fourth and 12th values in this data set are 4 and 7, respectively. The semiquartile distance can then be calculated as

$$\mathrm{QD} = \frac{7-4}{2} = 1.5$$

There are times when it is desired to describe the relative variability of one or more sets of data. The most common way of doing this is to compute the coefficient of variation (CV), which is calculated simply as the ratio of the SD to the mean, or

$$\mathrm{CV} = \frac{\mathrm{SD}}{\bar{X}}$$

A CV of 0.2% or 20% thus means that the SD is 20% of the mean. In toxicology the CV is frequently between 20% and 50% and may at times exceed 100%.

Applying Statistics to Toxicology

To successfully apply statistics to toxicology it is critical to understand the biological dimensions of a problem, as well as the unique characteristics of toxicological data analysis. These characteristics include the following:

1. The need to work with a relatively small sample set of data collected from the members of a population (laboratory animals) that are not actually the population of interest (i.e., humans or a target animal population).
2. The need to frequently deal with data resulting from a sample that was censored on a basis other than the investigator's design. By censoring, of course, is meant that not all desired data points were collected. This censoring could be the result of either a biological factor (the test animal being dead or too debilitated to manipulate) or a logistic factor (equipment being inoperative or a tissue being missed in necropsy).
3. The conditions under which experiments are conducted are extremely varied. In pharmacology (the closest cousin to at least classical toxicology), the possible conditions of interaction of a chemical or physical agent with a person are limited to a small range of doses via a single route over a short course of treatment to a defined patient population. In toxicology however, all these variables (dose, route, time span, and subject population) are limited only by the investigator.
4. The timeframes available to solve toxicological problems are limited by practical and economic factors. This frequently means that there is no time to repeat a critical study if the first attempt fails. So a true iterative approach is often not possible.

The training of most pathologists in statistics remains limited to a single introductory course which concentrates on some theoretical basics. As a result, the armentarium of statistical techniques of most toxicologists is limited and the tools that are normally employed (*t*-tests, chi-square, analysis of variance, and linear regression) are neither fully developed nor well understood.

To appreciate the biological dimensions of analyzing data and the difference between biological significance and statistical significance, it is useful to

consider the four possible combinations of these two different types of significance as shown in the table given below:

Biological significance	*Statistical significance*	
	No	*Yes*
No	Case I	Case II
Yes	Case III	Case IV

Cases I and IV give no problems, for the answers are the same statistically and biologically. But cases II and III present problems. In case II (the 'false positive'), we have a circumstance where there is a statistical significance in the measured difference between treated and control groups, but there is no true biological significance to the finding. This is not an uncommon happening, for example, in the case of clinical chemistry parameters. This is called a type I error by statisticians, and the probability of this happening is called the α level. In case III (the 'false negative'), there is no statistical significance, but the differences between groups are biologically and toxicologically significant. This is called a type II error by statisticians, and the probability of such an error happening by random chance is called the β level. An example of this second situation is when there are few of a very rare tumor type in treated animals. In both of these latter cases, numerical analysis, no matter how well done, is no substitute for professional judgment. Along with this, however, it is critical to have a feeling for the different types of data and for the value or relative merit of each. Note that the two error types interact, and in determining sample size it is necessary to specify both α and β levels. **Table 2** demonstrates this interaction in the case of tumor or specific lesion incidence.

The reasons that biological and statistical significance are not identical are multiple, but a central one is certainly causality. Through this consideration of statistics, it should be kept in mind that just because a treatment and a change in an observed organism are seemingly or actually associated with each other does not 'prove' that the former caused the latter. Though this fact is now widely appreciated for correlation (e.g., the fact that the number of storks' nests found each year in England is correlated with the number of human births that year does not mean that storks bring babies), it is just as true in the general case of significance. Proof that treatment causes an effect requires an understanding of the underlying mechanism and proof of its validity. At the same time, it is important to realize that not finding a good correlation or suitable significance associated with a treatment and an effect likewise does not prove that the two are not associated, that a treatment does not cause an effect. At best, it gives a certain level of confidence that under the conditions of the current test, these items are not associated.

Bias and Chance

Any toxicological study aims to determine whether a treatment elicits a response. An observed difference in response between a treated and control group need not necessarily be a result of treatment. There are, in principle, two other possible explanations: *bias*, or systematic differences other than treatment between the groups, and *chance*, or random differences. A major objective of both experimental design and analysis is to try to avoid bias. Wherever possible, treated and control groups to be compared should be alike in respect of all other factors. Where differences remain, these should be corrected for in the statistical

Table 2 Sample size required to obtain a specified sensitivity at $p<0.05$ treatment group incidence

Background tumor incidence (%)	*p*[a]	*Required sample size*									
		Incidence rate (%)									
		0.95	*0.90*	*0.80*	*0.70*	*0.60*	*0.50*	*0.40*	*0.30*	*0.20*	*0.10*
0.30	0.90	10	12	18	31	46	102	389			
	0.50	6	6	9	12	22	32	123			
0.20	0.90	8	10	12	18	30	42	88	320		
	0.50	5	5	6	9	12	19	28	101		
0.10	0.90	6	8	10	12	17	25	33	65		
	0.50	3	3	5	6	9	11	17	31	68	
0.05	0.90	5	6	8	10	13	18	25	35	76	464
	0.50	3	3	5	6	7	9	12	19	24	147
0.01	0.90	5	5	7	8	10	13	19	27	46	114
	0.50	3	3	5	5	6	8	10	13	25	56

[a] Power for each comparison of treatment group with background incidence.

analysis. Chance cannot be wholly excluded, as identically treated animals will not respond identically. While even the most extreme difference might in theory be due to chance, a proper statistical analysis will allow the experimenter to assess this possibility The smaller the probability of a 'false positive', the more confident the experimenter can be that the effect is real. Good experimental design improves the chance of picking up a true effect with confidence by maximizing the ratio between 'signal' and 'noise'.

Hypothesis Testing and Probability (*p*) Values

A relationship of treatment to some toxicological end point is often stated to be 'statistically significant ($p < 0.05$)'. What does this really mean? A number of points have to be made. First, statistical significance need not necessarily imply biological importance, if the end point under study is not relevant to the animal's well-being. Second, the statement will normally be based only on the data from the study in question and will not take into account prior knowledge. In some situations, for example, when one or two of a very rare tumor type are seen in treated animals, statistical significance may not be achieved but the finding may be biologically extremely important, especially if a similar treatment was previously found to elicit a similar response. Third, the *p*-value does not describe the probability that a true effect of treatment exists. Rather, it describes the probability of the observed response, or one more extreme, occurring on the assumption that treatment actually had no effect whatsoever. A *p*-value that is not significant is consistent with a treatment having a small effect, not detected with sufficient certainty in this study. Fourth, there are two types of *p*-value. A 'one-tailed' (or one-sided) *p*-value is the probability of getting by chance a treatment effect in a specified direction as great as or greater than that observed. A 'two-tailed' *p*-value is the probability of getting, by chance alone, a treatment difference in either direction which is as great as or greater than that observed. By convention, *p*-values are assumed to be two-tailed unless the contrary is stated. Where, which is unusual, it is possible to rule out in advance the possibility of a treatment effect except in one direction, a one-tailed *p*-value should be used. Often, however, two-tailed tests are to be preferred, and it is certainly not recommended to use one-tailed tests and not report large differences in the other direction. In any event, it is important to make it absolutely clear whether one- or two-tailed tests have been used.

It is a great mistake, when presenting results of statistical analyses, to mark, as do some laboratories, results simply as significant or not significant at one defined probability level (normally $p < 0.05$). This practice does not allow the reader any real chance to judge whether or not the effect is a true one. Some statisticians present the actual *p*-value for every comparison made. While this gives precise information it can make it difficult to assimilate results from many variables. One recommended practice to mark *p*-values routinely using plus signs to indicate positive differences (and minus signs to indicate negative differences): $+++$ $p < 0.001$, $++$ $0.001 \leqslant p < 0.01$, $+$ $0.01 \leqslant p < 0.05$, $\pm$ $0.05 \leqslant p < 0.1$. This highlights significant results more clearly and also allows the reader to judge the whole range from 'virtually certain treatment effect' to 'some suspicion'. Note that using two-tailed tests, bracketed plus signs indicate findings that would be significant at the conventional $p < 0.05$ level using one-tailed tests but are not significant at this level using two-tailed tests. This 'fiducial limit' ($p < 0.05$) implies a false-positive incidence of one in 20, and though now imbedded in regulation, practice, and convention, was somewhat an arbitrary choice to begin with. In interpreting *p*-values it is important to realize they are only an aid to judgment to be used in conjunction with other available information. A $p < 0.01$ increase might be due to chance when it was unexpected, occurred only at a low dose level with no such effect seen at higher doses, and was evident in only one subset of the data. In contrast, a $p < 0.05$ increase might be convincing if it occurred in the top dose and was for an end point that was expected to show an increase from known properties of the chemical or closely related chemicals.

Multiple Comparisons

When a *p*-value is stated to be < 0.05, this implies that, for that particular test, the difference could have occurred by chance less than one time in 20. Toxicological studies frequently involve making treatment-control comparisons for large numbers of variables and, in some situations, also for various subsets of animals. Some statisticians worry that the larger the number of tests the greater is the chance of picking up statistically significant findings that do not represent true treatment effects. For this reason, an alternative 'multiple comparisons' procedure has been proposed in which, if the treatment was totally without effect, then 19 times out of 20 all the tests should show nonsignificance when testing at the 95% confidence level. Automatic use of this approach cannot be recommended. Not only does it make much more difficult to pick up any real effects, but also there is something inherently unsatisfactory

about a situation where the relationship between a treatment and a particular response depends arbitrarily on which other responses happened to be investigated at the same time. It is accepted that in any study involving multiple end points there will inevitably be a gray area between those showing highly significant effects and those showing no significant effects, where there is a problem distinguishing chance and true effects. However, changing the methodology so that the gray areas all come up as nonsignificant can hardly be the answer.

Estimating the Size of the Effect

It should be clearly understood that a *p*-value does not give direct information about the size of any effect that has occurred. A compound may elicit an increase in response by a given amount, but whether a study finds this increase to be statistically significant will depend on the size of the study and the variability of the data. In a small study, a large and important effect may be missed, especially if the end point is imprecisely measured. In a large study, on the contrary, a small and unimportant effect may emerge as statistically significant.

Hypothesis testing tells us whether an observed increase can or cannot be reasonably attributed to chance, but not how large it is. Although much statistical theory relates to hypothesis testing, current trends in medical statistics are toward confidence interval estimation with differences between test and control groups expressed in the form of a best estimate, coupled with the 95% confidence interval (Q). Thus, if one states that treatment increases response by an estimated 10 units (95% CI 3–17 units), this would imply that there is a 95% chance that the indicated interval includes the true difference. If the lower 95% confidence limit exceeds 0, this implies that the increase is statistically significant at $p < 0.05$ using a two-tailed test. One can also calculate, for example, 99% or 99.9% confidence limits, corresponding to testing for significance at $p < 0.01$ or $p < 0.001$.

In screening studies of standard design, the tendency has been to concentrate mainly on hypothesis testing. However, presentation of the results in the form of estimates with confidence intervals can be a useful adjunct for some analyses and is very important in studies aimed specifically at quantifying the size of an effect.

Two terms refer to the quality and reproducibility of the measurements of variables. The first, accuracy, is an expression of the closeness of a measured or computed value to its actual or 'true' value in nature. The second, precision, reflects the closeness or reproducibility of a series of repeated measurements of the same quantity.

If all of the measurements of a particular variable are arranged in order as points on an axis marked as to the values of that variable, and if the sample were large enough, the pattern of distribution of the data in the sample would begin to become apparent. This pattern is a representation of the frequency distribution of a given population of data; that is, of the incidence of different measurements, their central tendency, and dispersion. The most common frequency distribution, and one that will be discussed throughout this article, is the normal (or Gaussian) distribution. The normal distribution is such that two-thirds of all values are within 1 SD of the mean (or average value for the entire population) and 95% are within 1.96 SD of the mean. Symbols used are μ for the mean and σ for the SD. Other common frequency distributions, such as the binomial, Poisson and chi-square, are sometimes encountered.

Statistical Principles in Experimental Toxicology

Toxicological experiments generally have a twofold purpose. The first purpose is to answer the question whether or not an agent results in an effect on a biological system. The second purpose is to determine how much of an effect is present. It has become increasingly desirable that the results and conclusions of studies aimed at assessing the effects of environmental agents be as clear and unequivocal as possible. It is essential that every experiment and study yield as much information as possible, and that the results of each study have the greatest possible chance of answering the questions it was conducted to address. The statistical aspects of such efforts, so far as they are aimed at structuring experiments to maximize the possibilities of success, are called experimental design.

The four basic statistical principles of experimental design are replication, randomization, concurrent (local) control and balance. In abbreviated form, these may be summarized as follows:

1. *Replication*. Any treatment must be applied to more than one experimental unit (animal, plate of cells, litter of offspring, etc.). This provides more accuracy in the measurement of a response than can be obtained from a single observation, as underlying experimental errors tend to cancel each other out. It also supplies an estimate of the experimental error derived from the variability

among each of the measurements taken (or replicates). In practice, this means that an experiment should have enough experimental units in each treatment group (i.e., a large enough *N*) so that reasonably sensitive statistical analysis of data can be performed. The estimation of sample size is addressed in detail later in this article.

2. *Randomization*. This is practiced to ensure that every treatment shall have its fair share of extreme high and extreme low values. It also serves to allow the toxicologist to proceed as if the assumption of 'independence' is valid. This assumption is that there is no avoidable (known) systematic bias in obtaining data.
   Random allocation of animals to treatment groups is a prerequisite of good experimental design. If not carried out, it is not possible to be sure whether treatment versus control differences are due to treatment or to 'confounding' by other relevant factors. The ability to randomize easily is a major advantage animal experiments have over epidemiology.
   While randomization eliminates bias (as least in expectation), simple randomization of all animals may not be the optimal technique for producing a sensitive test. If there is another major source of variation (e.g., sex or batch of animals), it will be better to carry out stratified randomization (i.e., carry out separate randomizations within each level of the stratifying variable).
   The need for randomization applies not only to the allocation of the animals to the treatment, but also to anything that can materially affect the recorded response. The same random number that is used to apply animals to treatment groups can be used to determine cage position, order of weighing, order of bleeding for clinical chemistry, order of sacrifice at terminations and so on.
3. *Concurrent control*. Comparisons between treatments should be made to the maximum extent possible between experimental units from the same closely defined population. Therefore, animals used as a control group should come from the same source, lot, age, and so on as test group animals. Except for the treatment being evaluated, test and control animals should be maintained and handled in exactly the same manner.
   While historical control data can, on occasion, be useful, a properly designed study demands that a relevant concurrent control group be included with which results for the test group can be compared. The principle that like should be compared with like, apart from treatment, demands that control animals should be randomized from the same source as treatment animals. Careful consideration should also be given to the appropriateness of the control group. Thus, in an experiment involving treatment of a compound in a solvent, it would often be inappropriate to include only an untreated control group as any differences observed could only be attributed to the treatment–solvent combination. To determine the specific effects of the compound a comparison group given the solvent only, by the same route of administration, would be required.
   It is not always generally realized that the position of the animal in the room in which it is kept may affect the animal's response. An example is the strong relationship between incidence of retinal atrophy in albino rats and closeness to the lighting source. Systematic differences in cage position should be avoided, preferably via randomization.
4. *Balance*. If the effects of several different factors are being evaluated simultaneously, the experiment should be laid out in such a way that the contributions of the different factors can be separately distinguished and estimated. There are several ways of accomplishing this using one of several different forms of design, as will be discussed later.

In addition, there are a number of facets of any study which may affect its ability to detect an effect of a treatment. These relate to either minimizing the role of chance or avoiding bias.

Choice of Species and Strain

Ideally, the responses of interest should be rare in untreated control animals but should be reasonably readily evoked by appropriate treatments. For example, some species or specific strains, perhaps because of inappropriate diets, have high background tumor incidences which make increases both difficult to detect and difficult to interpret when detected.

Sampling

Sampling – the selection of which individual data points will be collected, whether in the form of selecting which animals to collect blood from or to remove a portion of a diet mix from for analysis – is an essential step upon which all other efforts toward a good experiment or study are based.

There are three assumptions about sampling which are common to most of the statistical analysis techniques that are used in toxicology. These are that the sample is collected without bias, that each member of a sample is collected independently of the others,

and that members of a sample are collected with replacements. Precluding bias, both intentional and unintentional, means that at the time of selection of a sample to measure, each portion of the population from which that selection is to be made has an equal chance of being selected. Ways of precluding bias are discussed in detail in the section on experimental design.

Independence means that the selection of any portion of the sample is not affected by and does not affect the selection or measurement of any other portion.

Finally, sampling with replacement means that in theory, after each portion is selected and measured, it is returned to the total sample pool and thus has the opportunity to be selected again. This is a corollary of the assumption of independence. Violation of this assumption (which is almost always the case in toxicology and all the life sciences) does not have serious consequences if the total pool from which samples are selected are sufficiently large (say 20 or greater) so that the chance of reselecting that portion is small anyway.

There are four major types of sampling methods: random, stratified, systematic, and cluster. Random is by far the most commonly employed method in toxicology. It stresses the fulfillment of the assumption of avoiding bias. When the entire pool of possibilities is mixed or randomized (procedures for randomization are presented in a later section), then the members of the group are selected in the order that are drawn from the pool.

Stratified sampling is performed by first dividing the entire pool into subsets or strata, then doing randomized sampling from each strata. This method is employed when the total pool contains subsets that are distinctly different but in which each subset contains similar members. An example is a large batch of a powdered pesticide in which it is desired to determine the nature of the particle size distribution. Larger pieces or particles are on the top, while progressively smaller particles have settled lower in the container and at the very bottom, the material has been packed and compressed into aggregates. To determine a timely representative answer, proportionally sized subsets from each layer or strata should be selected, mixed and randomly sampled. This method is used more commonly in diet studies.

In systematic sampling, a sample is taken at set intervals (such as every fifth container of reagent). This is most commonly employed in quality assurance or (in the clinical chemistry laboratory) in quality control.

In cluster sampling, the pool is already divided into numerous separate groups (such as bottles of tablets), and small sets of groups (such as several bottles of tablets) are first selected and then a few members from each set are selected. The result is a cluster of measures. Again, this is a method most commonly used in quality control or in environmental studies when the effort and expense of physically collecting a small group of units is significant.

In classical toxicology studies sampling arises in a practical sense in a limited number of situations. The most common of these are:

1. Selecting a subset of animals or test systems from a study to make some measurement (which either destroys or stresses the measured system, or is expensive) at an interval during a study. This may include such cases as doing interim necropsies in a chronic study or collecting and analyzing blood samples from some animals during a subchronic study.
2. Analyzing inhalation chamber atmospheres to characterize aerosol distributions with a new generation system.
3. Analyzing diet in which test material has been incorporated.
4. Performing quality control on an analytical chemistry operation by having duplicate analyses performed on some materials.
5. Selecting data to audit for quality assurance purposes.

Dose Levels

This is a very important and controversial area. In screening studies aimed at hazard identification it is normal, in order to avoid requiring huge numbers of animals, to test at dose levels higher than those to which man will be exposed, but not so high that marked toxicity occurs. A range of doses is normally tested to guard against the possibility of an inappropriate selection of the high dose as the metabolic pathways at the high doses may differ markedly from those at lower doses and, also, to ensure no large effects occur at dose levels in the range to be used by humans. In studies aimed at risk estimation, more and lower doses may be tested to obtain fuller information on the shape of the dose–response curve.

Number of Animals

This is obviously an important determinant of the precision of the findings. The calculation of the appropriate number depends on: (1) the critical difference, that is, the size of the effect it is desired to detect; (2) the false-positive rate, that is, the probability of an effect being detected when none exists

(equivalent to the 'α level' or 'type I error'), (3) the false-negative rate, that is, the probability of no effect being detected when one of exactly the critical size exists (equivalent to the 'β level' or 'type II error'), and (4) some measure of the variability in the material.

Tables and/or formulas relating numbers of animals required to obtain values of critical size, α and β are available in many statistics texts and software is also available for this purpose. As a rule of thumb, to reduce the critical difference by a factor of n for a given α and β the number of animals required will have to increased by a factor of n^2.

Duration of the Study

It is obviously important not to terminate the study too early for fatal conditions, which are normally strongly age-related. Less obviously, going on for too long in a study can be a mistake, partly because the last few weeks or months may produce relatively few extra data at a disproportionate cost, and partly because diseases of extreme old age may obscure the detection of tumors and other conditions of more interest. For nonfatal conditions, the ideal is to sacrifice the animals when the average prevalence is $\sim 50\%$.

Stratification

To detect a treatment difference with accuracy, it is important that the groups being compared are as homogeneous as possible with respect to other known causes of the response. In particular, suppose that there is another known important cause of the response for which the animals vary, so that the animals are a mixture of hyper- and hypo-responders from this cause. If the treated group has a higher proportion of hyperresponders it will tend to have a higher response even if treatment has no effect. Even if the proportion of hyperresponders is the same as in the controls, it will be more difficult to detect an effect of treatment because of the increased between animal variability.

Given that this other factor is known, it will be sensible to take it into account in both the design and analysis of the study. In the design, it can be used as a 'blocking factor' so that animals at each level are allocated equally (or in the correct proportion) to control and treated groups. In the analysis, the factor should be treated as a stratifying variable, with separate treatment–control comparisons made at each level, and the comparisons combined for an overall test of difference. This is discussed later, where the factorial design is addressed as one example of the more complex designs that can be used to investigate the separate effect of multiple treatments.

Statistics and Experimental Protocols in Toxicology

It is now routine to develop exhaustively detailed protocols for an experiment or study prior to its conduct. *A priori* selection of statistical methodology (as opposed to the *post hoc* approach) is as significant a portion of the process of protocol development and experimental design as any other and can measurably enhance the value of the experiment or study. Prior selection of statistical methodologies is essential for proper design of other portions of a protocol such as the number of animals per group or the sampling intervals for body weight. Implied in such a selection is the notion that the toxicologist has both an in-depth knowledge of the area of investigation and an understanding of the general principles of experimental design, for the analysis of any set of data is dictated to a large extent by the manner in which the data are obtained.

A second concept and its understanding are essential to the design of experiments in toxicology, that of censoring. Censoring is the exclusion of measurements from certain experimental units, or indeed of the experimental units themselves, from consideration in data analysis or inclusion in the experiment at all. Censoring may occur either prior to initiation of an experiment (where, in modern toxicology, this is almost always a planned procedure), during the course of an experiment (when they are almost universally unplanned, resulting from such as the death of animals on test), or after the conclusion of an experiment (when data are excluded because of being identified as some form of outlier).

In practice, *a priori* censoring in toxicology studies occurs in the assignment of experimental units (such as animals) to test groups. The most familiar example is in the common practice of assignment of test animals to acute, subacute, subchronic, and chronic studies, where the results of otherwise random assignments are evaluated for body weights of the assigned members. If the mean weights are found not be comparable by some preestablished criterion (such as a 90% probability of difference by analysis of variance) then members are reassigned (censored) to achieve comparability in terms of starting body weights. Such a procedure of animal assignment to groups is known as a *censored randomization*.

The first precise or calculable aspect of experimental design encountered is determining sufficient test and control group sizes to allow one to have an adequate level of confidence in the results of a study

(i.e., in the ability of the study design with the statistical tests used to detect a true difference, or effect, when it is present). The statistical test contributes a level of power to such a detection. Remember that the power of a statistical test is the probability that a test results in rejection of a hypothesis, H_0 say, when some other hypothesis, H, say, is valid. This is termed the power of the test 'with respect to the (alternative) hypothesis H'.

If there is a set of possible alternative hypotheses, the power, regarded as a function of H, is termed the *power function* of the test. When the alternatives are indexed by a single parameter θ, simple graphical presentation is possible. If the parameter is a vector θ, one can visualize a power surface.

If the power function is denoted by $\beta(\theta)$ and H_0 specifies $\theta = \theta_0$, then the value of β(II), the probability of rejecting H_0 when it is in fact valid, is the significance level. A test's power is greatest when the probability of a type II error is the least. Specified powers can be calculated for tests in any specific or general situation.

Some general rules to keep in mind are:

- The more stringent the significance level, the greater the necessary sample size. More subjects are needed for a 1% level test than for a 5% level test.
- Two-tailed tests require larger sample sizes than one-tailed tests. Assessing two directions at the same time requires a greater investment.
- The smaller the critical effect size, the larger the necessary sample size. Subtle effects require greater efforts.
- Any difference can be significant if the sample size is large enough.
- The larger the power required, the larger the necessary sample seize. Greater protection from failure requires greater effort. The smaller the sample size, the smaller the power; that is, the greater the chance of failure.
- The requirements and means of calculating necessary sample size depend on the desired (or practical) comparative sizes of test and control groups.

This number (N) can be calculated, for example, for equal-sized test and control groups, using the formula:

$$N = \frac{(t_1 + t_2)^2}{d^2} S$$

where t_1 is the one-tailed t-value with $N-1$ degrees of freedom corresponding to the desired level of confidence, t_2 is the one-tailed t-value with $N-1$ degrees of freedom corresponding to the probability that the sample size will be adequate to achieve the desired precision, S is the sample SD, derived typically from historical data and calculated as:

$$S = \sqrt{\frac{1}{N-1} \sum (V_1 - V_2)^2}$$

There are a number of aspects of experimental design which are specific to the practice of toxicology. Before discussing the step-by-step development of experimental designs, these aspects should first be considered.

1. Frequently, the data gathered from specific measurements of animal characteristics are such that there is wide variability in the data. Often, such wide variability is not present in a control or low-dose group, but in an intermediate dosage group variance inflation may occur. That is, there may be a large SD associated with the measurements from this intermediate group. In the face of such a set of data, the conclusion that there is no biological effect based on a finding of no statistically significance effect might well be erroneous.
2. In designing experiments, it is important to keep in mind the potential effect of involuntary censoring on sample size. In other words, though a study might start with five dogs per group, this provides no margin should any die before the study is ended and blood samples are collected and analyzed. Just enough experimental units per group frequently leaves too few at the end to allow meaningful statistical analysis, and allowances should be made accordingly in establishing group sizes.
3. It is certainly possible to pool the data from several identical toxicological studies. One approach to this is meta-analysis, considered in detail later in this chapter. For example, if an acute inhalation study was performed where only three treatment group animals survived to the point at which a critical measure (such as analysis of blood samples) was taken, there would not be enough data to perform a meaningful statistical analysis. In such a case, the protocol could be repeated with new control and treatment group animals from the same source. At the end, after assurances that the two sets of data are comparable, the data from survivors of the second study could be combined (pooled) with those from the first.
4. Another frequently overlooked design option in toxicology is the use of an unbalanced design, that is, of different group sizes for different levels of treatment. There is no requirement that each group in a study (control, low dose, intermediate

dose and high dose) have an equal number of experimental units assigned to it. Indeed, there are frequently good reasons to assign more experimental units to one group than to others, and, all the major statistical methodologies have provisions to adjust for such inequalities, within certain limits. Most commonly in the unbalanced design larger groups are assigned to either the highest dose, to compensate for losses due to possible deaths during the study, or to the lowest dose to give more sensitivity in detecting effects at levels close to an effect threshold or more confidence to the assertion that no effect exists.

5. A common problem is the existence of an undesired variable that is influencing the experimental results in a nonrandom fashion. Such a variable is called a confounding variable; its presence, as discussed earlier, makes the clear attribution and analysis of effects at best difficult, and at worst impossible. Sometimes such confounding variables are the result of conscious design or management decisions, such as the use of different instruments, personnel, facilities, or procedures for different test groups within the same study. Occasionally, however, such confounding variables are the result of unintentional factors or actions, in which there is, as it is called, a lurking variable. Such variables almost always arise as the result of standard operating procedures being violated: water not being connected to a rack of animals over a weekend, a set of racks not being cleaned as frequently as others, or a contaminated batch of feed being used.
6. Finally, some thought must be given to the clear definition of what is meant by experimental unit and concurrent control.

The experimental unit in toxicology encompasses a wide variety of possibilities. It may be cells, plates of microorganisms, individual animals, litters of animals, and so on. The importance of clearly defining the experimental unit is that the number of such units per group is the *N*, which is used in statistical calculations or analyses and critically affects such calculations. The experimental unit is the unit, which receives treatments and yields a response which is measured and becomes a datum.

A true concurrent control is one that is identical in every manner with the treatment groups except for the treatment being evaluated. This means that all manipulations, including gavaging with equivalent volumes of vehicle or exposing to equivalent rates of air exchanges in an inhalation chamber, should be duplicated in control groups just as they occur in treatment groups.

The goal of experimental design is statistical efficiency and the economizing of resources. The single most important initial step in achieving such an outcome is to clearly define the objective of the study: get a clear statement of what questions are being asked.

For the reader who would like to further explore experimental design, there are a number of more detailed texts available which include more extensive treatments of the statistical aspects of experimental design.

Experimental Design Types in Toxicology

There are four basic experimental design types used in toxicology. These are the randomized block, latin square, factorial design, and nested design. Other designs that are used are really combinations of these basic designs, and are very rarely employed in toxicology. Before examining these four basic types, however, we must first examine the basic concept of blocking.

Blocking is, simply put, the arrangement or sorting of the members of a population (such as all of an available group of test animals) into groups based on certain characteristics which may (but are not sure to) alter an experimental outcome. Such characteristics, which may cause a treatment to give a differential effect, include genetic background, age, sex, overall activity levels and so on. The process of blocking then acts (or attempts to act), so that each experimental group (or block) is assigned its fair share of the members of each of these subgroups.

Remember that randomization is aimed at spreading out the effect of undetectable or unsuspected characteristics in a population of animals or some portion of this population. The merging of the two concepts of randomization and blocking leads to the first basic experimental design, the randomized block. This type of design requires that each treatment group have at least one member of each recognized group (such as age), the exact members of each block being assigned in an unbiased (or random) fashion.

The second type of experimental design assumes that it is possible to characterize treatments (whether intended or otherwise) as belonging clearly to separate sets. In the simplest case, these categories are arranged into two sets which may be thought of as rows (for, say, source litter of test animal, with the first litter as row 1, the next as row 2, etc.) and the secondary set of categories as columns (for, say, ages of test animals, with 6–8 weeks as column 1, 8–10 weeks as column 2 and so on). Experimental units are then assigned so that each major treatment

(control, low dose, intermediate dose, etc.) appears once and only once in each row and each column. If the test groups are denoted as A (control), B (low), C (intermediate), and D (high), such an assignment would appear as shown in the table below:

Source litter	*Age (weeks)*			
	6–8	*8–10*	*10–12*	*12–14*
1	A	B	C	D
2	B	C	D	A
3	C	D	A	B
4	D	A	B	C

The third type of experimental design is the factorial design, in which there are two or more clearly understood treatments, such as exposure level to test chemical, animal age, or temperature. The classical approach to this situation (and to that described under the latin square) is to hold all but one of the treatments constant; and at any one time to vary just that one factor. Instead, in the factorial design all levels of a given factor are combined with all levels of every other factor in the experiment. When a change in one factor produces a different change in the response variable at one level of a factor than at other levels of this factor, there is an interaction between these two factors which can then be analyzed as an interaction effect.

The last of the major varieties of experimental design are the nested designs, where the levels of one factor are nested within (or are subsamples of) another factor. That is, each subfactor is evaluated only within the limits of its single larger factor.

See also: Carcinogenesis; Toxicity Testing, Carcinogenesis.

Further Reading

Bickis MG (1990) Experimental design. In: Arnold DL, Grice HC, and Krewski DR (eds.) *Handbook of In Vivo Toxicity Testing*, pp. 113–166. San Diego, CA: Academic Press.

Gad SC (2004) *Statistics and Experimental Design for Toxicologists*, 4th edn. Boca Raton, FL: CRC Press.

Lee PN and Lovell D (1999) Statistics for toxicology. In: Ballantyne B, Marrs T, and Syversen T (eds.) *General and Applied Toxicology*, 2nd edn., pp. 291–302. New York: Grone's Dictionaries.

Sokal RR and Rohlf FJ (1995) *Biometry*, 3rd edn. New York: W.H. Freeman and Company.

Glossary

Some frequently used statistical terms and their meanings

Term	Meaning
95% confidence interval	A range of values (above, below or above and below) the sample (mean, median, mode, etc.) has a 95% chance of containing the true value of the population (mean, median, mode). Also called the fiducial limit equivalent to the $p < 0.05$
Bias	Systemic error as opposed to a sampling error. For example, selection bias may occur when each member of the population does not have an equal chance of being selected for the sample
Degrees of freedom	The number of independent deviations, normally abbreviated *df*
Independent variables	Also known as predictors or explanatory variables
p-value	Another name for significance level; normally 0.005
Power	The effect of the experimental conditions on the dependent variable relative to sampling fluctuation. When the effect is maximized, the experiment is more powerful. Power can also be defined as the probability that there will not be a type 11 error $(I - \beta)$. Conventionally, power should be at least 0.07
Random	Each individual member of the population has the same chance of being selected for the sample
Robust	Having inferences or conclusions little effected by departure from assumptions
Sensitivity	The number of subjects experiencing each experimental condition divided by the variance of scores in the sample
Significance level	The probability that a difference has been erroneously declared to be significant, typically 0.005 and 0.001 corresponding to 5% and 1% chance of error
Type I error (false positives)	Concluding that there is an effect when there really is not an effect. Its probability is the α level
Type II error (false negatives)	Concluding there is no effect when there really is an effect. Its probability is the β level

Stoddard Solvent

Richard D Phillips

- Representative Chemicals: A mixture of saturated aliphatic and alicyclic C_7–C_{12} hydrocarbons with a content of 15–20% (by weight) of aromatic C_7–C_{12} hydrocarbons. The C_9–C_{11} hydrocarbons are most abundant, constituting $\geqslant$80% (by weight) of the total
- Chemical Abstracts Service Registry Numbers: CAS 8052-41-3 (Stoddard Solvent); CAS 64742-48-9 (White Spirit Type 3); CAS 64742-82-1 (White Spirit Type 1); 64742-88-7 (White Spirit Type 0); 64741-92-0 (White Spirit Type 2)
- Synonyms: White spirit; Petroleum spirits; Solvent naphtha

Uses

Stoddard Solvent is used mainly in paints and varnishes, in cleaning products, and as a degreasing and extraction solvent. It may also be used in some types of photocopier toners, printing inks, and adhesives.

Background Information

There are different grades of Stoddard Solvent depending upon the type and level of posttreatment. These treatments include hydro-desulfurization, solvent extraction, and hydrogenation. These successive treatments result in lower and lower aromatics in the final product.

Exposure Routes and Pathways

Humans are predominately, exposed to Stoddard Solvent through the inhalation of vapor. Exposure can occur through the skin if one comes into contact with Stoddard Solvent or a product containing it.

Toxicokinetics

Studies in humans and animals have shown that Stoddard Solvent is readily absorbed through the lungs. In general, the aromatic components are likely to be more completely absorbed due to their higher blood/gas solubility. It is expected that volatile components or metabolites of Stoddard Solvent that have low blood solubility would be most easily excreted in exhaled breath. Aromatic components would be expected to be excreted primarily in urine as metabolites.

Mechanism of Toxicity

Stoddard Solvent is a slight to severe skin irritant depending on the exposure condition and duration. This is related to the defatting properties of the solvent. Little is known regarding the specific mechanisms of action for systemic toxicity to Stoddard Solvent. Its effect on the nervous system at high exposure levels may be due to its general solvent properties and anesthetic effect of hydrocarbons in general.

Acute and Short-Term Toxicity (or Exposure)

Animal

Stoddard Solvent possesses low acute toxicity for mammals. Thus, an LC_{50} for rats was not achieved with 8 h exposure to 8200 mg m^{-3} (1400 ppm).

Stoddard Solvent was determined to be a slight to moderate irritant.

In short- and long-term toxicity studies on Stoddard Solvent, the central nervous system (CNS), respiratory system, liver, and kidney were generally found to be the target of Stoddard Solvent toxicity.

Several inhalation studies with Stoddard Solvent have shown that male rats develop hyalin droplet nephropathy which is believed to be associated with the α-2u-globulin in male rats. Stoddard Solvent did not cause developmental toxicity based on studies in rats.

Human

The odor threshold of Stoddard Solvent is quite low, and vapors can be detected at levels of 0.5–5 mg m^{-3}. Tolerance of the odor may be developed.

Eye irritation has been reported in connection with acute exposure down to a level of 600 mg m^{-3} (100 ppm). At higher levels, respiratory irritation and more pronounced eye irritation occur. Acute CNS systems such as headache, 'drunkenness', dizziness, and fatigue have been reported in several cases of occupational exposure.

Ingestion of Stoddard Solvent has been reported to produce gastrointestinal irritation with pain, vomiting, and diarrhea as well as damage to the gastrointestinal tract.

Owing to its low viscosity and low surface tension, Stoddard Solvent poses a risk of aspiration into the lungs following oral exposure. A few milliliters of solvent aspirated into the lungs are able to

produce serious bronchopneumonia and 10–30 ml may be fatal.

Prolonged dermal exposure to Stoddard Solvent, for example, resulting from wearing clothes that have been soaked or moistened by white spirit for hours, may produce irritation and dermatitis.

There are a number of reports associating exposure to Stoddard Solvent in painters with neurological effects. However, in many of these instances, the exposure to Stoddard Solvent and associated confounding exposures are not clear. Many of the reported effects are also subjective in nature and the role of Stoddard Solvent is difficult to decipher.

Chronic Toxicity (or Exposure)

Animal

The National Toxicology Program recently conducted chronic inhalation studies with Stoddard Solvent in rats and mice. The reports are still in review, and details of the draft are summarized here. Groups of 50 male and 50 female rats and mice were exposed to Stoddard Solvent by inhalation at concentrations of 0, 138 (male rats), 550, 1100, or 2200 $mg\,m^{-3}$ (female rats and mice), 6 $h\,day^{-1}$, 5 $day\,week^{-1}$ for 105 weeks. For rats, survival in the top exposure concentration groups of males and females was significantly less than that of the chamber controls.

At 2 years, adrenal medulla tumors occurred with positive trends in male rats, and the incidences in the 550 and 1100 $mg\,m^{-3}$ groups were significantly increased. Also, a slightly increased incidence of renal adenomas occurred in the 1100 $mg\,m^{-3}$ group. Nonneoplastic lesions related to Stoddard Solvent exposure occurred in the kidney of male rats.

Survival of exposed mice was similar to that of the chamber controls. Mean body weights of exposed female mice were greater than those of the chamber controls. The incidences of hepatocellular adenoma occurred with a positive trend in female mice, and the incidence of multiple hepatocellular adenoma in female mice exposed to 2200 $mg\,m^{-3}$ was significantly increased.

In Vitro Toxicity Data

Stoddard Solvent was tested for mutagenicity in *Salmonella typhimurium* and found to be negative with and without 59 metabolic activation.

Clinical Management

Gastric emptying by either lavage or emesis is contraindicated since there is a danger of pulmonary aspiration and subsequent pneumonitis. If a person is overexposed to vapor of Stoddard Solvent, the victim should be moved to fresh air as quickly as possible. If acute effects of central nervous system depression are present, the appropriate treatment may be indicated.

Washing with soapy water is suggested following dermal contact, and ocular washing with water following eye contact.

Environmental Fate

The transport and partitioning of Stoddard Solvent is dependent on the environmental fate of its hydrocarbon components.

Sorption to organic matter in soil or water is a major partitioning process for all hydrocarbon classes (alkanes, cycloalkanes, and aromatics) with partitioning to the soil-vapor phase being relatively unimportant. At low concentrations, the aromatic constituents of Stoddard Solvent, particularly the alkyl benzenes, are more water soluble than alkanes and cycloalkanes and may dissolve in infiltrating water with a minimum of volatilization. As such, they may be transported through soil into the underlying groundwater, although sorption to soil organic matter will retard this leaching process. For saturated deep soils that contain no oxygen and little organic matter, the model predicts that some (20%) aromatic hydrocarbons will not undergo biodegradation, but will be dissolved in the soil-water phase, and subsequently will be transported to underlying groundwater.

If a release of Stoddard Solvent exceeds the sorptive capacity of the soil, large quantities of Stoddard Solvent may move through the soil with gravity as bulk fluid and enter the groundwater. At the soil/groundwater interface, the soluble components can dissolve in the water, while insoluble components with specific gravities of less than 1 will float on top of the water table and move horizontally along the soil/water interface.

Alkanes are likely to be sorbed to organic matter in the soil and are, therefore, unlikely to be dissolved in water moving through soil. However, some of these compounds may volatilize more quickly than they will bind to organic matter. Most aliphatic hydrocarbons have low water solubilities, but those with higher water solubilities are likely to be dissolved in water and may be transported through soil more rapidly, although the extent may be reduced by sorption to organic matter or volatilization.

No information was found on the bioaccumulation potential of Stoddard Solvent in either aquatic or terrestrial ecosystems. However, the potential for bioaccumulation of Stoddard Solvent in either

ecosystem is dependent on the bioaccumulation potential of the individual hydrocarbon components. In general, lower molecular weight alkanes do not tend to bioaccumulate, aromatics may have a moderate tendency to bioaccumulate, and the higher molecular weight alkanes, such as cycloalkanes, tend to bioaccumulate. However, these bioaccumulation tendencies may be offset by the metabolic capabilities of the organisms toward hydrocarbons.

Ecotoxicology

The few studies on the aquatic toxicity of Stoddard Solvent and related hydrocarbon mixtures indicate moderate toxicity to freshwater and marine organisms. The toxicity is probably due to the dissolved fraction and leads to 96 h LC_{50} values of the order of 0.5–5.0 mg l^{-1}.

These results are likely to overestimate the effects of Stoddard Solvent in the field, given its volatility and lowered bioavailability following sorption to soil/sediment.

Further Reading

Agency for Toxic Substances and Disease Registry (ATSDR) (1995) *Toxicological Profile for Stoddard Solvent*, US Department of Health and Human Services.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic substances and Disease Registry. Toxicological Profile for Stoddard Solvent.

Strontium

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 7440-24-6
- Chemical/Pharmaceutical/Other Class: Alkaline earth metal
- Chemical Formula: Sr^{2+}

Uses

Strontium compounds are used in making ceramics and glass products, pyrotechnics, paint pigments, fluorescent lights, and medicines.

Strontium can also exist as several radioactive isotopes, the most common is ^{90}Sr. Strontium-90 is formed in nuclear reactors or during the explosion of nuclear weapons. Radioactive strontium generates beta particles as they decay. One of the radioactive properties of strontium is half-life, or the time it takes for half of the isotope to give off its radiation.

It is used in fireworks, red signal flares, and tracer bullets. It also can be added to alloys of tin and lead to add hardness and durability, and it can be used as a deoxidizer in copper and bronze. In addition, it can be used as an igneous coloring agent, a material for the cathode of vacuum bulbs, a material for condensers and optical glass, and as a lead and iron removing agent. Small amounts of strontium are added to molten aluminum to improve the castability of the metal, making it more suitable for casting items that have been traditionally made from steel. The radioactive form (^{89}Sr) is used as an antineoplastic (radiation source). Strontium compounds are used as agricultural chemicals.

Background Information

Strontium is a naturally occurring element found in rocks, soil, dust, coal, and oil. Naturally occurring strontium is not radioactive and is referred to as stable strontium. Stable strontium in the environment exists in four stable isotopes, ^{84}Sr (read as strontium 84), ^{86}Sr, ^{87}Sr, and ^{88}Sr. Twelve other unstable isotopes are known to exist. Its radioactive isotopes are ^{89}Sr and ^{90}Sr. Strontium is chemically similar to calcium. It was discovered in 1790. The isotope ^{90}Sr is a highly radioactive poison, and was present in fallout from atmospheric nuclear explosions and is created in nuclear reactors. Atmospheric tests of nuclear weapons in the 1950s resulted in deposits and contaminations. ^{90}Sr has a half-life of 28 years and is a high-energy beta emitter. Its common cationic salts are water soluble; it forms chelates with compounds such as ethylenediaminetetraacetic acid; strontium coordination compounds are not common. Powdered metallic strontium may constitute an explosion hazard when exposed to flame.

Exposure Routes and Pathways

Oral, ingestion, and inhalation are possible exposure routes. Radiation also penetrates the body.

Toxicokinetics

Strontium tends to replace calcium in bone. Radioactive isotopes of strontium, mainly ^{90}Sr, released into the environment due to nuclear accidents may contribute significantly to the internal radiation exposure of members of the public after ingestion of strontium with contaminated foodstuffs. The committed radiation dose is significantly dependent on the fraction of the ingested activity that crossed the gut wall; sodium alginate is a potent agent for reducing strontium absorption with high efficiency and virtually no toxicity. The data obtained show that the uptake of ingested strontium from milk was reduced by a factor of nine when alginate was added to milk.

Mechanism of Toxicity

Its inherent toxicity and that of its compounds resembles that of calcium. The state of calcium nutrition of exposed individuals is a major determinant of toxicity. The radioactive isotope, when ingested or inhaled, is processed by the body and resides in bones. Strontium ionizes molecules in the body by the emission of beta particles. It increases the risk of cancer.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute poisoning in laboratory animals leads to excess salivation, vomiting, colic, and diarrhea. In rats, death is due to respiratory failure; in cats, it is due to cardiac arrest.

Human

No toxic effects from industrial use of nonradioactive strontium have been recorded.

Chronic Toxicity (or Exposure)

Animal

Leukemia and cancers of the bone, nose, lung, and skin have been observed in laboratory animals exposed to radioactive strontium.

Human

Leukemia has been seen in humans exposed to relatively large amounts of radioactive strontium. The International Agency for Research on Cancer has determined that radioactive strontium is a human carcinogen. ^{89}Sr has been explored as an anticancer treatment, for example, for prostate cancer, and has been used as palliative treatment for patients with bone pain from osseous metastases. Excellent clinical responses for bone pain treatment have been observed (acceptable hematologic toxicity; and clinical results rival those of external beam radiation therapy).

Environmental Fate

Stable strontium is a dust in air. It eventually settles over land and water. Stable strontium dissolves in water and moves deeper in soil to underground water.

Ecotoxicology

Strontium-90 pollutes water and soil at some reprocessing plants. Atmospheric contamination can occur from nuclear fallout. A study in the United States has concluded that high concentrations of strontium in eggshells of some passerine birds may be associated with lower hatching success.

See also: Aluminum; Carcinogenesis.

Further Reading

Hollriegl V, Rohmuss M, Oeh U, and Roth P (2004) Strontium biokinetics in humans: Influence of alginate on the uptake of ingested strontium. *Health Physics* 86: 193–196.

Mora MA (2003) Heavy metals and metalloids in egg contents and eggshells of passerine birds from Arizona. *Environmental Pollution* 125: 393–400.

Siegel HJ, Luck JV Jr., and Siegel ME (2004) Advances in radionuclide therapeutics in orthopaedics. *Journal of the American Academy of Orthopaedic Surgeons* 12: 55–64.

Relevant Websites

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

http://risk.lsd.ornl.gov – US Oak Ridge National Laboratory. Toxicity summary for Strontium-90 (from the Risk Assessment Information System).

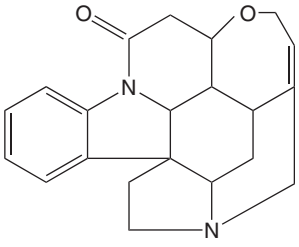
Structure–Activity Relationship *See* Toxicity Testing, Modeling.

Strychnine

Fermin Barrueto Jr.

This article is a revision of the previous print edition article by Janice Reeves and Carey Pope, volume 3, pp. 201–202,

- Chemical Abstracts Service Registry Number: CAS 57-24-9
- Synonyms: Kwik-Kil; Mouse-Rid; Mouse-Tox; Strychnos
- Chemical/Pharmaceutical/Other Class: Naturally occurring alkaloid
- Chemical Formula: $C_{21}H_{22}N_2O_2$
- Chemical Structure:



Uses

Strychnine is used chiefly in poison baits for rodents and sometimes birds.

Exposure Routes and Pathways

The primary pathways for unintentional or intentional exposure to strychnine are inhalation and ingestion. Ocular and dermal exposures can also occur.

Toxicokinetics

Strychnine is rapidly absorbed from the gastrointestinal tract, nasal mucosa, and parenteral sites. It is readily metabolized in the liver by microsomal enzymes. The highest concentrations of strychnine are found in the liver, kidneys, and blood. About 15% will appear unchanged in the urine within 24 h. Strychnine has an elimination half-life of $\sim$10 h.

Mechanism of Toxicity

The exact mechanism of strychnine's action in the nervous system is unclear but it is thought that the inhibitory action of the neurotransmitter glycine at Crenshaw cell-motor axon synapses is blocked by strychnine. This essentially decreases excitatory thresholds and produces tetanic convulsions in response to sensory stimuli. While the main locus for strychnine's neurotoxicity is the spinal cord, the medulla also appears affected. Effects on other organ systems appear to be secondary to these actions in the central nervous system (CNS). Strychnine competitively blocks the binding of glycine to membranes isolated from spinal cord.

Acute and Short-Term Toxicity (or Exposure)

Animal

Strychnine is a compound of high acute toxicity. The oral LD value in rats is $\sim 15\,mg\,kg^{-1}$. Parenteral routes of exposure are more toxic; LD_{50} values in laboratory rodents range from 1 to $4\,mg\,kg^{-1}$.

Human

Within 15–30 min after ingestion of strychnine, the patient will experience restlessness, apprehension, heightened acuity of perception, hyperreflexia, and muscle stiffness of the face and legs. Violent convulsions can follow these symptoms or occur in the absence of these previous symptoms. As poisoning progresses, the convulsions become more violent and the intervals between convulsions become shorter. The LD (oral) for humans has been estimated to be $30\,mg\,kg^{-1}$. Toxicity has been reported at 0.1 mg per 100 ml in blood concentrations.

Chronic Toxicity (or Exposure)

Animal

Toxicity of strychnine varies by sex in rats. Twenty-eight day feeding studies showed that males were able to tolerate up to $8\,mg\,kg^{-1}\,day^{-1}$ without adverse effects; females were able to tolerate $2.5\,mg\,kg^{-1}\,day^{-1}$.

Human

Significant cumulative toxicity has not been described because both detoxification and excretion are comparatively rapid.

In Vitro Toxicity Data

Strychnine is frequently used as a research tool due to its glycine inhibitory effects.

Clinical Management

Treatment is symptomatic and supportive with emphasis on controlling neuromuscular hyperactivity. For patients exposed to strychnine fumes, the patient should be moved to fresh air, and eyes and skin should be decontaminated immediately with water. For patients with strychnine ingestions, emesis is not recommended because of the violent convulsive activity and increased risk of aspiration. Activated charcoal should be used immediately to minimize absorption. Once convulsions have been controlled, efforts to correct fluid, electrolyte, and acid–base abnormalities caused by repeated convulsions should be made.

Environmental Fate

Strychnine has been used as a rodenticide and pesticide for decades. It is believed that strychnine may undergo direct photolysis in the atmosphere, on soil surfaces, and in surface water.

See also: Pesticides.

Further Reading

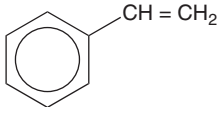
Flood RG (1999) Strychnine poisoning. *Pediatric Emergency Care* 15: 286–287.

Oberpaur B, Donoso A, and Claveria C (1999) Strychnine poisoning: An uncommon intoxication in children. *Pediatric Emergency Care* 15: 264–265.

Styrene

Ralph J Parod

- Chemical Abstracts Service Registry Number: CAS 100-42-5
- Synonyms: Ethenylbenzene; Phenylethylene; Vinylbenzene
- Chemical/Pharmaceutical/Other Class: Hydrocarbon
- Chemical Structure:



Uses

Styrene is produced by the alkylation of benzene with ethylene followed by catalytic dehydrogenation. It is used in the manufacture of general-purpose and high-impact polystyrene plastics (~50%), expanded polystyrene (~7%), copolymer resins with acrylonitrile and butadiene (~7%) or acrylonitrile only (~1%), styrene–butadiene latex (~6%) and synthetic rubber (~5%), unsaturated polyester resins (~6%), and as a chemical intermediate.

Exposure Routes and Pathways

The closed system techniques currently used in styrene monomer and copolymer resin production limit worker exposures to a time-weighted average (TWA) exposure of generally <10 ppm. However, in open systems used to manufacture some polyester reinforced plastics (e.g., shower stalls and boats), worker exposures to styrene and resins via inhalation and to a lesser extent dermal contact may pose a health hazard. It is unlikely that the residual styrene monomer contained in consumer products (200–800 $mg\,kg^{-1}$) poses a significant health risk to the general public due to the slow (0–0.03% $year^{-1}$) and dispersive nature of its release from these sources. Styrene is present in ambient air due to emissions from industrial production, coal-fired power plants, cigarette smoke, and gasoline engine exhaust. Styrene is also present in certain cheeses and fish products where it is produced by microorganisms acting on natural or added substances in these foods, and in several raw agricultural products that have not contacted styrenic based storage containers. The total exposure of the general population to styrene from these background sources is 0.3–0.8 $\mu g\,kg^{-1}\,day^{-1}$ with inhalation being the major route of exposure.

Toxicokinetics

Styrene is absorbed by all routes of exposure. Absorption through the respiratory tract is rapid and the major route of human exposure. Once absorbed, styrene is rapidly distributed throughout the body. Studies in rats and mice indicate that styrene or its metabolites are distributed to the liver, kidneys, heart, subcutaneous fat, lung, brain, and spleen. In both species, fat contained the highest concentration of styrene, suggesting that fat may act as a modest reservoir for these compounds. While there are qualitative similarities in the metabolism of styrene among species, quantitative differences have been noted.

Mice, rats and rabbits exhibit a much greater capacity than humans to metabolize styrene (via cytochrome P450) to styrene-7,8-epoxide in both the respiratory tract and liver. In these species, styrene-7,8-epoxide is inactivated by metabolism to hippuric acid (via epoxide hydrolase) and hydroxyphenylethyl mercapturic acid (via glutathione *S*-transferase). The latter pathway is much more prevalent in rodents than humans. In humans, styrene is metabolized via cytochrome P450 to styrene-7,8-epoxide, which is rapidly metabolized via epoxide hydrolase to mandelic acid ($\sim$60%) and phenylglyoxylic acid ($\sim$25%), with very minor amounts of 4-vinylphenol, hippuric acid, and the glucuronide of styrene glycol. Metabolism occurs primarily in the liver and to a lesser extent in extrahepatic tissues (e.g., kidney, intestine, and lung). Approximately 90–97% of the styrene absorbed by humans is eliminated as urinary metabolites. Urinary elimination of the primary metabolites is biphasic, with half-lives of 4–9 and 17–26 h (mandelic acid) and 10 and 26 h (phenylglyoxylic acid). Only a small fraction of the absorbed dose is eliminated in expired air or urine as the parent compound.

Mechanism of Toxicity

There has been a general belief that styrene-7,8-epoxide is responsible for the carcinogenicity and nasal toxicity associated with styrene. However, more recent data indicate that other mechanisms may be operative or even predominant (i.e., site-specific metabolism of styrene to ring-oxidized metabolites that are toxic to the lung and induce lung cell proliferation (e.g., 4-vinylphenol), depletion of pulmonary glutathione). Such effects occur to a greater extent in mice than rats and are thought to lead to cytotoxicity, cell proliferation, and the slow development of noninvasive tumors in the mouse lung. These stresses are even less prevalent in humans than rats, suggesting that humans are less susceptible to the development of lung tumors than mice. Work in this area is ongoing. The mechanism for the neurotoxic effects of styrene has not been established.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} for styrene in male and female rats is $\sim 5000\,mg\,kg^{-1}$. The LC_{50} in rats exposed to styrene for 4 h is 2770 ppm; the LC_{50} in mice exposed to styrene for 2 h is 4940 ppm. Repeated daily exposures over a period of weeks to months to 500–1300 ppm styrene can result in irritation of the eyes and upper respiratory tract, ototoxicity, and central nervous system (CNS) depression. Liver toxicity noted in mice given daily exposures to 200 ppm styrene over a period of two weeks was associated with the depletion of liver glutathione levels. Studies of clastogenicity have been negative.

Human

Acute inhalation exposures may result in irritation of the nasal mucosa and eyes ($\geqslant$50 ppm), irritation of the skin, and CNS depression ($>$100 ppm). Symptoms of CNS depression include nausea, drowsiness, and ataxia. The disagreeable odor of styrene, which is detectable at $\sim$0.04–0.3 ppm, serves as a good warning aid. However, olfactory fatigue may occur at high concentrations.

Chronic Toxicity (or Exposure)

Animal

In male mice, lifetime inhalation exposures to styrene at concentrations of 40, 80, or 160 ppm (but not 20 ppm) significantly increased the incidence of bronchiolar adenomas (benign) but not bronchial carcinomas. In female mice similar exposures resulted in a significant elevation in the incidences of bronchiolar adenomas (benign) at 20, 40, or 160 ppm (but not 80 ppm) and bronchiolar carcinomas at 160 ppm. The tumors occurred late in life and were found in the terminal bronchioles originating most likely from Clara cells. In contrast, lifetime exposure of rats to between 50 and 1000 ppm styrene did not cause tumors in the lungs or any other tissue. These chronic exposures also resulted in toxicity to the nasal epithelium of mice and rats at the lowest concentrations studied. Styrene has not been shown to cause reproductive or developmental toxicity. Chronic inhalation exposures to multiple species that produced clear evidence of toxicity did not affect the reproductive organs of treated animals. Inhalation exposures up to 500 ppm styrene did not affect either fertility or reproduction in a rat two-generation reproduction study or development of the nervous system in second generation offspring. In addition, inhalation of 300–600 ppm styrene by pregnant rats and rabbits was not embryo- or fetotoxic.

Human

Styrene exposures between 50 and 100 ppm have been associated with neurological effects including decrements in color discrimination, nerve conduction, and neurobehavioral performance. These changes appear to be transient, with improvement

occurring between 1 and 24 months postexposure. Styrene is unlikely to be toxic to the human nasal epithelium since, unlike in rodents, this tissue does not metabolize detectable amounts of styrene and contains metabolic pathways capable of efficiently eliminating metabolites if formed. Epidemiological studies have not provided a clear link between styrene exposure and adverse pregnancy outcomes. Regarding cancer, epidemiological studies have been performed in three industrial settings, including the reinforced plastics industry where styrene exposures tend to be the higher and are less confounded by other chemicals. On balance, the data do not suggest a causal association between styrene exposure and any form of cancer. Increased frequencies of chromosomal aberrations in peripheral lymphocytes have been reported in some but not all studies of workers from the reinforced plastics industry, but the potential relationship of these effects to styrene exposure is still under debate.

In Vitro Toxicity Data

In vitro studies of mutagenicity and chromosomal aberrations in bacterial and mammalian cells have generally produced negative results, although positive results have sometimes been observed in the presence of metabolic activation.

Clinical Management

Acute exposures are likely to be associated with CNS depression and, at very high doses, pulmonary irritation. Removal from exposure and ventilatory support are the initial priorities. Alert individuals ingesting >2 or $3\,mg\,kg^{-1}$ should be given syrup of ipecac. Because hydrocarbon pneumonitis is a significant risk with styrene ingestion, intubation should precede lavage in those individuals at risk of aspiration due to a reduced level of alertness.

Environmental Fate

Styrene is a liquid and will partition to the atmosphere when released to the environment due to its volatility. In the atmosphere, styrene is rapidly eliminated due to its reaction with hydroxyl radicals (7 h half-life) or tropospheric ozone (10 h half-life). Water does not provide a significant sink for styrene due to its low water solubility ($300\,mg\,l^{-1}$), rapid volatilization from water to air (half-life of 1–3 h), and biodegradation (15 days half-life). In soil, styrene rapidly volatilizes from the surface (1 min half-life) but more slowly from deeper strata. Styrene biodegrades in soil and sediment with half-lives of 30 and 300 days, respectively.

Ecotoxicology

Although of limited relevance to real world exposures, a series of guideline studies have evaluated the intrinsic ecotoxicity of styrene under conditions that minimized volatilization. In these investigations that incorporated analytical verification, the 96 h LC_{50} value for fish was $10\,mg\,l^{-1}$ (fathead minnow); the no-observed-effect level (NOEL) was $4\,mg\,l^{-1}$. For the freshwater invertebrates, the 48 and 96 h LC_{50} values were $4.7\,mg\,l^{-1}$ (daphnids) and $9.5\,mg\,l^{-1}$ (amphipods), respectively; the NOELs were 1.9 and $4.1\,mg\,l^{-1}$, respectively. For green algae, the 96 h EC_{50} was $0.72\,mg\,l^{-1}$ and the NOEL was $0.063\,mg\,l^{-1}$; the effects noted were algistatic, not algicidal. Styrene is infrequently detected in surface and drinking water around the world; and when it is detected, levels are typically $<0.01\,mg\,l^{-1}$. For earthworms in soil, styrene has a 14 day LC_{50} of $120\,mg\,kg^{-1}$ and a NOEL of $44\,mg\,kg^{-1}$.

Other Hazards

Styrene is explosive in the range of 1.1–6.1% and has a vapor density of 3.6.

Exposure Standards and Guidelines

International occupational exposure limits (OELs) generally range between 20 and 100 ppm as an 8 h TWA; short-term exposure limits (STELs), typically a 15 min TWA, range between 40 and 250 ppm. The US Occupational Safety and Health Administration (OSHA) and the American Conference of Governmental Industrial Hygienists (ACGIH) have established an 8 h TWA OEL for styrene of 100 and 20 ppm, respectively; the ACGIH STEL is 40 ppm. OSHA and the styrene industry have an enforceable voluntary agreement to keep exposures under 50 ppm. Styrene has been judged possibly carcinogenic to humans (group 2B) by the International Agency for Research on Cancer. Styrene is not listed in the 10th edition of the Report on Carcinogens published by the US National Toxicology Program (NTP); however, styrene-7,8-oxide is listed by NTP as reasonably anticipated to be a human carcinogen. The National Institute for Occupational Safety and Health lists 700 ppm styrene as being immediately dangerous to life or health.

See also: Respiratory Tract.

Further Reading

Alexander M (1997) Environmental fate and effects of styrene. *Critical Reviews in Environmental Science And Technology* 27: 383–410.

Cohen JT, Carlson G, Charnley G, *et al.* (2002) A comprehensive evaluation of the potential health risks associated with occupational and environmental exposure to styrene. *Journal of Toxicology and Environmental Health, Part B* 5: 1–263.

Cushman JR, Rausina GA, Cruzan G, *et al.* (1997) Ecotoxicity hazard assessment of styrene. *Ecotoxicology and Environmental Safety* 37: 173–180.

Subchronic Toxicity *See* Toxicity, Subchronic.

Sudan Grass

Julie Weber

- SYNONYMS: *Sorghum sudanense*; *Sorghum vulgare* var *sudanense*; *Holcus sudanensis*; Grass sorghum; Shattercane; Sudan; Family Gramineae (Poaceae)

Uses

Sudan grass is native to Sudan. It is cultivated and naturalized widely in the United States, but is most common in the southern states. Although common to the southern states, it is also grown from southern Texas to Minnesota and North Dakota in the central grassland regions. Sudan grass only develops fibrous roots and does not become a noxious weed. As seedlings, virtually all species of sorghum resemble one another and are frequently confused with young corn plants. The sorghum species are used for forage, hay, silage, and grain.

Exposure Routes and Pathways

Ingestion of any part of the plant would be the common route of exposure. The green, aerial portions, especially the leaves, stems, roots, and canes are toxic.

Toxicokinetics

Limited information on absorption is available. After ingestion, the onset of symptoms of cyanide toxicity is expected in 30 min to 2 h; however, symptoms could be delayed. The delay in symptoms may be due to the rate at which the hydrocyanic acid is yielded when hydrolyzed by the enzymes during digestion. The major route for detoxification of cyanide is the conversion to thiocyanate by an enzymatic reaction catalyzed by rhodanese, an enzyme widely distributed in tissues, with the highest concentration in the liver. The rhodanese system can detoxify large amounts of cyanide but cannot respond quickly enough to prevent fatalities.

Mechanism of Toxicity

The sorghum group contains the cyanogenic glycoside dhurrin. The glycoside itself is harmless. When the plant is torn, chewed, or damaged, the glycosidase comes in contact with and hydrolyzes the glucoside to an α-hydroxynitrile aglycone and glucose. The aglycone further dissociates to *p*-hydroxybenzaldehyde and hydrocyanic acid. Hydrolysis is complete in 10 min at 20°C. The reaction takes place slowly in an acidic pH, but an alkaline medium hastens the process. A delay in symptoms after ingestion would be explained by a slow hydrolysis when transportation occurs from the acidic stomach medium to the alkaline medium of the duodenum. Released and absorbed hydrocyanic acid forms a stable complex with ferric iron and cytochrome oxidase; inhibiting the activity of the enzyme and aerobic metabolism. Cells with cytochrome oxidase are unable to utilize the available oxygen and suffer from hypoxia. Therefore, the nerve cells can no longer obtain oxygen and the respiratory center ceases to function.

Forage sorghum can accumulate levels of nitrates that can also produce poisoning. The nitrite ion readily reacts with hemoglobin in red blood cells, oxidizing it to methemoglobin which cannot transport oxygen.

Another disease syndrome of ataxia/cystitis/teratogenesis has been seen especially in horses with a slight variation of effects in cattle and possibly sheep. Both cyanide and nitriles are suspected, but the specific cause is unknown.

The last syndrome reported sporadically in sheep is a primary photosensitization. The toxin responsible has not been identified.

Acute and Short-Term Toxicity (or Exposure)

Animal

Livestock cyanide poisoning may result from plant consumption. All animal species are susceptible to the toxic effects of cyanide. Ruminants appear to be more susceptible to cyanogenic glycosides in plants because the favorable conditions prevailing in the rumen facilitate rapid hydrolysis and absorption. Mucous membranes of the eyes and mouth may appear congested. Gastric contents, if examined immediately, have a characteristic benzaldehyde odor, resulting from benzaldehyde production from aglycone breakdown of certain cyanogenic glycosides. Potential symptoms expected are hyperpnea to dyspnea, excitation, gasping, staggering, paralysis, prostration, tremors, convulsions, coma, and death. Cherry red blood may be noted.

Nitrate intoxication is a serious problem for livestock where sorghums are used for forage. In ruminant digestion, nitrates are converted to nitrites after ingestion of plants containing high levels of nitrates. The nitrites begin to increase in the rumen, peaking in 3–4 h. The nitrites are $\sim$10 times more toxic and are the more immediate cause of poisoning. Symptoms of nitrite toxicity include discoloration of the mucous membranes, depression, rapid respiration, ataxia, apprehension, severe dyspnea, trembling, and weakness with a chocolate brown discoloration of the blood.

Some environmental factors that increase the cyanogenic potential of the plant are high nitrogen and low phosphorus in soil, periods of drought that wilt plants or delay growth and age of plants (young growth has the highest potential for toxicity). Many years of selective breeding have resulted in hybrids having lower potential for developing hydrogen cyanide.

Human

Sudan grass poisonings in humans are not reported. Symptoms are expected to be similar to those exhibited in animals. Patients would be treated as for other cyanogenic plant ingestions.

Chronic Toxicity (or Exposure)

Animal

Animals eating Sudan grass containing low levels of cyanogenic glycosides for prolonged periods (usually several weeks or months) may be at risk to develop chronic cystitis, ataxia, and teratogenicity. Mares and cows that chronically eat Sudan grass with low levels of the cyanogenic glycoside are at risk to develop ataxia, urinary incontinence (dribbling urine), and abortion. The offspring may develop musculoskeletal deformities. Sublethal doses of nitrate also may induce abortion because nitrates readily crosses the placenta and causes fetal methemoglobinemia and death. Chronic ingestion of nitrate in the diet is suspected of affecting vitamin A metabolism, thyroid function, reproduction, and milk production.

Clinical Management

In symptomatic patients, decontamination should be deferred until other basic and advanced life-support measures have been instituted. Cyanide toxicity usually progresses so quickly that treatment may not be available in a time period to be effective. Induction of emesis is not recommended. Subtoxic amounts do not require emesis; moreover, the potentially rapid progression of clinical course contraindicates it. Activated charcoal may be effective if administered soon after the ingestion. The cyanide antidote kit should only be administered in those persons with significant symptoms (impaired consciousness, seizures, acidosis, and unstable vital signs). The mainstay of treatment in ruminants is sodium thiosulfate. Sodium nitrite may be used to enhance the effects of the sodium thiosulfate. Arterial blood gases, electrolytes, serum lactate and pyruvate, hemoglobin, glucose, creatinine, whole blood cyanide levels, and methemoglobin levels should be monitored and treated as necessary. Hyperbaric oxygen can be used for those with severe symptoms not responding to normal supportive and antidotal treatment.

Miscellaneous

Sudan grass is an annual grass with stems up to 9 ft tall that branch from the base. Leaf blades are broad to narrow up to 0.5 in. wide and 12 in. long; panicles open. The plant flowers in a 12 in. long, erect, loose panicle that is approximately half as wide as it is tall. It also forms a grass or grain-like glume around seed buds with bristle-shaped tips.

See also: Cyanide.

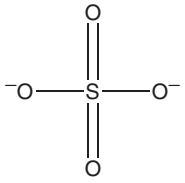
Further Reading

Hall AH and Rumack BH (1986) Clinical toxicology of cyanide. *Annals of Emergency Medicine* 15: 1067–1074.

Sulfates

J R Clarkson, Lu Yu, and Lance Fontenot

- Representative Chemicals: Barite ($BaSO_4$); Epsomite ($MgSO_4 \cdot 7H_2O$); Gypsum ($CaSO_4 \cdot 2H_2O$); Magnesium ($MgSO_4$); Sodium ($NaSO_4$); Calcium ($CaSO_4$); Lead ($PbSO_4$); Barium ($BaSO_4$); Strontium ($SrSO_4$)
- Chemical Abstracts Service Registry Number: CAS 14808-79-8
- Synonym: Sulfate anion
- Chemical Formula: SO_4^{2-}
- Chemical Structure:



Uses

Sulfates and sulfuric acid products are used in the production of fertilizers, chemicals, dyes, glass, paper, soaps, textiles, fungicides, insecticides, astringents, and emetics. They are also used in the mining, wood-pulp, metal, and plating industries, in sewage treatment, and in leather processing. Aluminum sulfate (alum) is used as a sedimentation agent in the treatment of drinking water. Copper sulfate has been used for the control of algae in raw and public water supplies.

Sulfate, a soluble, divalent anion (SO_4^{2-}), is produced from the oxidation of elemental sulfur, sulfide minerals, or organic sulfur. Sulfate is ubiquitous in the environment because of the abundance of sulfur on earth. Anthropogenic sources of sulfate include the burning of sulfur-containing fossil fuels, household wastes including detergents, and industrial effluents from tanneries, steel mills, sulfate-pulp mills, and textile plants. Sulfate is also used in pickle liquor (sulfuric acid) for steel and metal industries, as a feedstock or reagent in manufacturing processes, in some fertilizers, and exists as an end-product in the form of copper sulfate in its use as a fungicide and algicide.

Exposure Routes and Pathways

Humans may be exposed to sulfate from a variety of sources that include drinking water, food, ambient air, occupational settings, and consumer products. Ingestion of drinking water containing sulfate is the most common exposure route. Exposure through inhalation is not very important, although it may be an irritant to mucous membranes, such as eyes, nose, and respiratory tract. Dermal exposure is also not considered a major exposure route because sulfates are poorly absorbed through the skin.

Toxicokinetics

Absorption of sulfate from the intestine depends on the amount of sulfate ingested and the type of cation associated with the sulfate. Inorganic sulfate distributed in blood and serum levels are affected more by ingestion of dietary protein, and less by drinking water containing high concentrations of sulfate. Inorganic sulfate does not accumulate in tissues. Inorganic sulfate is incorporated into several types of biomolecules, such as glycoproteins, glycosaminoglycans, and glycolipids. Metabolism in the body could be affected by the presence of drugs, such as acetaminophen. Sulfates are usually eliminated in the urine as free unbound form or as conjugates of various chemicals. In humans, ~30–62% of an oral dose is excreted in the urine within 24–72 h, whereas rats excrete ~56–90% of the dose. At high sulfate doses that exceed intestinal absorption, sulfate is excreted in the feces. There are no data in literature that indicate that sulfate is accumulated, even when there is chronic ingestion of above-normal sulfate levels.

Mechanism of Toxicity

The cathartic effect of sulfate is mainly due to the osmotic activity of unabsorbed sulfate salts in the intestine. The laxative effect that results from sulfate is an osmotic diarrhea. Whether or not this laxative effect occurs depends on the amount of sulfate and other osmotically active materials that are present in the intestines; these materials include magnesium, sodium, and some sugars.

An osmotic-induced diarrhea ceases once the osmotically active gastrointestinal contents are excreted. In the case of sulfate, adults appear to adapt within 1 or 2 weeks and are no longer affected by the sulfate in their drinking water supply. However, dehydration may be the consequence of these same osmotic forces; therefore, populations such as infants or the elderly consuming formula or powdered nutritional supplements that have been mixed with water containing high concentrations of sulfate may be more sensitive. Persons with renal diseases may be more sensitive to effects of high sulfate ingestion because excess sulfate is excreted through the kidney.

Since humans appear to develop a tolerance to drinking water with high sulfate concentrations, chronic exposures do not appear to produce the same laxative effect as seen in acute exposures. While it is not known when this acclimation occurs in adults, researchers believe that acclimation occurs within 7–10 days.

Acute and Short-Term Toxicity (or Exposure)

Data in humans and animals suggest that acute oral exposures to sulfate exert a laxative effect (loose stool) and sometimes diarrhea (unusually frequent or unusually liquid bowel movements) following exposures to high concentrations. However, these effects are not observed for longer-term exposures. This may be because of acclimation to sulfate over time.

High sulfate concentrations do not appear to exert adverse reproductive or developmental effects. There is little to no data available on the mutagenic or teratogenic effects of sulfate.

Animal

The oral LD_{50} values of ammonium sulfate and potassium sulfate in the rat are 3000–4000, 2140, and 6600 mg kg^{-1}, respectively.

The oral LD_{50} of sodium sulfate in the mouse is 5989 mg kg^{-1}. Sulfate administered to young pigs at 1800 mg l^{-1} in drinking water for 28 days developed loose and watery stools. A study on the effect of inorganic sulfate on bowel function in piglets reported that concentrations greater than 1200 mg l^{-1} increased the incidence of diarrhea. Concentrations greater than 1800 mg l^{-1} resulted in a persistent diarrhea. No adverse developmental effects were observed following the administration of 2800 mg kg^{-1} day^{-1} of sulfate to pregnant ICRISIM mice on gestation days 8–12. No reproductive effects were observed following the ingestion of drinking water containing up to 5000 mg l^{-1} of sulfates by ICRISIM mice and 3298 mg l^{-1} of sulfates by Hampshire × Yorkshire × Duroc pigs. On the basis of these studies, sulfate does not appear to be a reproductive or a developmental toxicant.

Human

Most data on human responses to sulfate are based on short-term exposures that are obtained from controlled settings (i.e., studies and experimental trials). The risk of adverse health effects to the general population is limited and acute, and such effects occur only at high drinking water concentrations (>500 mg l^{-1}, and in many cases >1000 mg l^{-1}). The data from human studies demonstrated that sulfate induces a laxative effect following acute exposures of concentrations greater than 500 mg l^{-1}. However, the severity of the laxative effect may depend on the sulfate salt, as well as the dose administered. Subpopulations sensitive to sulfate ingested through drinking water include formula-fed infants, the elderly, or invalids who use powdered nutritional supplements, and visitors who are not acclimated to high sulfate concentrations in drinking water.

In a case-control investigation to assess the association between infant diarrhea and ingestion of water containing elevated sulfate levels, a total of 274 mothers of infants born in 19 South Dakota counties with high sulfate concentrations in tap water were identified and interviewed using a telephone questionnaire or in person. No significant association existed between exposure to sulfate from tap water and subsequent diarrhea in infants. The average sulfate concentration in drinking water for cases was 416 versus 353 mg l^{-1} for controls. In another study to determine the effects of high sulfate concentrations in transient populations, there were no statistically significant differences in the mean number of bowel movements among dose groups, and there was also no apparent trend in the percentage of subjects that reported diarrhea during the exposure period.

Chronic Toxicity (or Exposure)

Animal

Data from animal studies on the reproductive, developmental, and carcinogenic effects are available for long-term exposures to sulfate. In a 90 day study, rats administered mineral waters containing up to 1595 mg l^{-1} of sulfate showed no soft feces or diarrhea, indicating rapid acclimation. High sulfate concentrations do not appear to exert adverse reproductive or developmental effects. Following the ingestion of drinking water containing up to 5000 mg l^{-1} of sulfates by mice and pigs, no reproductive effects were observed. Furthermore, no adverse developmental effects were observed following the administration of 2800 mg kg^{-1} day^{-1} of sulfate to pregnant mice.

Human

Since humans appear to develop a tolerance to drinking water with high sulfate concentrations, chronic exposures do not appear to produce the same laxative effect as seen in acute exposures. Some reports have shown that chronic exposure to high sulfate concentrations in drinking water does not have laxative effects in human.

A survey conducted in North Dakota showed a slight increase in the percentage of people who reported that their drinking water had a laxative effect when the drinking water contained 500–1000 $mg\,l^{-1}$ sulfate compared to the percentage of people who reported a laxative effect from drinking water that contained $<500\,mg\,l^{-1}$. Sixty-eight per cent of people who consumed water with 1000–1500 $mg\,l^{-1}$ reported a laxative effect. Analysis of data from North Dakota showed that drinking water containing $\geqslant 750\,mg\,l^{-1}$ sulfate was associated with a self-reported laxative effect whereas drinking water containing $\leqslant 600\,mg\,l^{-1}$ was not. These data were reanalyzed and found that most people experienced a laxative effect when they drank water that contained $>1000\,mg\,l^{-1}$ sulfate.

Clinical Management

The available toxicological data indicate that sulfate may cause adverse health effects in humans and animals. Sulfate has a laxative effect in high doses, but adverse health effects arc temporary and recovery is rapid. Subpopulations sensitive to sulfate ingested through drinking water include formula-fed infants, the elderly, or invalids who use powdered nutritional supplements, and visitors who are not acclimated to high sulfate concentrations in drinking water. Persons with renal diseases may also be more sensitive to effects of high sulfate ingestion.

Environmental Fate

Sulfates are discharged into water from mines and smelters, and from kraft pulp and paper mills, textile mills, and tanneries. Atmospheric sulfur dioxide, formed by the combustion of fossil fuels and by metallurgical roasting processes, may contribute to the sulfate content of surface waters. Sulfur trioxide, produced by the photolytic or catalytic oxidation of sulfur dioxide, combines with water vapor to form dilute sulfuric acid, which falls as 'acid rain'. The environmental fate and transport of sulfate are inextricably linked to the physical and chemical processes active in the earth's sulfur cycle.

Ecotoxicology

With respect to the propagation of fish and wildlife, there is no recommended ambient water quality criterion for the protection of aquatic life for sulfate because sulfate is not generally considered a significant ecological concern, except perhaps where it is a dominant component of total dissolved solids, when sulfate would contribute significantly to excessive salinities (greater than $1000\,mg\,l^{-1}$). There are several sources where published ecotoxicological data are available. These include US Environmental Protection Agency's (EPA) Aquatic Information Retrieval System, the Hazardous Substances Databank, and published scientific literature. Reported chronic toxicity effect levels for sulfate range from 361 to $1488\,mg\,l^{-1}$. The acute toxicity threshold is assumed to be $450\,mg\,l^{-1}$.

Exposure Standards and Guidelines

The US EPA established a Secondary Maximum Contaminant Level for sulfate of $250\,mg\,l^{-1}$, based on taste properties.

A US EPA health-based advisory for acute effects (absence of laxative effects) of 500 mg of sulfate per liter is recommended. In situations, where the water contains high concentrations of total dissolved solids and/or other osmotically active ions, laxative-like effects may occur if mixed with concentrated infant formula or powdered nutritional supplement; therefore, an alternate low-mineral-content water source is advised. Infants are more susceptible to diarrhea water loss than adults because of differences in gastrointestinal structure and function.

The Association for the Advancement of Medical Instrumentation suggests a maximum concentration of $100\,mg\,l^{-1}$ of sulfate in water used for dialysis.

In case of sulfuric acid, time-weighted average is $1\,mg\,m^{-3}$, whereas National Institute for Occupational Safety and Health/Occupational Safety and Health Administration establish $80\,mg\,m^{-3}$ to be immediately dangerous to life and health.

See also: Gastrointestinal System; Pollution, Air; Respiratory Tract; Sulfur Dioxide; Sulfuric Acid.

Further Reading

US Environmental Protection Agency (1992) Drinking Water Criteria Document for Sulfate, Final Report.

US Environmental Protection Agency (1999) Health Effects from Exposure to High Levels of Sulfate in Drinking Water Study. EPA 815-R-99-001.

US Environmental Protection Agency (1999) Health Effects from Exposure to Sulfate in Drinking Water Workshop. EPA 815-R-99-002.

US Environmental Protection Agency (2003) Contaminant Candidate List Regulatory Determination Support Document for Sulfate. EPA-815-R-03-16.

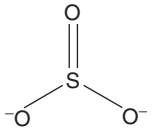
Relevant Website

http:// www.epa.gov – US Environmental Protection Agency (2003) Drinking Water Advisory: Consumer Acceptability Advice and Health Effects Analysis on Sulfate, EPA 822-R-03-007.

Sulfites

Shayne C Gad

- Representative Chemicals: Sulfur dioxide, SO_2; Sodium metabisulfite, $Na_2S_2O_5$; Sodium bisulfite, $NaHSO_3$; Sodium sulfite, Na_2SO_3; Potassium metabisulfite, $K_2S_2O_5$; Potassium bisulfite, $KHSO_3$
- Chemical Abstracts Service Registry Number: Sulfite (CAS 14265-45-3)
- Synonyms: Sulfite; Sulfite anion
- Chemical/Pharmaceutical/Other Class: Inorganic sulfites and bisulfites
- Chemical Structure:



Uses

Inorganic sulfites and bisulfites (such as sodium sulfite, Na_2O_3S) are used in photography, the bleaching of wool, the preserving of foods (e.g., meats and egg yolks), beverages, and medications. They act as effective antioxidant compounds and are also used for pulp making. Their preservative properties include controlling microbial growth, preventing browning and spoilage. Under the (US) Federal Food, Drug, and Cosmetic Act, sulfites are permitted for use as preservatives in food. Like other ingredients, sulfites must be declared in the ingredient statement when added to a food product. In addition, sodium sulfite, ammonium sulfite, sodium bisulfite, potassium bisulfite, ammonium bisulfite, sodium metabisulfite, and potassium metabisulfite are inorganic salts that function as reducing agents in cosmetic formulations. All except sodium metabisulfite also function as hair-waving/straightening agents. In addition, sodium sulfite, potassium sulfite, sodium bisulfite, and sodium metabisulfite function as antioxidants in cosmetics. All except ammonium sulfite are widely used in hair care products.

Background Information

Endogenous sulfite is generated as a consequence of the body's normal processing of sulfur-containing amino acids. In addition, as discussed below, sulfite can be produced by neutrophils. Sulfites occur as a consequence of fermentation and also naturally in a number of foods and beverages. As food additives, sulfating agents were first used in 1664, and approved for use in the United States in the 1800s. Sulfite is also noted as a water treatment additive, for example, to control oxygen levels in power plant boiler water. Further, sulfur dioxide is a common air pollutant, and may enter the body via inhalation. Sulfur dioxide has been reported to react with water in the ambient air and in the respiratory tract's mucous membranes to form sulfite and bisulfite ions.

Exposure Routes and Pathways

The pathways of exposure are oral, dermal, and through inhalation.

Toxciokinetics

Sulfites that enter mammals via ingestion, inhalation, or injection are metabolized by sulfite oxidase to sulfate.

Mechanism of Toxicity

Although the physiological basis for sulfite sensitivity is still poorly understood, clinical observations have established that certain medical conditions are associated with a predisposition to sulfite hypersensitivity. Approximately 500 000 individuals in the United States ($<0.05\%$ of the population) are at risk because they are asthma sufferers, who are steroid-dependent or who have airway hypersensitivity. Completed studies suggest that sulfite in the form of sulfur dioxide is the agent that causes the physiological response. It is hypothesized that sulfur dioxide causes bronchoconstriction, and that sulfur dioxide acts on tracheobronchial receptors to induce a cholinergic reflex. Inhaled sulfur dioxide elicited a stronger reaction in sulfite oxidase-deficient rats than endogenously accumulated sulfites and *S*-sulfocysteine (a reaction product of sulfite with cystine residues in proteins).

Acute and Short-Term Toxicity (or Exposure)

Animal

In oral-dose animal toxicity studies, hyperplastic changes in the gastric mucosa were the most common findings at high doses. Ammonium sulfite aerosol had an acute LC_{50} of $>400\,mg\,m^{-3}$ in guinea pigs. A single exposure to low concentrations of a

sodium sulfite fine aerosol produced dose-related changes in the lung capacity parameters of guinea pigs. A 3 day exposure of rats to a sodium sulfite fine aerosol produced mild pulmonary edema and irritation of the tracheal epithelium. Severe epithelial changes were observed in dogs exposed for 290 days to $1\,mg\,m^{-3}$ of a sodium metabisulfite fine aerosol. These fine aerosols contained fine respirable particle sizes that are not found in cosmetic aerosols or pump sprays. None of the cosmetic product types, however, in which these ingredients are used are aerosolized. Sodium bisulfite (tested at 38%) and sodium metabisulfite (undiluted) were not irritants to rabbits following occlusive exposures. Sodium metabisulfite (tested at 50%) was irritating to guinea pigs following repeated exposure.

In rats, sodium sulfite heptahydrate at large doses (up to $3.3\,g\,kg^{-1}$) produced fetal toxicity but not teratogenicity. Sodium bisulfite, sodium metabisulfite, and potassium metabisulfite were not teratogenic for mice, rats, hamsters, or rabbits at doses up to $160\,mg\,kg^{-1}$. Generally, sodium sulfite, sodium metabisulfite, and potassium metabisulfite were negative in mutagenicity studies. Sodium bisulfite produced both positive and negative results. In evaluating the positive genotoxicity data obtained with sodium bisulfite, the Cosmetic Ingredient Review Expert Panel established by the Cosmetic, Toiletry & Fragrance Association noted that the equilibrium chemistry of sulfurous acid, sulfur dioxide, bisulfite, sulfite, and metabisulfite suggests that some bisulfite may have been present in the genotoxicity tests involving the other ingredients and vice versa. Thus, the genotoxicity data were concluded to not give a clear, consistent picture of the genotoxic potential of these chemicals. Further, the bisulfite form is used in very low concentrations (0.03–0.7%) in most cosmetic products except wave sets. In wave sets, the pH ranges from 8 to 9 where the sulfite form would predominate. Skin penetration would be low due to the highly charged nature of these particles and any sulfite that did penetrate would be converted to sulfate by the enzyme sulfate oxidase. As used in cosmetics, therefore, these ingredients would not present a genotoxicity risk. The Cosmetic Ingredient Review Expert Panel concluded that sodium sulfite, potassium sulfite, ammonium sulfite, sodium bisulfite, ammonium bisulfite, sodium metabisulfite, and potassium metabisulfite are safe as used in cosmetic formulations.

Human

Clinical oral and ocular-exposure studies found no adverse effects for the sulfites used in cosmetics. Sodium sulfite was not irritating or sensitizing in clinical tests. These ingredients, however, may produce positive reactions in dermatologic patients under patch test conditions.

Sulfite-induced bronchospasm (sometimes leading to asthma) was first noticed as an acute sensitivity to metabisulfites, which were sprayed on restaurant salads (and salad bars) and used in wine. Emergency room admissions confirm that ingestion of sulfites can lead to asthmatic attacks, rashes, and abdominal upset. An alert physician observed that six patients, who had been admitted to the emergency room, had consumed the same brand of salsa. Two of the patients had asthma flare-ups, two experienced coughing and tightness of the throat, and two required mechanical ventilation. It was discovered that the offending salsa had a sulfite content of 1800 ppm, well above the level of $\sim$700 ppm found in other brands of salsa. One of the patients, fully aware of her sensitivity to sulfites, thought it was safe to eat the salsa because it was improperly labeled as 'fresh'.

The US Food and Drug Administration Center for Food Safety and Applied Nutrition (CFSAN) has monitored reports of adverse reactions to sulfites since 1980. As of June 1999, CFSAN has received 1132 consumer complaints describing adverse reactions thought to be due to the ingestion of foods with sulfites. Out of 799 reports with adequate information about the intensities of the reaction, 388 (48.6%) were classified as severe.

Recently, it has been shown that sulfite is actively produced from neutrophils by stimulation with the bacterial endotoxin, lipopolysaccharide (LPS), and that the serum sulfite concentration is increased in a rat model of sepsis induced by systemic injection of LPS. The serum concentration of sulfite was determined in patients with acute pneumonia, and was significantly higher than that in control subjects. Further, serum sulfite was serially determined before and after antibiotic therapy, and the levels were significantly reduced during the recovery phase compared with those during the acute phase. Moreover, neutrophils obtained from three patients during the acute phase of pneumonia spontaneously produced higher amounts of sulfite *in vitro* than those obtained after recovery. There was a close positive correlation between serum sulfite and C-reactive protein in patients with pneumonia. These findings suggest that serum sulfite increases during systemic inflammation in humans, and that sulfite may act as a mediator in inflammation.

See also: Food Additives; Food and Drug Administration, US; Sensitivity Analysis.

Further Reading

Lester MR (1995) Sulfite sensitivity: Significance in human health. *Journal of the American College of Nutrition* 14: 229–232.

Mitsuhashi H, Ikeuchi H, Yamashita S, *et al.* (2004) Increased levels of serum sulfite in patients with acute pneumonia. *Shock* 21: 99–102.

Nair B, Elmore AR, and Cosmetic Ingredients Review Expert Panel. (2003) Final report on the safety assessment of sodium sulfite, potassium sulfite, ammonium sulfite, sodium bisulfite, ammonium bisulfite, sodium metabisulfite and potassium metabisulfite. *International Journal of Toxicology* 22(Suppl. 2): 63–88.

Pelletier M, Lavastre V, and Girard D (2002) Activation of human epithelial lung a549 cells by the pollutant sodium sulfite: Enhancement of neutrophil adhesion. *Toxicological Sciences* 69: 210–216.

Ratthe C, Pelletier M, Roberge CJ, and Girard D (2002) Activation of human neutrophils by the pollutant sodium sulfite: Effect on cytokine production, chemotaxis, and cell surface expression of cell adhesion molecules. *Clinical Immunology* 105: 169–175.

Relevant Website

www.cfsan.fda.gov – US Food and Drug Administration. Sulfites: An Important Food Safety Issue.

Sulfonylureas *See* Hypoglycemics, Oral.

Sulfur Dioxide

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 7446-09-5
- Synonyms: Sulfurous anhydride; Sulfurous oxide
- Chemical/Pharmaceutical/Other Class: Oxidant gas
- Chemical Structure: O═S═O

Uses

Sulfur dioxide (SO_2) is a colorless and nonflammable gas with a pungent odor. It is used commercially to preserve fruits and vegetables and as a disinfectant in food production. It is also used in bleaching straw and textiles. Liquid SO_2 can be produced by pressurizing the gaseous form and then used as a solvent. Sulfur dioxide is also a primary component of air pollution.

Exposure Routes and Pathways

Contact with mucous membranes (eyes and nose) and inhalation are possible routes of exposure.

Toxicokinetics

Absorption is dependent on the level of exposure. Sulfur dioxide is soluble in both water and biological tissues. It is readily absorbed and distributed throughout the body. Most inhaled SO_2 is detoxified by sulfite oxidase in the liver. It is excreted through urine and through exhalation in expired air, although elimination from the respiratory tract is slow. Sulfur dioxide absorbed into the body may persist 1 week after exposure.

Mechanism of Toxicity

On moist skin or mucous membranes, SO_2 is converted to sulfurous acid, a direct irritant. This mechanism accounts for its ability to cause inflammation, burning sensation, and tissue damage (described below) in the eyes, throat, nose, and other respiratory tissues experiencing direct contact. Bronchoconstriction and other related effects may be mediated by release of leukotrienes, prostaglandins, or other inflammatory factors. How SO_2 causes any of the other systemic and clastogenic effects reported below is unclear. Some evidence suggests that free radicals and oxidative stress may play a role, and that metabolites of SO_2 (sulfites) may be responsible for clastogenicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

In rats, SO_2 accelerates aging and produces heart, lung, and kidney damage. The inhalation LD_{50} is $2520\,\text{ppm}\,\text{h}^{-1}$ in rats. The LD_{50} is 3000 ppm per 30 min in mice.

Human

Sulfur dioxide is irritating to the eyes, mucous membranes, and respiratory tract. High levels of exposure produce cardiac arrest. Moderate exposure produces pulmonary edema. Low exposure results in systemic acidosis. Individuals who have hyperactive airway disease, including asthma, may be particularly sensitive.

Chronic Toxicity (or Exposure)

Animal

No significant adverse reproductive or developmental effects have been noted in animal studies.

A 2 year cancer bioassay in mice that employed a single dose of 500 ppm for 5 min day^{-1}, 5 days week^{-1} for the duration of the study reported seeing an increase in lung tumors. This study is not considered adequate to conduct a meaningful assessment of cancer risk from SO_2 in human populations, and its relevance to human risk is not known.

Human

Lung function can be altered, but there is no clear epidemiologic evidence that chronic exposure to SO_2 has more serious (e.g., cancer-causing) effects in respiratory tissues in exposed populations. Workers and others known to have had significant exposures to SO_2 have manifested evidence of clastogenicity (e.g., chromosome aberrations and sister chromatid exchange in lymphocytes); however, the clinical significance of this is not clear and the possibility that this was a result of exposure to other agents cannot be ruled out.

In Vitro Toxicity Data

Sulfur dioxide produced weak increases in micronuclei after activation in a plant assay (*Tradescantia* spp.). Mammalian (cow, ewe) oocytes exposed *in vitro* had higher levels of chromosome aberrations when exposed to SO_2 without metabolic activation. The *Saccharomyces cerevisae* yeast test for gene mutations was also positive without activation.

Clinical Management

If skin or eye exposure occurs, the affected areas should be flushed with water for ~15 min. If ingested, the stomach contents should be diluted with water or milk. Gastric lavage or emesis should not be attempted. Pain should be treated without numbing the central nervous system. Open airways and steady blood pressure should be maintained. Prednisolone (2 mg kg^{-1} day^{-1}) should be given for 10 days.

Exposure Standards and Guidelines

Occupational/Occupational Safety and Health Administration: The Lowest Lethal Concentration (LLC) is 1000 ppm per 10 min and the permissible exposure limit/time-weighted average is 5 ppm per 8 h.

Environmental Protection Agency/environmental: The National Ambient Air Quality Standards annual arithmetic mean standard is 0.03 ppm; the 24 h limit is 0.14 ppm.

International Agency for Research on Cancer carcinogen classification: 3 (possible human carcinogen, based on a single mouse study employing only one air concentration; see details above).

See also: Absorption; Pollution, Air; Respiratory Tract.

Further Reading

Bingham E, Cohrssen B, and Powell CH (2001) *Patty's Toxicology*, 5th edn., vol. 3, pp. 491–494. New York: Wiley.

Dart RC (2004) *Medical Toxicology*, 3rd edn. Baltimore, MD: Lippincott.

Meng Z, Qin G, Zhang B, *et al.* (2003) Oxidative damage of sulfur dioxide inhalation on lungs and hearts of mice. *Environmental Research* 93(3): 285–292.

Relevant Websites

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

http://www.epa.gov – SO_2 – How Sulfur Dioxide Affects the Way we Live and Breathe (from the US Environmental Protection Agency).

Sulfuric Acid

Heriberto Robles

- Chemical Abstracts Service Registry Number: CAS 7664-93-9
- Synonyms: Acid mist; Dipping acid; Hydrogen sulfate; Sulfur acid; Sulfuric
- Chemical/Pharmaceutical/Other Class: Strong inorganic acid; Corrosive agent
- Chemical Formula: H_2SO_4

Uses

Sulfuric acid is a highly reactive compound and is extensively used in industry as a chemical intermediate and as a component of many industrial and commercial products. For example, it is used in fertilizers, lead-acid batteries, pigments and dyes, and as an industrial reagent in the paper, petroleum, and metal industries. It is also used in pharmaceuticals, as a food additive, and in toilet bowl cleaners.

Background Information

Sulfuric acid can be found in many strengths and formulations. Toxicological and chemical properties of sulfuric acid solutions are dependent on the sulfuric acid content of the solution. For example, solutions containing less than 10% sulfuric acid are highly irritating, while solutions containing more than 10% sulfuric acid are corrosive. Sulfuric acid solutions used in industry can be up to 98% in concentration, while consumer products such as toilet bowl cleaners may contain up to 8% sulfuric acid.

Exposure Routes and Pathways

Sulfuric acid is corrosive to living tissue. It can damage and destroy living cells and tissue upon contact. Therefore, pathways of exposure include all surface tissues (skin, eyes, mucus membranes) as well as internal surfaces exposed to outside elements such as the digestive and respiratory tracts.

Toxicokinetics

Sulfuric acid can cause tissue damage and destruction upon contact. Therefore, it is not absorbed into the systemic circulation. The reaction of sulfuric acid with body tissues produces irritation and chemical burns at the site of contact. Ingestion of sulfuric acid can cause internal tissue damage and associated secondary systemic effects such as gastrointestinal hemorrhaging, ischemia, hypoxia, shock, and necrosis.

Mechanism of Toxicity

Sulfuric acid is a highly reactive chemical. It can react with cells and tissues upon contact. Damage caused by sulfuric acid can range from tissue irritation to chemical burns and necrosis. Signs and symptoms of exposure include tissue damage at point of contact. Tissue injury appears within seconds of exposure and can continue for hours and even days if not properly treated. The tissue damage extent and severity is dependent on the dose received, exposure interval, and strength (molar concentration) of the sulfuric acid solution. Highly concentrated sulfuric acid solutions (usually found in industrial chemicals) are more dangerous than diluted acid solutions (as those found in consumer products).

The mode of action of sulfuric acid is the same in humans and animals. Therefore, acute and chronic effects are expected to be the same for animals and humans.

Acute and Short-Term Toxicity (or Exposure)

The major hazard associated with exposure to sulfuric acid is direct irritation and corrosion of internal and external tissue surfaces. Signs and symptoms associated with potential routes of exposure include:

- *Inhalation:* Nose and throat irritation, coughing, sneezing, difficulty in breathing, and pulmonary edema. Death may result from esophageal edema (caused by chemical burns to the esophagus) or sudden circulatory collapse (caused by generalized lung tissue destruction).
- *Ingestion:* Throat irritation, difficulty in swallowing, hemorrhaging, perforation, and necrosis of digestive tract.
- *Skin:* Damage can range from dermatitis and irritation to necrosis and scarring. Extensive and severe chemical burns can be life threatening.
- *Eye:* Eyes are especially susceptible to acid burns. Signs and symptoms in increasing severity include: irritation, lacrimation, conjunctivitis, corneal burns and perforation, visual loss, and perforation of the eye.

Chronic Toxicity (or Exposure)

Chronic exposure to diluted solutions of sulfuric acid can produce chronic tissue damage. Adverse effects

seen following chronic exposure are usually due to repeated and sustained tissue damage and repair. Signs and symptoms associated with chronic exposure include: decreased lung capacity and function, recurrent respiratory infections, bronchitis, and possibly cancer. The International Agency for Research on Cancer has determined that chronic, occupational exposure to sulfuric acid mist may cause cancer of the upper respiratory tract. Repeated ingestion of dilute sulfuric acid solutions may cause perforation of teeth enamel and gastritis.

Clinical Management

Basic life support measures should be implemented and further absorption 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 made and gastric lavage performed 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, esophagus, and lung damage. Patients should be monitored and treated in an intensive care unit. Monitor vital signs and blood chemistry at least once a day. Institute life support as needed.

Environmental Fate

Sulfuric acid is found in nature in the vicinity of volcanoes. It is also used in industry for manufacturing numerous consumer products. Therefore, the chemical may be released to the environment as a waste product or from unintentional, accidental releases. If released to soil, it will dissolve in soil moisture and migrate with either soil moisture or groundwater flow. If released to water, it will dissolve or create sulfate salts. Dissolved sulfuric acid will react with calcium and magnesium to produce sulfate salts. Sulfuric acid can contribute to the 'weathering' of soil and rocks by reacting with calcium and carbonates contained in soil and rocks.

Exposure Standards and Guidelines

The US Occupational Safety and Health Administration has established $1\,mg\,m^{-3}$ as the 8 h time-weighted average permissible level for sulfuric acid in workplace air.

Miscellaneous

Special precautions must be taken when working with sulfuric acid. Personnel handling this chemical must follow industrial hygiene and health protection requirements for handling potentially corrosive substances. At a minimum sulfuric acid exposure should be minimized through the use of engineering controls, work practices, and personal protective equipment, including impervious and disposable gowns and gloves as well as eye and respiratory protection. In addition, working areas and working instruments must be especially designed for handling potentially harmful substances.

See also: Acids; Corrosives; Great Smog of London.

Further Reading

Amdur MO (1989) Health effects of air pollutants: Sulfuric acid, the old and the new. *Environmental Health Perspectives* 81: 109–113 (discussion 121–122).

Ellenhorn MJ and Barceloux DG (eds.) (1988) *Medical Toxicology, Diagnosis and Treatment of Human Poisoning*. New York: Elsevier.

Goldfrank LR, Fromenbaum NE, Lewin NA *et al.* (eds.) (1994) *Goldfrank's Toxicologic Emergencies*, 5th edn. Norwalk, CT: Appleton & Lange.

Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, and Gilman AG (eds.) (1996) *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 9th edn. New York: McGraw-Hill.

Rossoff IS (2002) *Encyclopedia of Clinical Toxicology*. Boca Raton, FL: The Parthenon Publishing Group.

Relevant Website

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

Surfactants *See* Surfactants, Anionic and Nonionic; Surfactants, Perfluorinated; Detergent.

Surfactants, Anionic and Nonionic

Gerald L Kennedy

This article is a revision of the previous print edition article by Anne E Bryan, volume 3, pp. 209–210, © 1998, Elsevier Inc.

- Representative Chemicals:
  - Anionic: Alkyl aryl polyester sulfates and sulfonates (Triton); Alkyl aryl sodium sulfonates; Alkylated sodium phosphates, sulfates (sodium lauryl sulfate, Tergitol) or sulfonates; Linear alkyl benzene sulfonates, soaps, and diethanolamine oleate
  - Nonionic: Synthetic detergents (Joy, Cascade); Alkyl ethoxylates; Alkyl phenoxy polyethoxy ethanols (lgepal); Glyceryl stearate; Pluronics polyoxyethylene sorbitols (Tween), and polysorbates
- Chemical/Pharmaceutical/Other Class: Surfactants (detergents)

Uses

These materials are used primarily for removing dirt from either soft or hard surfaces as in washing clothes or dishes, removing wax from floors, or removing grease from metals. Although the classes are based on the ionization of the surface active moiety at neutral pH (anionic, nonionic, or cationic), within a given class a wide range of chemical structures and toxicities is recognized. This section will consider what is felt to be the most generally applicable toxicities of these classes but reminds the reader that each of the chemicals might have its own particular toxicities and potencies.

Exposure Routes and Pathways

Most of these chemicals (or products) will come in contact with the skin in their normal intended use. However, the primary route for poisonings is the accidental ingestion of these chemicals by small children; so, special care needs to be taken to minimize this potential. Most of these agents are irritants to the eye (reference, soap in the eye), so contact with ocular tissues should be avoided.

Inhalation is a less likely route of contact but, as these materials are irritating to mucous membranes, contact with tissues of the upper respiratory tract (nose) will be irritating.

Toxicokinetics

The materials are not readily absorbed from the skin or gastrointestinal tract. The specifics of adsorption, distribution, metabolism, and excretion vary with the individual surfactants hence further generalization is unwarranted.

Mechanism of Toxicity

Detergents are not readily absorbed and most reported toxicities come from contact with surface tissues such as skin and eye. Hand dishwashing detergents tend to be less irritating than machine dishwashing agents. Where alkyl chain length vary, those of shorter chain length tend to be more irritating (and somewhat more toxic) when given orally.

Relatively little systemic toxicity has been reported except in poisoning situations. The irritating properties of these chemicals, to some extent, serve as a warning mechanism to avoid the exposed organism against further contact.

Acute and Short-Term Toxicity (or Exposure)

Animal

As in the human overexposure cases, gastrointestinal irritation and ocular irritation would be expected in animal tests. Oral doses required to produce mortality in rats are generally very high, in the greater than 1–2 $g\,kg^{-1}$ range. For example, the oral LD_{50} for sodium lauryl trioxyethylene sulfate is 1.8 $g\,kg^{-1}$. Dogs and monkeys appear less sensitive to oral doses than rodents perhaps because they tend to vomit more readily. With alkyl polyethylene glycol ether, monkeys showed emesis following single oral doses above 5 $g\,kg^{-1}$ along with signs of central nervous system depression. Again, in general these materials are irritants that are low in acute toxicity and show systemic responses only under highly exaggerated exposure conditions.

Human

Skin irritation has been encountered after prolonged occupational dermal contact. Skin dryness, irritation, and contact dermatitis have all been seen after varying degrees of exposure. Eye exposure to most anionic and nonionic detergents results in momentary eye irritation with no permanent eye damage. Eye exposure to low-phosphate detergents, which tend to be more alkaline may produce eye injury.

If ingested (and depending on the amount), nausea, vomiting, and diarrhea are the most common manifestations of toxicity. Persistent effects rarely result but dehydration, electrolyte imbalance (most notable hypochloremic metabolic acidosis) have been reported.

In the workplace, occupational asthma has been reported. Aspiration may result in upper airway edema and considerable respiratory distress. Again, low phosphate detergents will produce oral, esophageal, and respiratory tract burns due to their alkaline nature.

Chronic Toxicity (or Exposure)

Animal

Systemic responses have been shown to occur only after treatment with high doses for relatively long periods of time. Sodium lauryl trioxyethylene sulfate fed to rats at 0.5% (50 000 ppm) for 2 years showed no gross anatomical, biochemical, or tissue histopathologic lesions (including no increase in tumors). Mice painted twice weekly applications of a 5% aqueous solution developed no skin tumors. Again, the effects of concern with these chemicals tend to be short term relating to their irritation properties.

Human

There is no useful experimental data on the long-term effects of these chemicals in man. Clearly the fast-acting short-term irritation effects would be expected to allow exposed individuals to be aware of and restrict long-term contact (not necessarily repeated contact as in the use of detergents, dishwashing agents, etc.).

In Vitro Toxicity Data

In general, the surfactant properties of these chemicals tend to make *in vitro* testing difficult both qualitatively and quantitatively. The physical characteristics of surfactants tend to keep the molecule at the water/oil, water/air interface thus making meaningful contact with the *in vitro* organ/tissue systems difficult. Both mechanistic-related effects and noneffects, can result from attempts to test these chemicals in *in vitro* aqueous systems hence caution is advised when attempting to conduct, evaluate, or interpret such information.

Clinical Management

For oral exposure, immediate dilution with either water or milk should be employed. Spontaneous emesis frequently occurs (if not, it is unlikely that significant ingestion has occurred). Patients should be observed for signs of esophageal or gastrointestinal tract irritation or burns. If inhaled, move the patient to fresh air and monitor for respiratory distress. If coughing or difficulty breathing develops, evaluate the patient for respiratory tract irritation, bronchitis, or pneumonia. Oxygen and assisted ventilation can be used in extreme cases. Bronchospasms which occur rarely (and again following significant exposure) can be treated with $\beta 2$ agonists and oral or parenteral corticosteroids. Following eye exposures, irrigate the eye with copious amounts of room temperature water for at least 15 min. If irritation, pain, swelling, lacrimation, or photophobia persists, seek the attention of a physician. Dermal exposures should be treated by removal of contaminated clothing and jewelry and washing the exposed area with copious amounts of water. As before, persistent irritation or pain should be referred to health care professionals.

In summary, ingestion of nonionic or anionic detergents alone is not generally serious. Ingestion of automatic dishwater soaps or low-phosphate detergents, which are more alkaline, may result in burns of the mouth, pharynx, and esophagus. Ingestion of hard soap bars is generally associated with emesis and mild diarrhea. Eye contact injuries may occur with these agents causing varying degrees of damage.

Environmental Fate

The hydrocarbon chains of these materials generally tend to break down in the environment relatively quickly. The business end of the molecule behaves differently hence no overarching statements can be made. Sodium lauryl sulfate can readily be removed from aqueous systems (filtering and aeration) and is readily biodegraded (42 of 45 Pseudomonas strains could degrade alkyl sulfates to the saturated and unsaturated fatty acid). In sewage, sludge, seawater, and by selected organisms from 75% to 100% degradation is reported for sodium lauryl sulfate.

Ecotoxicology

It is difficult to characterize the effects of surfactants as a class on environmental organisms. However, there appears to have been relatively little impact on the health of environmental organisms as a result of exposure to these chemicals. Again using sodium lauryl sulfate as an example, concentrations of from 1.2 to 600 $\mathrm{mg\,l^{-1}}$ are required to inhibit the activity

of microorganisms in sludge. In bacteria, effective concentration 50% (EC_{50}) of from 43 to $>9000\,mg\,l^{-1}$ have been reported. In invertebrates, EC50 values range from 1 to $118\,mg\,l^{-1}$. Thirty-eight different fish species have been studied for their acute lethality response and values range from 0.4 to $560\,mg\,l^{-1}$ with most of the species responding $\sim 5\,mg\,l^{-1}$. Algae appear to be a more sensitive organism with EC_{50} values ranging from 0.02 to $7\,mg\,l^{-1}$.

Other Hazards

Although not a hazard, these chemicals, as surfactants, produce foam at the air/water interface and direct discharge to waterways results in unsightly build-up of foam on/around the water.

See also: Sensory Organs; Shampoo; Skin.

Further Reading

Anon. (1991) Environmental and Human Safety of Major Surfactants. Volume 1: Anionic Surfactants. Part 1. Linear Alkylbenzene Sulfonates. Part 2. Alcohol Ethyl Sulfates. Part 3. Alkyl Sulfates. Part 4. Alpha Olgin Sulfonates. Volume 2: Nonionic Surfactants, Alcohol Ethoxylates and Alkylphenol Ethoxylates. Government Reports Announcements & Index (GRA&I).

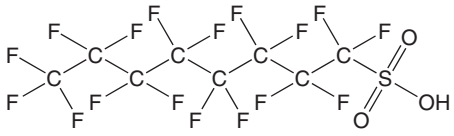
Lewis MA (1990) Chronic toxicities of surfactants and detergent builders to algae: A review and risk assessment. *Ecotoxicology and Environmental Safety* 20: 123–140.

Oba K and Takei R (1992) Carcinogenic, mutagenic/genetic toxicity, and teratogenic properties. Anionic Surfactants: Biochemistry, Toxicology, Dermatology. Surfactant Science Series, vol. 43. 2: 331–409.

Surfactants, Perfluorinated

John Newsted and Paul Jones

- Representative Chemicals: Perfluorooctane sulfonic acid (PFOS); Perfluorooctanesulfonyl fluoride (POFS); *N*-Methylperfluorooctane sulfonamidoethanol (N-MeFOSE); *N*-Ethylperfluorooctane sulfonamidoethanol (N-EtFOSE)
- Chemical Abstracts Service Registry Numbers: Perfluorooctanesulfonate, acid (CAS 1763-23-1); Perfluorooctanesulfonyl fluoride (CAS 307-35-7); *N*-Methylperfluorooctane sulfonamidoethanol (CAS 24448-09-7); *N*-Ethylperfluorooctanesulfonamidoethanol (CAS 1691-99-2)
- Synonyms: Perfluorooctane sulfonic acid; 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-1-Octanesulfonic acid; Heptadecafluoro-1-octanesulfonic acid; Perfluorooctylsulfonic acid
- Chemical/Pharmaceutical/Other Class: Perfluorinated surfactants consist of a broad class of fluorinated chemicals of differing structures, physical–chemical properties, and modes of toxic action. The focus in this article is on sulfonyl-perfluorinated surfactants
- Chemical Formulas:
  - Perfluorooctane sulfonate: $C_8F_{17}SO_3H$
  - Perfluorooctanesulfonyl fluoride: $C_8F_{17}SO_2F$
  - *N*-Methylperfluorooctanesulfonamidoethanol: $C_8F_{17}SO_2N(CH_3)CH_2CH_2OH$
  - *N*-Ethylperfluorooctanesulfonamidoethanol: $C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2OH$
- Chemical Structure:



PFOS

Uses

Perfluorinated surfactants are fully fluorinated organic compounds that due to their unique chemical properties can be used in a variety of industrial processes and products. Some characteristics of these chemicals are the ability to repel water and oil, reduce surface tension, catalyze oligomerization and polymerization, and maintain their properties under extreme conditions. Sulfonyl-based perfluorochemicals (PFOS) have been used in a variety of products that can be divided into three main categories: surface treatments (carpet and textile protection), paper protection (grease, oil, and water resistance), and performance chemicals (fire fighting foams, mining surfactants, electronic etching baths). However, due to the presence of PFOS in biota, in sites remote from production, and in human blood, PFOS has been voluntarily withdrawn from commercial production.

Exposure Routes and Pathways

Occupational exposure to perfluorinated chemicals (PFCs) may occur through inhalation of and dermal

contact with these compounds at workplaces where they are produced or used. Environmental monitoring data indicate that the general population may be exposed to PFCs such as PFOS via ingestion of contaminated fish and drinking water, and by dermal contact with products containing PFCs. The use of PFOS in food packaging as water and grease repellents also serves as a source of exposure to these compounds.

Toxicokinetics

PFOS is well absorbed from the digestive tract while dermal absorption appears to be limited. No quantitative data are available on absorption of PFOS via inhalation. Once absorbed, PFOS is bound to protein and is distributed primarily in blood and liver. Significant enterohepatic circulation of PFOS has been reported in several species. PFOS is not known to undergo further metabolism but other fluorochemicals such as perfluorooctanesulfonyl fluoride (POSF) and ethylperfluorooctane sulfonamidoethanol (Et-FOSE) may undergo metabolism to PFOS. Elimination from the body is slow with PFOS being found in both urine and feces. In addition, PFOS has also been shown to traverse the placenta and expose the fetus *in utero*. PFOS is also distributed into the milk of lactating females. The estimated serum half-life in humans is $\sim$1428 days (or 4 years).

Mechanism of Toxicity

The mechanisms governing the toxicity of PFOS to biological systems are still under investigation. Potential modes of action that have been identified include competition with fatty acids for carrier protein sites, cholesterol synthesis, and bioenergetics. Other studies suggest that PFOS may alter peroxisomal fatty acid β-oxidation.

Acute and Short-Term Toxicity (or Exposure)

Animal

PFOS has shown moderate acute toxicity in rats (LD_{50} of 251 mg kg^{-1}). In a 90 day repeat-dose–response study with rats, exposure to PFOS (6 mg kg^{-1} day^{-1}) resulted in hepatotoxicity and mortality. Adverse signs of toxicity included hepatic vacuolization and hepatocellular hypertrophy, gastrointestinal effects, hematological abnormalities, weight loss, convulsions, and death. Postnatal deaths and other developmental effects have been reported at low doses in offspring in a two-generation reproductive toxicity study with rats. At the highest dose (3.2 mg kg^{-1} day^{-1}) all pups died within a day after birth while $\sim$30% of the F1 pups died after 4 days in the 1.6 mg kg^{-1} day^{-1} group. The no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effect level (LOAEL) for the second-generation offspring (F2 pups) were 0.1 and 0.4 mg kg^{-1} day^{-1}, respectively. In a developmental study with rabbits exposed to PFOS, maternal toxicity was evident at 1.0 mg kg^{-1} day^{-1}. Developmental effects due to PFOS included reduced fetal body weight and reduced ossification of the sternum, hyoid, metacarpals, and pubis. The LOAEL for developmental effects was 2.5 mg kg^{-1} day^{-1} and the NOAEL was 1.0 mg kg^{-1} day^{-1}.

Chronic Toxicity (or Exposure)

Animal

Potential carcinogenicity of PFOS has been examined in a dietary 2 year bioassay with rats. In male and female rats exposed to 20 mg kg^{-1}, there was a significant increase in the incidence of hepatocellular adenomas while in females there was also an increase in hepatocellular carcinomas. In addition, there was a significant increase in thyroid follicular cell adenomas and carcinomas in males in a recovery group from the 20 mg kg^{-1} treatment group.

Human

PFOS has been detected in the serum of occupational and general populations. In occupational surveys, PFOS serum levels have been found to range from 0.1 to 12.83 ppm with an average of 1.32 ppm. In the general population the range of PFOS serum concentrations was from 4.3 to 1656 ppb. The mean PFOS level was determined to be 30–53 ppb. Occupational surveys have also shown some association between PFOS exposure and human health. In a survey of males, PFOS serum concentrations of 1.69–10.06 ppm were associated with increased serum triglycerides, alkaline phosphatase, total bilirubin, and alanine aminotransferase. Serum triiodothyronine was higher and the thyroid hormone binding ratio was lower in workers with the greatest PFOS serum levels. In a mortality study of workers, mortality risks for most cancer types and nonmalignant causes were not elevated. However, an increased risk of neoplasms of the male reproductive system and bladder cancer was associated with workers with the highest and longest exposures to fluorochemicals.

In Vitro Toxicity Data

PFOS was not mutagenic in *Salmonella* tests. PFOS also did not induce chromosomal aberrations in

human lymphocytes or micronuclei in bone marrow of mice. PFOS did inhibit gap junctional intercellular communication in a rat liver and dolphin kidney cell line, an effect that was both rapid and reversible.

Environmental Fate

As a result of the production and use of perfluorooctane sulfonic acid and its precursors, PFOS has been released to the environment through variety of waste streams. The environmental partitioning behavior of PFCs is unusual in that PFOS-based substances are both oleophobic and hydrophobic. As a result, an octanol/water partitioning (K_{ow}) coefficient for PFOS has not been determined. PFOS is persistent in the environment and does not hydrolyze, undergo direct or indirect photolysis, or biodegrade to any significant degree. While PFOS has low volatility, several PFOS precursors are considered volatile, including EtFOSE and MeFOSE alcohols. If released to soil, sediment or sludge, PFOS is expected to adsorb strongly to organic and inorganic components. Due to these properties, PFOS is expected to persist in soils, sediments, and sludge. If released into water, PFOS is expected to remain in the water compartment unless it is assimilated into organisms or adsorbed onto particulate matter and potentially deposited into sediments. Volatilization from water surfaces or biodegradation is not expected to be important fate processes. PFOS has the potential to bioaccumulate in aquatic organisms. Laboratory-based bioconcentration factors for PFOS range from 56 to over 1000 while field-based bioaccumulation factors range from 830 to 125 000. The field-based bioaccumulation factors for PFOS may be overestimated due to metabolism of accumulated perfluorinated derivatives of PFOS.

See also: Fluorine; Surfactants, Anionic and Nonionic.

Further Reading

OECD (2002) Hazard assessment of perfluorooctane sulfonate (PFOS) and its salts. ENV/JM/RD(2002)17/FINAL.

Olsen GW, Burris JM, and Mandel JH (2003) Human donor liver and serum concentrations of perfluorooctane sulfonate and other perfluorochemicals. *Environmental Science and Technology* 37: 888–891.

Seacat AM, Thomford PJ, Hansen KJ, *et al.* (2003) Subchronic dietary toxicity of potassium perfluorooctane sulfonate in rats. *Toxicology* 183: 117–131.

Synergism *See* Chemical Interactions.

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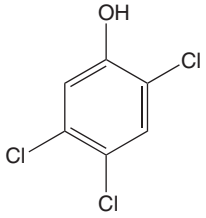
T

2,4,5-T

Lynn Weber

- Chemical Abstracts Service Registry Number: CAS 93-76-5
- Synonyms: 2,4,5-Trichlorophenol
- Chemical/Pharmaceutical/Other Class: Chlorinated phenoxyacetic acids. A closely related compound is 2,4-D (2,4-dichlorophenoxyacetic acid)
- Chemical Structure:



Uses

2,4,5-T is manufactured for use as a broad-spectrum herbicide. Its use in the United States has been suspended.

Background Information

A combination of the herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-T (Agent Orange), was used by the US military during the Vietnam War for defoliation. Long-term health consequences from exposure to Agent Orange, in particular to dioxin contaminants, have been suspected.

Exposure Routes and Pathways

Exposure to 2,4,5-T is by the oral, inhalation, and dermal routes.

Toxicokinetics

2,4,5-T is absorbed dermally in humans. Radioactivity was found in all tissues examined as well as in milk and fetuses after single oral administration of 0.17–41 mg kg^{-1} [^{14}C]2,4,5-T to pregnant rats. 2,4,5-T is eliminated largely unchanged. No metabolites are known, although it is possible that a small amount is eliminated as a glucuronide conjugate. The volume of distribution after a single oral dose of 5 mg kg^{-1} varies as follows: in humans, 0.079 l kg^{-1}; in rats, 0.14 l kg^{-1}; and in dogs, 0.22 l kg^{-1}. 2,4,5-T is bound extensively to the plasma protein, which could limit renal clearance of the herbicide; 2,4,5-T is also bound to renal cortex microsomal and cytosol fractions. 2,4,5-T given orally to volunteers (100–150 mg) was readily absorbed and gradually eliminated from blood plasma, showing a first-order elimination rate; more than 80% of a dose was excreted in urine in intact form within 72 h. Clearance of 2,4,5-T from plasma and body of dogs, mice, and humans is slower than that in rats.

Mechanism of Toxicity

The effect of 2,4,5-T was investigated in *in vitro* studies with sublethal concentrations of 2,4,5-T, which showed an inhibitory effect on calcium-dependent ATPase. *In vivo* exposure to various sublethal concentrations of 2,4,5-T during 96 h caused a significant inhibition of microsomal calcium-dependent ATPase. 2,4,5-T inhibits renal anion transport. Exposure of cells to 2,4,5-T resulted in a dose-dependent inhibition of DNA synthesis. Also, 2,4,5-T was shown to combine with choline to form 2,4,5-T-acetylcholine, a false neurotransmitter that inhibited muscle contraction. Since the false neurotransmitter could be formed at muscarinic as well as nicotinic synaptic sites, this interference with cholingeric neurotransmission may at least partially explain myotonia, ventricular fibrillation, and fetal growth retardation reported after 2,4,5-T exposure. Finally, 2,4,5-T is a well-known peroxisome proliferator agent, particularly in rodent liver.

Acute and Short-Term Toxicity (or Exposure)

Animal

2,4,5-T in pure form is considered to be of relatively low toxicity.

Oral LD_{50}s were as follows: mouse, 389 mg kg^{-1}; rat, 500 mg kg^{-1}; guinea pig, 381 mg kg^{-1}; dog, $>$100 mg kg^{-1}. The percutaneous LD_{50} in rats was $>$5000 mg kg^{-1}. Single oral doses of 100 mg kg^{-1} body weight of 2,4,5-T fed to pigs caused anorexia, vomiting, diarrhea, and ataxia; at autopsy, hemorrhagic enteritis and congestion of liver and kidney were found. 2,4,5-T (containing no detectable 2,3,7,8-tetrachlorodibenzo-*p*-dioxin) affected chromosomes of bone marrow cells of mongolian gerbil (*Meriones unguiculatus*) that received five consecutive daily intraperitoneal injections by causing significant increases in chromatid gaps, chromatid breaks, and fragments after total doses of 250 mg kg^{-1} or more but not after 150 mg kg^{-1} or less.

The no-effect levels for embryotoxicity for commercial 2,4,5-T were as follows: rat, 25 mg kg^{-1} day^{-1}; mouse, 20 mg kg^{-1} day^{-1}; hamster, 40 mg kg^{-1} day^{-1}; and monkey, 40 mg kg^{-1} day^{-1}.

Human

2,4,5-T in pure form is considered to be of relatively low toxicity. Limited data are available on exact toxic doses. Intravenous injection of up to 28 mg kg^{-1} of 2,4-D has been well tolerated, while a dose of 50 mg kg^{-1} produced toxicity. Death has resulted following ingestion of 80 mg kg^{-1}.

Common findings after acute ingestion included miosis, coma, fever, hypotension, emesis, tachycardia, and muscle rigidity. Complications may include respiratory failure, pulmonary edema, and rhabdomyolysis. Ingestions cause burning of the mouth, esophagus, and stomach. Irritation of skin, eyes, nose, and throat may also occur. Tachycardia is common. Cardiac arrhythmias occurred in one suicide case. Pulmonary edema has been reported. Respiratory paralysis and bradypnea are common in large ingestions. Vertigo, headache, malaise, and paresthesias have been reported occasionally in occupational handlers. Higher doses may produce muscle twitching and spasms, followed by profound muscle weakness and unconsciousness. Individual idiosyncrasies may be involved in reported neuropathies. Rhabdomyolysis may occur. Myotonia (stiffness of legs) has been observed in severely poisoned persons. Vomiting and diarrhea have been reported. Elevated LDH, SGOT (AST), and SGPT (ALT) (LDH, lactate dehydrogenase; SGOT, serum glutamic oxaloacetic transaminase; AST, aspartate aminotransferase; SGPT, serum glutamic pyruvic transaminase; ALT, alamine aminotransferase) have been reported. Albuminuria, hemoglobinuria, and azotemia may occur. Acute exposure may cause irritation of the skin. Chloracne from chlorodioxin contaminants in 2,4,5-T has been reported in heavily exposed workers.

Chronic Toxicity (or Exposure)

Animal

2,4,5-T itself is not believed to be carcinogenic or teratogenic in animals; these effects, produced by technical grades of the chemical, are believed to be due to the dioxin that is present as an impurity. In 2 year feeding trials no effect was observed in rats receiving 30 mg kg^{-1} diet or in 90 day trials in beagle dogs at 60 mg kg^{-1} diet.

Human

2,4,5-T itself is not believed to be carcinogenic or teratogenic in humans; these effects, produced by technical grades of the chemical are believed due to the dioxin that is present as an impurity.

Chronic 2,4,5-T exposure may reduce metabolic rate and subsequently lead to perinatal growth retardation. There may be an association of 2,4,5-T exposure with hydantidiform mole formation. However, the effects of 2,4,5-T and dioxin (an impurity in 2,4,5-T preparations) cannot be distinguished from each other in most studies.

Classification of Carcinogenicity

Evidence in humans is limited; overall summary evaluation of carcinogenic risk to humans is group 2B: the agent is possibly carcinogenic to humans. Recent studies conducted to clarify the carcinogenic potential found that although the closely related 2,4-D is mutagenic in a yeast test, cultured mammalian cells, and *in vivo* treated mice, 2,4,5-T appears to exhibit little or no mutagenicity.

Clinical Management

These herbicides can be measured in plasma and urine by high-performance liquid chromatography. Chlorophenoxy compounds do not affect blood cholinesterase activities.

Emesis may be indicated in recent substantial ingestion unless the patient is or could rapidly become obtunded, comatose, or convulsing. It is most effective if initiated within 30 min. For activated charcoal/cathartic, a charcoal slurry, aqueous or mixed with saline cathartic or sorbitol, should be administered. A baseline complete blood count (CBC), electrolytes, and renal and hepatic function test should be obtained. Urine should be tested for protein, RBCs, and myoglobin. Urine output should be monitored. LDH, SGOT (AST), and alkaline phosphatase should

be followed to detect liver injury, and creatine phosphokinase (CPK) should be followed to detect muscle damage. Urine pH, arterial pH, and bicarbonate should be measured to detect acidosis. Respiratory depression, hypotension, and metabolic acidosis should be treated. Adequate urine flow should be maintained with intravenous fluids if victim is dehydrated. The patient should be monitored closely for cardiac arrhythmias, hyperthermia, and seizures.

If exposed via inhalation, the victim should be moved to fresh air and monitored for respiratory distress. If cough or difficulty in breathing develop, evaluation for respiratory tract irritation, bronchitis, or pneumonitis should be performed. Humidified supplemental oxygen (100%) should be administered with assisted ventilation as required.

Exposed eyes should be irrigated with copious amounts of tepid water for at least 15 min. If irritation, pain, swelling, lacrimation, or photophobia persists, the patient should be seen in a health care facility.

If clothing is contaminated, it should be removed and discarded. Affected skin should be washed vigorously, including hair and nails; soap washings should be repeated.

Environmental Fate

Released 2,4,5-T exhibits mobility that ranges from high in sandy soils to slight in organic-rich soils and generally will not persist beyond one growing season. With a pK_a of 2.88, 2,4,5-T will be found in the dissociated form in most compartments of the environment and will be bound to humic acids, to sediment, or to fine droplets in air. Volatilization and bioaccumulation are not expected to be significant.

Ecotoxicology

Exposure to 2,4,5-T reduces fecundity and impairs larval development in honeybees (100–1000 ppm). It has also been reported to reduce arthropod counts in sprayed forested areas by up to 50%. Gill Ca-ATPase activity was reduced by 2,4,5-T exposure in rainbow trout (*Oncorhynchus mykiss*) and juveniles were more susceptible than adults. Studies conducted in birds (Japanese quail and mallard ducks) report liver abnormalities, proliferation of bile canaliculi, anorexia, wasting, and decreased fecundity. Many of these effects are likely attributable to dioxins that may also be present in 2,4,5-T preparations.

Exposure Standards and Guidelines

The chronic reference dose for 2,4,5-T is $0.01\,mg\,kg^{-1}\,day^{-1}$ whereas the acceptable daily intake is $0.03\,mg\,kg^{-1}\,day^{-1}$.

See also: Chlorophenoxy Herbicides; 2,4-D (2,4-Dichlorophenoxy Acetic Acid); Pesticides; Pollution, Water.

Relevant Website

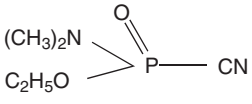
http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.

Tabun

Harry Salem and Frederick R Sidell*

Published by Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 77-81-6
- Synonyms: GA; Ethyl *N*,*N*-dimethylphosphoramidocyanidate ethyl dimethylphosphoramidocyanidate; Dimethylaminoethoxy-cyanophosphine oxide; Dimethylamidoethoxyphosphoryl cyanide; Ethyldimethylaminocyanophosphonate; Ethyl ester of dimethylphosphoramidocyanidic acid; Ethyl phosphorodimenthylamidocyanidate; G agent; Nerve gas; Nerve agent
- Chemical/Pharmaceutical/Other Class: Tabun is a non-persistent anticholinesterase liquid, or organophosphate (OP) nerve agent, colorless to brown, with a faint almond odor
- Chemical Formula: $C_5H_{11}N_2O_2P$
- Chemical Structure:



Uses

Tabun is a nerve agent used in chemical warfare.

*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.

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.

Toxicokinetics

Tabun is absorbed both through the skin and via respiration. Nerve agents inhaled as vapors or aerosols enter the systemic circulation resulting in toxic manifestations from seconds to 5 min following inhalation.

The enzyme organophosphate (OP) hydrolase hydrolyzes tabun, sarin, soman, and diisopropyl fluorophosphates at approximately the same rate.

Mechanism of Toxicity

Tabun and 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. 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 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 $\sim 1\%$ 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 reactivation 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.

Recently, investigations have focused on organophosphate nerve agent poisoning secondary to acetylcholine effects. These include the effects of nerve agents on y-amino butyric acid neurons and cyclic nucleotides. In addition, changes in brain neurotransmitters such as dopamine, serotonin, noradrenaline, 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

Following inhalation exposure, the median lethal dosage (LCt_{50}) in humans has been estimated to be 135 mg min m^{-3} at a respiratory minute volume (RMV) of 15 l min^{-1} for a duration of 0.5–2 min and 200 mg min m^{-3} at a resting RMV of 10 l min^{-1}. For percutaneous vapor, the LCt_{50} is estimated to be between 20 000 and 40 000 mg min m^{-3}, while for liquid tabun, the percutaneous human LD_{50} is estimated to be l–1.5 g per human. The permissible airborne exposure concentration of tabun for an 8 h workday or a 40 h workweek is an 8 h time-weighted average of 0.0001 mg m^{-3}. The number and severity of signs and symptoms following tabun exposure are dependent on the quantity, rate, and route of entry. Very small doses to the skin may cause local sweating and tremors with few other effects. Individuals intoxicated with tabun display approximately the same sequence of signs and symptoms regardless of the route of exposure. Signs and symptoms following vapor exposure include runny nose, tightness of chest, dimness of vision and miosis (pinpoint pupils), difficulty in breathing (dyspnea), drooling and excessive sweating, nausea, vomiting, cramps, involuntary defecation and urination, twitching, jerking, staggering, headache, confusion, drowsiness, coma, and convulsions. Death follows cessation of respiration. Death following inhalation and liquid in the eye occurs from 1 to 10 min following exposure. If skin absorption is sufficient to be lethal, death may occur within 1 or 2 min or be delayed for 1 or 2 h.

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 (i.v. or i.m.), which may be repeated two or three 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 (i.m.).

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. Pyridostigmine bromide is available as a pretreatment for nerve agent exposure. It is available in 30 mg tablets; tablets should be administered every 8 h. When used prior to exposure, it should be followed by atropine and pralidoxime chloride after exposure.

Supportive therapy may include ventilation via an endotracheal airway if possible and suctioning of excess secretions from the airways.

Table 1 Acute toxicities of tabun in various species by various routes of exposure

Route of exposure/species	*Toxicities*
Inhalation (10 min exposure)	LCt_{50} ($mg\,min\,m^{-3}$)
Guinea pig	3 930
Cat	2 500
Rat	3 040
Rabbit	8 400
Dog	4 000
Monkey	2 500
Mouse	450
Percutaneous	LD_{50} ($mg\,kg^{-1}$)
Rat	18
Rabbit	2.5
Dog	30
Monkey	9.3
Mouse	1.0
Guinea pig	35
Intravenous	LD_{50} ($\mu g\,kg^{-1}$)
Cat	47
Rat	66
Rabbit	63
Dog	85
Mouse	150
Intraperitoneal	LD_{50} ($\mu g\,kg^{-1}$)
Rat	490
Mouse	604
Subcutaneous	LD_{50} ($\mu g\,kg^{-1}$)
Dog	284
Rat	162
Rabbit	375
Mouse	250
Monkey	70
Guinea pig	120
Hamster	245
Intramuscular	LD_{50} ($\mu g\,kg^{-1}$)
Chicken	118
Monkey	34
Mouse	440
Rat	800
Oral	LD_{50} ($\mu g\,kg^{-1}$)
Rat	3 700
Dog	200
Rabbit	16 300

Animal Toxicity

Tabun is similar in action to sarin (GB); however, it is about have as toxic as sarin by inhalation and is more irritating to the eyes at low concentrations.

Small doses of nerve agents in animals can produce tolerance. They have also been demonstrated to produce neuropathies, myopathies, and delayed neurotoxicity in addition to their classical cholinergic effects. In rats, acute administration of nerve agents in subconvulsive doses produced tumors and hindlimb 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.

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 central nervous system 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.

Table 1 lists the LCt_{50} ($mg\,min\,m^{-3}$) values reported following the inhalation of tabun as well as acute toxicities by other routes of exposure in various animal species.

See also: G-Series Nerve Agents; Nerve Agents; Sarin; Soman; V-Series Nerve Agents: Other than VX; VX.

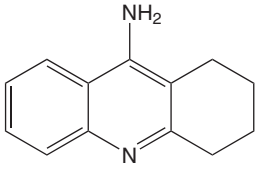
Relevant Websites

http://sis.nlm.nih.gov – Specialized Information Services, Division of the National Library of Medicine.
http://www.bt.cdc.gov – Centers for Disease Control and Prevention. Department of Health and Human Services, USA.

Tacrine

Ramesh C Gupta

- Chemical Name: 1,2,3,4-Tetrahydro-9-aminoacridine
- Chemical Abstracts Service Registry Number: CAS 321-64-2
- Synonym: Cognex
- Chemical and Physical Properties: Tacrine has an empirical formula of $C_{13}H_{14}N_2 \cdot HCl$ and a molecular weight of 234.73. Tacrine hydrochloride represents a white solid that is readily water soluble and soluble in organic solvents. This formulation has a melting point of 283–284°C and a bitter taste.
- Chemical Structure:



Uses

In the 1950s, tacrine was used experimentally to reverse cholinergic coma in animals. In the 1960s, tacrine was used to reverse the effects of phencyclidine-like drugs. It was also marketed for many years as a respiratory stimulant. In 1993, the Food and Drug Administration approved tacrine for the treatment of symptoms of mild to moderate Alzheimer's disease.

Exposure Routes and Pathways

Tacrine is therapeutically indicated by the oral route.

Pharmacokinetics

Tacrine is rapidly absorbed with a bioavailability of between 10% and 30%. Tacrine is about 55% bound to plasma proteins and has a clinical half-life of about 3–6 h following a single oral dose. In the body, tacrine can be metabolized to up to seven different products.

Mechanism of Toxicity

Tacrine has numerous mechanisms of action. The putative principle mechanism of action of tacrine for Alzheimer's disease is reversible inhibition of acetylcholinesterase (AChE), which thereby slows the breakdown of the chemical messenger acetylcholine (ACh) in the brain. In addition, tacrine blocks the sodium and potassium channels.

Acute and Short-Term Toxicity (or Exposure)

Animal

Tacrine causes elevation of serum enzymes indicative of liver cell damage with acute exposures. Studies also suggest that the neurotoxic actions of tacrine in the brain occur due to an increase in ACh levels, resulting in the overstimulation of muscarinic receptors.

Human

Tacrine can cause mild hepatotoxicity, which is self-resolving on discontinuation. Other side effects, including those related to cholinergic effects, are nausea, emesis, diarrhea, abdominal pain, dyspepsia, rhinitis, myalgia, tremors, and excessive urination. Overdose symptoms include seizures, muscle weakness, low blood pressure, severe nausea, vomiting, fast and weak pulse, irregular breathing, and slow heartbeat.

Chronic Toxicity (or Exposure)

Animal

Rats receiving tacrine at the doses of $10\,mg\,kg^{-1}$, i.p., twice daily for 4 days show signs of excess cholinergic stimulation. Tacrine at the doses of 7.5 or $10\,mg\,kg^{-1}$, i.p., two or three times daily for 4 days also induces myopathy in the diaphragm and leg muscles (soleus, gastrocnemius, and plantaris) of rats. As has been shown for other AChE inhibitors, tacrine-induced myopathy appears to result from increased ACh levels at the neuromuscular junction resulting in

the excessive stimulation of nicotinic ACh receptors on muscle cells. In addition, tacrine causes excessive production of free radicals in muscle cells, which can be attenuated by nitric oxide synthase inhibitors.

Human

Hepatotoxicity is the limiting side effect in tacrine therapy. About 50% of those patients given tacrine show elevated serum alanine aminotransferase (ALT) levels indicating some degree of hepatotoxicity. In almost all cases, these changes are noted within the first 12 weeks of treatment. Jaundice is a rare finding.

Clinical Management

In patients experiencing mild liver toxicity, it is often possible to continue at a lower dose or stop and then resume therapy at a lower dose. The addition of lecithin appears to reduce the severity of benign hepatic reaction. Other side effects are generally treated symptomatically.

See also: Anticholinergics; Cholinesterase Inhibition; Liver.

Further Reading

Jeyarassasingam G, Yeluashvili M, and Quilk M (2000) Tacrine, a reversible acetylcholinesterase inhibitor, induced myopathy. *Neuroreport* 11: 1173–1176.

Monteith DK, Theiss JC, Haskins JR, and de la Iglesia FA (1998) Functional and subcellular organelle changes in isolated rat and human hepatocytes induced by tetrahydroaminoacridine. *Archives of Toxicology* 72: 147–156.

Talc

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 14807-96-6
- Synonyms: Nytal 200; Nytal 400, TY 80, Mussolinite; Magnesium silicate hydroxide; Talcum; French chalk
- Chemical/Pharmaceutical/Other Class: Silicate
- Chemical Formula: $Mg_3Si_4O_{10}(OH)_2$

Uses

Talc is a primary ingredient in talcum powder, and is used in some antiperspirants and deodorants, and in cosmetics. It also can be used as a pigment in paints, primers, and enamels as well as a filler for paper, rubber, soap, and in household putty.

Exposure Routes and Pathways

Inhalation, dermal, perhaps some oral, and publications of uptake application to the genital area (uptake via the vagina). Potential occupational exposures include cosmetic workers, paint makers, paper makers, pottery makers, rubber cable coaters, rubber tire makers, talc millers, talc miners, and talc powder makers. Consumer exposures involve various talc-containing products.

Toxicokinetics

The toxic effects of talc are dependent on the route, dose, and properties of the talc involved. Talc commonly contains other minerals, including in some instances several forms of asbestos and silica. The lung deposition and the effects of subchronic exposure to talc were studied in rats and mice. Mathematical models simulating chronic talc exposure utilizing the data were used to predict long-term accumulations of talc in rodent and human lungs. Lungs from other animals were removed and examined for histopathological changes. No clinical signs of toxicity were seen. Talc accumulated in the lungs in a dose-dependent manner, and no exposure related lung lesions other than slight diffuse increases in the number of free macrophages containing talc particles within the alveolar spaces of rats and mice exposed to the highest doses were seen. Talc lung burdens of 2–3 $mg\,g^{-1}$ were predicted to occur in rats and mice exposed to 17 $mg\,m^{-3}$ talc for 2 years. In human lungs, equilibrium talc lung burdens of $\sim$2 $mg\,g^{-1}$ were predicted to occur after 4 years of exposure to 2 mg/m^{-3} talc, the occupational exposure threshold limit value (TLV). It was concluded that the predicted long-term talc lung burdens are significantly lower than those obtained experimentally, that this could reflect impaired pulmonary clearance, and that caution should be exercised when predicting lung burdens from short-term exposures to talc concentrations of 2 $mg\,m^{-3}$

or greater. Other studies have suggested that inert particles of talc can travel from the perineum (i.e., the general region between the anus and the genital organs) to the ovaries.

Acute and Short-Term Toxicity (or Exposure)

Human

Acute inhalation exposure can cause coughing, dyspnea, sneezing, vomiting, and cyanosis. Talc, which is water insoluble, dries up the mucous membranes of the tracheobronchial trees. This results in impairment of ciliary function. Inhaling large quantities of talc can result in obstruction of the small airways in addition to drying the mucous membranes, leading to respiratory distress syndrome, or death. Clinical studies of intravenous (IV) drug abusers have shown that IV injections of pills containing psychoactive agents and talc as a binder can result in microemboli forming in small pulmonary arteries, arterioles, and capillaries. This can result in granuloma formation, impaired pulmonary function, and death. IV injection of talc-containing formulations has been shown to predispose users to infections. Talc can induce severe granulomatous reactions when introduced into wounds or the operative field. See below for genotoxicity information.

Chronic Toxicity (or Exposure)

Animal

Long-term mouse and rat inhalation studies of talc found some evidence of carcinogenic activity of talc in male rats based on an increased incidence of benign or malignant pheochromocytomas of the adrenal gland. There was clear evidence of carcinogenic activity of talc in female rats based on increased incidences of alveolar/bronchiolar adenomas and carcinomas of the lung and benign or malignant pheochromocytomas of the adrenal gland. There was no evidence of carcinogenic activity of talc in male or female mice exposed to 6 or 18 mg m^{-3}. Hamsters, 4 weeks old, were exposed to an aerosol of talc baby powder for 3, 30, or 150 min day^{-1} for 5 days a week for 30 days. Two further groups of hamsters, 7 weeks old, were exposed to talc aerosol for 30 or 150 min day^{-1} for 300 days or until death. No primary neoplasm was found in the respiratory system of any hamster. The incidence of alveolar cell hyperplasia was 25% in the groups exposed to aerosol for 30 and 150 min day^{-1} for 300 days, compared with 10% in the control group. See below for genotoxicity and for other carcinogenicity information.

Human

Talc produces fibriotic pneumonitis. Four distinct forms of pulmonary disease caused by talc have been defined:

- Talcosilicosis is caused by talc mined with high silica content mineral. Findings in this form are identical with those of silicosis.
- Talcoasbestosis closely resembles asbestosis and is produced by crystalline talc, generally inhaled with asbestos fibers. Pathologic and radiographic abnormalities are virtually identical with those of asbestosis, including calcifications and malignant tumor formation.
- Talcosis, caused by inhalation of pure talc, may include acute or chronic bronchitis as well as interstitial inflammation; radiographically, it appears as interstitial reticulations or small, irregular nodules, typical of small airway obstruction.
- The fourth form, due to IV administration of talc, is usually associated with abuse of oral medications and production of vascular granulomas manifested by consolidations, large nodules, and masses.

Clinical and epidemiologic studies have suggested the existence of an association between ovarian carcinoma and talcum powder and deodorant sprays applied to the genital area. Talc particles have been detected in histologic sections of ovarian carcinomas; however, the results of epidemiologic investigations have varied, finding risks increased twofold to no significant risk detected. One recent review concluded that the concerns that cosmetic talc might be carcinogenic lack persuasive scientific support for the following reasons:

- These concerns are based on some epidemiological studies whose results were barely significant statistically and of questionable biological importance. (Their results lacked dose–response relationships, and were inconsistent and ambiguous. Further whether inanimate talc particles can translocate from the perineum to the ovaries, a precondition if they were to cause ovarian cancer, remains unresolved.)
- The results of the inhalation study in animals, which has raised concerns, "cannot be considered as relevant predictors of human risk" according to a panel of experts and other experts.
- The elevated incidence of lung cancer in pottery workers occurred several decades ago by exposure to air levels that now cannot be allowed to occur, and the exposures were to a multitude of industrial dusts.
- There is a lack of scientific support that pure cosmetic or pharmaceutical-grade talc poses a real risk

under consumer conditions. Talc is not genotoxic, is not carcinogenic when injected into ovaries of rats, does not cause cancer decades after pleurodesis, and induces apoptosis *in vitro* in human mesothelioma cells but not in normal mesothelial cells. There is no credible evidence of a cancer risk from inhalation of cosmetic talc by humans.

In Vitro Toxicity Data

The genotoxicity of talc has been determined using *in vitro* cell systems previously developed for testing asbestos fibers. The talc samples used consisted of particles of respirable size in order to test the effect of particles likely to be deposited in the lung. Genotoxicity was tested in cultures of rat pleural mesothelial cells using genotoxicity assays for unscheduled DNA synthesis and sister chromatid exchanges. The effects were compared with those obtained with negative controls (attapulgite and anatase) and positive controls (chrysotile and crocidolite asbestos). In contrast to asbestos, none of the talc samples, or the negative controls, induced enhancement of unscheduled DNA synthesis and sister chromatid exchanges in treated cultures in comparison with the untreated cultures.

Exposure Standards and Guidelines

Talc is listed as an A4 chemical (not classifiable as a human carcinogen) by the American Conference of Governmental Industrial Hygienists (ACGIH). Further, the ACGIH threshold limit value, 8 h time-weighted average (TWA) is $2\,mg\,m^{-3}$ (for talc containing no asbestos fibers; particulate matter containing no asbestos and $<1\%$ crystalline silica; respirable fraction). The (US) Occupational Safety and Health Administration permissible exposure limit, 8 h TWA is 20 million particles per cubic feet of air (mppcf) (for talc not containing asbestos, and containing less than 1% quartz).

See also: Asbestos; Cosmetics and Personal Care Products.

Further Reading

Carr CJ (1995) Talc: Consumer uses and health perspectives. *Regulatory Toxicology and Pharmacology* 21: 211–215.

Gertig DM (2000) Prospective study of talc use and ovarian cancer. *Journal of the National Cancer Institute* 92: 249–252.

Nasreen N (2000) Talc induces apoptosis in human malignant mesothelioma cells *in vitro*. *American Journal of Respiratory and Critical Care Medicine* 161: 595–600.

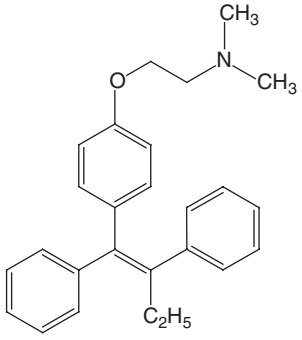
Oberdörster G (1995) The NTP talc inhalation study: A critical appraisal focused on lung particle overload. *Regulatory Toxicology and Pharmacology* 21: 233–241.

Wehner AP (2002) Cosmetic talc should not be listed as a carcinogen: Comments on NTP's deliberations to list talc as a carcinogen. *Regulatory Toxicology and Pharmacology* 36: 40–50.

Tamoxifen

Teresa Dodd-Butera and Molly Broderick

- Chemical Abstracts Service Registry Number: CAS 10540-29-1
- Synonyms: Nolvadex; Tamoxifen citrate
- Chemical/Pharmaceutical/Other Class: Nonsteroidal antiestrogen
- Chemical Structure:



Uses

Tamoxifen is appropriate for use as a palliative treatment in patients with an estrogen receptor-positive tumor. Additionally, it is used to prevent disease recurrence. Tamoxifen is the only agent approved by the United States Food and Drug Administration for breast cancer reduction and is generally administered for 5 years.

Exposure Routes and Pathways

The common mode of exposure to tamoxifen is through ingestion. It is available in oral dosage form (10 and 20 mg tablets) and may also be inhaled. Vapors may produce explosive dust clouds. Hazardous products include carbon monoxide, carbon dioxide, and nitrous oxide.

Toxicokinetics

Tamoxifen is absorbed orally, with peak concentrations in 4–7 h, biphasic decline in plasma concentration, and a terminal half-life of 7 days. The predominant metabolite is *N*-desmethyltamoxifen, which has a half-life of 14 days. However, a minor metabolite, 4-hydroxytamoxifen, is also generated. Both of these are further metabolized to 4-hydroxy-*N*-desmethyltamoxifen. *In vitro* studies showed that erythromycin, cyclosporin, nifedipine, and kiltiazem competitively inhibited formation of the latter metabolite. Steady-state levels are achieved after approximately 4 weeks of treatment. Tamoxifen is enterohepatically recirculated and excreted primarily in the stool.

Mechanism of Toxicity

Tamoxifen competitively blocks estradiol binding to the estrogen receptor.

Acute and Short-Term Toxicity (or Exposure)

Animal

Tamoxifen produced impairment of fertility and conception in female rats. No genotoxic potential was found in conventional *in vivo* and *in vitro* tests.

Human

Adverse reactions include nausea, vomiting, and hot flashes. Vaginal bleeding, menstrual irregularities, and skin rash occur with less frequency. Hypercalcemia, edema, anorexia, depression, and thromboembolic events are uncommon but have been reported.

Chronic Toxicity (or Exposure)

Animal

Studies in rats found a significant increase in hepatocellular cancer at doses higher than that administered to humans.

Human

Tamoxifen increases the risk of two types of cancer that can develop in the uterus: endometrial cancer, which arises in the lining of the uterus; and uterine sarcoma, which arises in the muscular wall of the uterus. Women taking tamoxifen had three times the chance of developing a pulmonary embolism, deep vein thrombosis, and increased chance of stroke. Women taking tamoxifen appear to be at increased risk for developing cataracts. Other eye problems, such as corneal scarring or retinal changes, have been reported. Tamoxifen may cause fetal harm when administered to a pregnant woman. It is unknown whether or not this drug is excreted in human milk when women taking tamoxifen are breastfeeding.

Clinical Management

Acute overdosage in humans has not been reported. If very high doses are administered, which manifest acute neurotoxicity and prolonged QT interval on an electrocardiogram, symptomatic treatment and cessation of the drug is required.

See also: Estrogens I: Estrogens and Their Conjugates.

Further Reading

Boelsterli UA (2003) *Mechanistic Toxicology*. London and New York: Taylor and Francis.

Goldfrank L, Flomenbaum N, Lewin N, *et al*. (eds.) (2002) *Goldfrank's Toxicologic Emergencies*, 7th edn. New York: McGraw-Hill Medical Publishing.

Klaassen C (ed.) (2001) *Casarett & Doull's Toxicology: The Basic Science of Poisons*, 6th edn. New York: McGraw-Hill.

White IN (2003) Tamoxifen: Is it safe? Comparison of activation and detoxication mechanisms in rodents and in humans. *Current Drug Metabolism* 4(3): 223–239.

Relevant Website

http://ntp-server.niehs.nih.gov – National Toxicology Program, Department of Health and Human Services, Tenth Report on Carcinogens: Tamoxifen.

Tannic Acid

Heriberto Robles

- Chemical Abstracts Service Registry Number: CAS 1401-55-4
- Synonyms: Digallic acid; Chinese tannin; Gallotannic acid; Galloylglucose; Glycerite; Digalloyl glucose; Tannin; Tannins
- Chemical/Pharmaceutical/Other Class: Plant polyphenol

Uses

Tannic acid is used as a fixative of dyes and as a chemical intermediate and reagent in the manufacture of inks, rubber, and imitation horns and tortoise shells. Tannic acid is also used to clarify beer and wine; in photography; as a chemical reagent in analytical laboratories; and in pharmaceutical preparations.

Background Information

Tannic acid is a naturally occurring plant polyphenol and can be found in practically all aerial plant tissues.

Exposure Routes and Pathways

The most significant route of exposure for tannic acid is via ingestion. However, inhalation and dermal exposure may also occur in industrial settings.

Toxicokinetics

Tannic acid is not consistently absorbed from intestinal mucosa or from the skin. Enhanced absorption rates can be seen in denuded skin and mucus membranes. Tannic acid can cause hardening of the gastrointestinal mucosa. This hardening can result in reduced gastrointestinal absorption of nutrients as well as of xenobiotics. Tannic acid has been experimentally shown to be able to reduce the carcinogenic potency of some amine derivatives and polycyclic aromatic hydrocarbons in laboratory animals. Tannic acid's anticarcinogenic properties appear to be mediated through the modulation of enzymes involved in xenobiotic metabolism.

Mechanism of Toxicity

Tannic acid causes centralobular liver necrosis following absorption from gastrointestinal tract, mucus membranes, or from denuded skin surfaces. Liver metabolism of tannic acid requires methyl-group donors. Therefore, methyl-group donors can be depleted following excessive tannic acid absorption.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals dosed with tannic acid by oral administration presented hemorrhagic gastritis, colic, jaundice, hemolytic anemia, necrosis of gastric mucosa, nephritis, and liver alterations. The LD_{50} in rats and mice has been reported to be 5 and $6\,g\,kg^{-1}$, respectively.

Tannic acid proved to be lethal to bovines after daily dosage of 50 g for 16 days. However, a daily dose of 25 g for 28 days did not produce observable adverse effects.

Human

Tannic acid is moderately toxic by the inhalation and ingestion exposure pathways. Acute, high-dose ingestion and absorption may cause nausea, vomiting, constipation, abdominal pain, and liver damage. Severe intoxications may result in centralobular liver necrosis.

Chronic Toxicity (or Exposure)

Animal

Foraging animals consuming oak tree leaves may consume potentially toxic doses of tannic acid. Excessive chronic consumption has been shown to decrease iron and thiamin absorption as well as decreased growth rate in juvenile animals.

In a chronic toxicity study, male and female rats were injected subcutaneously with an aqueous solution of tannic acid every fifth day for 290 days. Some dosed animals presented hepatomas and/or cholangiomas at the end of the study (after 388 days). Although tumor incidence in the control group was rare, no clear dose–response was evident in the tannic acid treated animals. In another study, no liver damage was observed in seven male rats fed tannic acid at a dose of $60\,mg\,kg^{-1}$ body weight per day during 152 days.

Human

An unusually high incidence of esophageal cancer has been noted in areas of South Africa where a sorghum rich in tannins is consumed. A positive relation has been observed between the tannin content of the sorghum and the incidence of esophageal cancer.

Clinical Management

Basic life-support measures should be implemented. Further absorption can be prevented by removing contaminated clothing and washing the affected area. If ingested, activated charcoal may be given to reduce absorption. A careful examination should be performed and gastric lavage instituted only if esophagus is not damaged and it is believed that lavage may be effective at removing the ingested material. If inhaled, respiratory distress should be monitored and oxygen administered or assisted ventilation given as needed.

There is no specific treatment for tannic acid toxicity. Supportive and symptomatic treatment is recommended. Liver function should be monitored in patients with gastrointestinal symptoms.

Environmental Fate

Tannins and tannic acid occur naturally in plants. Essentially all wood and plant tissue contain tannins. Therefore, biodegradation is expected to be the major environmental fate process for tannic acid.

Ecotoxicology

Tannic acid given in the diet of chicks at a concentration of 0.5% caused growth rate reductions. Tannic acid doses as high as 5.0% resulted in 70% mortality in dosed chicks.

Tannic acid given orally to rabbits produced hemorrhagic gastritis. Horses given doses ranging from 50 to 300 g by stomach tube presented colic and jaundice with hemolytic anemia. Upon autopsy, some horses presented necrosis of gastric mucosa, degeneration of heart muscle, nephritis, and liver changes.

See also: Gastrointestinal System; Plants, Poisonous.

Further Reading

Chen SC and Chung KT (2000) Mutagenicity and antimutagenicity studies of tannic acid and its related compounds. *Food and Chemical Toxicology* 38(1): 1–5.

Krajka-Kuzniak V and Baer-Dubowska W (2003) The effects of tannic acid on cytochrome P450 and phase II enzymes in mouse liver and kidney. *Toxicology Letters* 143(2): 209–216.

Rossoff IS (2002) *Encyclopedia of Clinical Toxicology.* Boca Raton, FL: The Parthenon Publishing Group.

Relevant Website

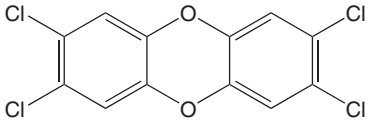
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Tannic Acid.

Taste *See* Sensory Organs.

TCDD (2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin)

Robert Kapp

- Chemical Abstracts Service Registry Number: CAS 1746-01-6
- EINECS No: 217-122-7
- Synonyms: 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin; 2,3,7,8-TCDD; 2,3,7,8-Tetra polychlorinated dibenzo-*p*-dioxin; 2,3,7,8-Tetrachlorodibenzo(*b*,*e*)(1,4)dioxin; 2,3,7,8-Tetrachlorodibenzo-1,4-dioxin; 2,3,7,8-Tetrachlorodibenzodioxin; Dibenzo(*b*,*e*)(1,4)dioxin, 2,3,7,8-tetrachloro-; Dibenzo-*p*-dioxin, 2,3,7,8-tetrachloro-; Dioxin; Dioxin (herbicide contaminant); Dioxine; TCDBD; Tetrachlorodibenzo-*p*-dioxin; Tetrachlorodibenzodioxin; Tetradioxin
- Related Compounds: Dioxins is a general term that is used to describe a group of hundreds of chemicals that are found in the environment and are derived from polychlorinated dibenzodioxins. All dioxins contain two benzene rings joined by two oxygen atoms. The polychlorinated dibenzofurans are a closely related family of compounds. There are ~75 known polychlorinated dibenzo-*p*-dioxins and ~135 dibenzofurans. Since the environment has many of the different dioxins at extremely low levels, chemical analysis is difficult.
- Chemical/Pharmaceutical/Other Class: Chlorinated dioxins
- Chemical Structure:



Uses

TCDD has no commercial uses but is found as a contaminant. TCDD is present in certain herbicide and fungicide formulations such as 1,4,5-T and in pentachlorophenols. As noted above, it is an unwanted contaminant created by incineration and was a contaminant in Agent Orange.

Background Information

TCDD is a colorless to white crystalline solid. The molecular formula is $C_{12}H_4Cl_4O_2$. The molecular weight is 322. Dioxin began accumulating in the environment about 1900 when Dow Chemical discovered a way to split NaCl into sodium and

chlorine atoms. Subsequently, Dow and other chemical manufacturers began attaching chlorine atoms to various petroleum hydrocarbons, which has produced a vast array of pesticides, solvents, and plastics. When these materials are manufactured or are burned in an incinerator, they release dioxin into the environment. Other sources of dioxins include sawmills, wire and scrap metal reclamation incinerators, cement kilns, cofiring wastes, transformer fires, wood stoves, fireplaces, and agricultural burning. When released into the air, many dioxins may be transported long distances. When dioxins are released into wastewater, some are broken down; however, most attach to soil and settle to the bottom sediment in a relatively stable form. During the Vietnam War, a broadleaf defoliant called Agent Orange was used by the US Troops to destroy enemy food crops and areas of cover in the jungle. Agent Orange was a 50–50 mix of two chemicals, known conventionally as 2,4,D ((2,4-dichlorophenoxy)acetic acid) and 2,4,5,T ((2,4,5-trichlorophenoxy)acetic acid). The combined product was mixed with kerosene or diesel fuel and dispersed by aircraft, vehicle, and hand spraying. An estimated 19 million gallons of Agent Orange were used in South Vietnam during the war. Agent Orange used in Vietnam was later found to be contaminated with TCDD. Hence, the personnel who were dispersing Agent Orange were possibly exposed to high levels of TCDD. While many believe that man is the sole source of dioxins, dioxins have been found worldwide in very remote areas leading others to believe that some of the primary sources are not yet known.

TCDD has an important history in the field of toxicology. In the 1970s, dioxin-contaminated oil was spread along roadways in the community of Times Beach, MO, in the United States to control dust. In December of 1982, the Meremac River flooded Times Beach and contaminated the entire town with dioxin. The US government bought the entire area and initiated cleanup as a Superfund site. A total of 265 000 tons of contaminated dirt was incinerated. Today, Times Beach, MO, no longer exists. Other incidents including potential health effects on veterans exposed to dioxin in Agent Orange have highlighted the history of this most toxic synthetic chemical known to man. An extensive number of medical and scientific studies on Agent Orange and dioxin have been conducted. The consensus of those studies suggests that even in military personnel exposed to the pesticide, there are no defined, consistent adverse health effects, even though many Vietnam veterans are at risk for a variety of health problems due to their military experience in Vietnam in general.

Exposure Routes and Pathways

The primary pathways for TCDD exposure appear to be inhalation and ingestion. Eating meat, fish, and dairy products makes up more than 90% of the intake of dioxins. Close proximity to an uncontrolled hazardous waste site or working in industries involved in producing pesticides containing dioxins can be sources of inhalation exposure for the general public and workers alike. Skin exposure can occur through contact with contaminated soils.

Toxicokinetics

Dioxins are absorbed by inhalation, oral, and dermal routes of exposure. Absorption is less efficient by the dermal than by the inhalation and oral routes. Absorption is vehicle-dependent and congener-specific. Hepta- and octachlorinated congeners exhibit decreased absorption. Dioxins can be carried in the blood by serum lipids and lipoproteins. Liver and fat are the major storage sites for dioxins. Tissue deposition is congener-specific, dose-dependent, and influenced by factors including route of exposure and age. Dioxins are slowly metabolized by microsomal enzymes to polar metabolites that undergo conjugation with glucuronic acid and glutathione. The major route of excretion is feces with smaller amounts being excreted in the urine. In mammals, lactation is an effective route of elimination. Dioxins can induce xenobiotic metabolizing enzymes. The induction of these enzymes (such as the CYP1A1 in the mouse) increases the metabolic processing of lipophilic chemicals to water-soluble derivatives, facilitating their elimination in the urine. TCDD is a poor substrate for detoxification enzymes and it tends to persist in the body for long periods of time.

Mechanism of Toxicity

Much of the activity initiated by the presence of small amounts of dioxin occurs at the Ah receptor, which is important to the body's ability to detoxify foreign substances. The dioxin molecule binds to the Ah receptor forming the 'receptor–dioxin complex'. TCDD's toxic actions depend on the formation of this complex. Once the complex is formed it moves to the cellular DNA (with the aid of a translocating protein, ARNT) where it activates genes to a number of biotransformation-related enzymes or other genes involved in growth and division of cells. Examples of enzymes induced by activation of the Ah receptor include CYP1A1, CYP1A2, glutathione transferase, NADPH quinine oxidoreductase, UDP-glucuronosyltransferase abd aldehyde dehydrogenase. If the

dioxin remains bound to the receptor, the receptor remains on the DNA so that enzymes are produced continuously. Once dioxin enters the body, a small amount is metabolized and usually eliminated. The majority of the dioxin, however, bioaccumulates in the body fat. As the fat is metabolized, stored dioxin is slowly released and excreted primarily in feces. The approximate half-life of dioxin in humans is estimated to range from 6 to 10 years.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals given a single oral dose of 0.1–25 $\mu g\,kg^{-1}$ have shown hyperkeratosis, facial alopecia, inflammation of the eyelids, increased liver weight, lipid accumulation, thymic atrophy, and histopathologic changes in the liver and thymus. There is a wide species difference in acute toxicity of TCDD, with oral LD_{50} ranging from 0.6 to 5000 μg TCDD kg^{-1}. Additional acute effects include the stimulation of aminolevulinic acid synthetase, which is a rate-limiting enzyme in porphyrin and heme synthesis.

Human

Acute exposure of humans to excessive amounts of dioxins has caused chloracne, liver toxicity, skin rashes, nausea, vomiting, and muscular pains.

Chronic Toxicity (or Exposure)

Animal

Animals exposed to chronic doses of dioxin have shown altered immune systems, thymic atrophy, with changes also noted in the spleen, lymph nodes, and bone marrow. Rodent reproductive studies have noted signs of developmental toxicity, however, including cleft palates and kidney abnormalities at 0.125–3.0 μg TCDD g^{-1} body weight. Impairment of reproduction including decreased fertility, litter size, number of pups alive at birth, postnatal survival, and postnatal body weight of pups was noted at doses of 0.01 μg TCDD $kg^{-1}\,day^{-1}$. TCDD also caused immunological deficits expressed by decreased thymus to body weight ratios in nursing newborn rats exposed through mother's milk. Male rats fed dosages of 0.001 μg TCDD per kilogram of body weight per week for 78 weeks showed ear duct carcinoma, lymphocytic leukemia, kidney adenocarcinoma, malignant peritoneal histiocytoma, skin angiosarcoma, and carcinomas of the tongue and nasal turbinates. Female rats fed dosages of 0.1 μg TCDD $kg^{-1}\,day^{-1}$ developed carcinomas of the liver as well as squamous cell carcinomas of the lung, tongue, and nasal turbinates. Male and female rats fed dosages of 0.5 μg TCDD $kg^{-1}\,week^{-1}$ for 2 years developed neoplastic nodules in the liver and thyroid adenomas.

Human

Chronic exposure to dioxins has resulted in splenic and testicular atrophy, elevated gamma-glutamyl transpeptidase levels, elevated cholesterol levels, and abnormal neurologic findings. Additional effects include enzyme induction, diabetes, and endocrine changes. The chronic, noncancer reference exposure level of $3.5 \times 10^{-6}\,\mu g\,m^{-3}$ is listed for TCDD or 2,3,7,8-equivalents by the California Air Pollution Control Officers Association Air Toxics 'Hot Spots' Program.

The human reproductive data available on TCDD are inconclusive. A study based on the accidental exposure of the population of Seveso, Italy, found no mutagenic, teratogenic, or fetotoxic effects in 30 elective abortions. Carcinogenicity studies examining humans exposed to TCDD have been inconclusive because of the small sample sizes and the concomitant exposures to other substances. Notwithstanding, TCDD is listed with the International Agency for Research on Cancer as Group 1 – carcinogen to humans. It is also listed by the Environmental Protective Agency (evidence that dioxin may have the potential to cause cancer from a lifetime exposure at levels above the maximum contaminant level); the National Toxicology Program (K – known to be a human carcinogen); the National Institute for Occupational Safety and Health (Ca – potential carcinogen with no further categorization); and the German MAK Commission (4 – substances with carcinogenic potential for which genotoxicity plays no role).

Clinical Management

Upon ocular exposure to TCDD, the eye should be immediately washed well with plenty of tap water. Skin exposed to TCDD should be immediately washed with soap and water. Get immediate medical attention in the event of ingestion or inhalation of TCDD.

Environmental Fate

Dioxins are ubiquitous environmental contaminants in air, water, and soil. Lower levels are found in less industrial regions compared to areas with heavy industry. Heptachloro- and octachloro-isomers are most common. Environmental fate of the dioxins involves volatilization, atmospheric distribution, wet

and dry deposition, photolysis, bioaccumulation, and biodegradation. Dioxins strongly adsorb to soils and sediments and are generally immobile. Photolysis of many dioxins is relatively robust. The half-life of TCDD was estimated to be 9–15 years on the surface, but 25–100 years below the surface. Dioxins can bioaccumulate in aquatic and terrestrial biota. Dioxin concentrations in urban air are around $2\,\mathrm{pg\,m^{-3}}$, with octachloro- and heptachlorodibenzodioxins being predominant. Urban-air concentration of 2,3,7,8-TCDD in the United States was estimated at <0.04–$0.18\,\mathrm{pg\,m^{-3}}$ but it is typically not detectable in air samples from rural communities. TCDD concentration can be much higher around contaminated sites. Because of tight adsorption to sediment, conventional water treatment appears to be effective in removing dioxins. TCDD has not been detected in drinking water. Concentrations of 2,3,7,8-TCDD in most soils are <12 ppt but considerably higher levels can be found in contaminated soils. 2,3,7,8-TCDD and other dioxins have also been detected in sediments of industrialized water bodies throughout the United States. The most frequently detected dioxin in fish tissues is 1,2,3,4,6,7,8,-heptachlorodibenzodioxin, which was found in fish tissues at 89% of the sites. Fish collected near pulp and paper mill operations using chlorine had the highest levels of 2,3,7,8-TCDD.

Ecotoxicology

Relatively little is known about the effects of dioxins in invertebrates. Controlled laboratory studies with dioxin-contaminated sediments reported no effects on amphipod mortality. Some studies have shown reduced reproductive success in worms and snails. Some invertebrates have been shown to express Ah receptors, but these receptors do not appear to bind dioxins, thus invertebrates are less sensitive to dioxin toxicity.

Fish exposed to dioxins exhibit alterations in development, reduced feeding, lethargy, and 'head-up' swimming. In general, dioxins are more toxic in the early-life stages. Toxicity in fish is higher for congeners containing 4–6 chlorines. Concentration of dioxins in fish eggs has been demonstrated. Deformities in chicks of cormorants, terns, and other fish-eating species from the Great Lakes area in the United States were correlated with dioxin contamination, a correlation between TCDD-toxic equivalents and reduced egg hatching, embryotoxicity, structural deformities, and altered parental behavior. These effects in birds may, however, have been due to polychlorinated biphenyls (PCBs) and not dioxins.

Controlled studies have shown some birds to be susceptible to dioxins, showing reduced egg production, embryotoxicity, and cardiovascular and brain malformations. Mink eating contaminated fish show listlessness, anorexia, reduced red blood cell counts, and enlarged spleens, livers, and lungs. Again, the contamination by PCBs often makes the discrimination between dioxins and PCBs difficult.

Exposure Standards and Guidelines

There is no reference dose for dioxins. The acceptable daily intake is 1–$10\,\mathrm{pg\,kg^{-1}\,day^{-1}}$. A threshold limit value for TCDD has not been established.

See also: Dioxins; Pesticides; Polychlorinated Biphenyls (PCBs).

Further Reading

Cole P, Trichopoulos D, Pastides H, Starr T, and Mandel JS (2003) Dioxin and cancer: A critical review. *Regulatory Toxicology and Pharmacology: RTP* 38: 378–388.

Young AL (2004) TCDD biomonitoring and exposure to Agent Orange: Still the gold standard. *Environmental Science and Pollution Research International* 11: 143–146.

Relevant Websites

http://europa.eu.int – The European Union. Many documents available on Dioxin Exposure and Health.

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

Teflon *See* Perfluorooctanoic Acid (PFOA).

Tellurium

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 13494-80-9
- Synonyms: Aurum paradoxium; Mettalum problematum
- Chemical/Pharmaceutical/Other Class: A rare metal with many properties similar to selenium and sulfur
- Chemical Formulas: Te^{4+}; Te^{6+}; Te^{2-}

Uses

The largest use for tellurium is as an additive to free-machining steel. Tellurium is used as a catalyst, in semiconductors and in 'daylight' vapor lamps. It is also used in the manufacturing of rubber and certain metal alloys and as a coloring agent in chinaware, porcelains, enamels, glass, and for producing black finish on silverware.

Background Information

Tellurium is one of the rarest elements on earth, and was discovered in 1782.

Exposure Routes and Pathways

Tellurium is ingested with foods such as nuts, fish, and certain dairy products. Many fatty foods contain tellurium, and some plants, like garlic, accumulate tellurium from the soil. Neither drinking water nor ambient air contains significant amounts of tellurium. Skin contact is not a significant exposure pathway.

In industrial settings, inhalation may be a significant exposure pathway. Airborne concentrations of tellurium are higher than the vicinity of metallurgical industries. Like selenium, tellurium is obtained as a by-product of copper, lead, and zinc refining. It is produced mainly from the tailings of bismuth.

Toxicokinetics

Tellurium is poorly absorbed from the gastrointestinal tract. Tellurous acid (soluble tellurium) can be absorbed through the skin, although ingestion or inhalation of fumes presents the greatest industrial hazard. A metallic taste in the mouth may result from excessive absorption. Tellurium concentrates in a variety of organs, primarily in the bones and kidneys, followed by the liver and the adipose tissue. Tellurium is metabolized in the body by the reduction to tellurides, and can then be biotransformed to dimethyltelluride (a reaction similar to the biotransformation of selenium), which is volatile and can be exhaled. Most tellurium is excreted in urine and bile. The characteristic sign of absorption is the garlic-like odor in the breath and sweat from dimethyltelluride.

Mechanism of Toxicity

Tellurium has a low toxicity in its elemental form, but dimethyltelluride is formed in the body. Tellurium caused highly synchronous primarily demyelination of peripheral nerves, related to the inhibition of squalene epoxidase, which blocks cholesterol synthesis. The sequence of metabolic events in sciatic nerve following tellurium treatment initially involves inhibition of the conversion of squalene to 2,3-epoxysqualene, and that this block in the cholesterol biosynthesis pathway results, either directly or indirectly, in the inhibition of the synthesis of myelin components and breakdown of myelin. The efficacy of garlic as a lipid-lowering agent has been recognized, but the biochemical mechanisms underlying this action are currently unknown. It is possible that organic tellurium compounds, which are found in high concentration in fresh garlic buds, may contribute to this action by inhibiting squalene epoxidase, the penultimate enzyme in the synthetic pathway of cholesterol. Weanling rats fed a diet rich in tellurium develop a demyelinating polyneuropathy due to inhibition of this enzyme in peripheral nerves. Chronic exposure to small amounts of tellurium found in garlic might reduce endogenous cholesterol production through inhibition of hepatic squalene epoxidase and so reduce cholesterol levels. Tellurium may also contribute to the characteristic odor of garlic.

Acute and Short-Term Toxicity (or Exposure)

Animal

The mouse, guinea pig, rabbit, and rat oral LD_{50} values ranged from 20 to 83 mg kg^{-1}. The temporal relationships of blood nerve barrier breakdown, to metabolic and morphological changes in tellurium neuropathy were investigated in rats fed diets containing 0% or 1.3% tellurium. Animals were observed for clinical signs of toxicity and were killed 12,

24, 48, 72, or 96 h after starting the diet. Tellurium-treated rats developed a garlic odor within 48 h and usually developed hind-limb paresis within 72 h. Progressive increases in blood–nerve barrier permeability occurred between 24 and 72 h in rats given tellurium; however, the blood–brain barrier was not affected by tellurium. Tellurium induced increased numbers of intracytoplasmic lipid droplets, intracytoplasmic membrane delimited clear vacuoles, and cytoplasmic excrescences within myelinating Schwann cells after 24 h, axon demyelination after 48 h, and endoneurial edema after 72 h. Cholesterol synthesis was sharply inhibited after 12 h, and squalene began accumulating in sciatic nerve segments at that time. It was concluded that the initial Schwann cell injury seen in tellurium neuropathy may be due to factors other than blood–nerve barrier breakdown and vasogenic endoneurial edema. Breakdown of the blood–nerve barrier could have a synergistic effect on tellurium induced Schwann cell injury.

Human

The toxicity of tellurium is dependent on the oxidation state. The tellurites $(TeO_3)^{2-}$, are the most toxic compared to tellurates $(TeO_4)^{2-}$, or elemental tellurium. Only a few cases of nonoccupational poisoning to tellurium have been reported so far, and toxic effects are rare. Severe poisoning results in respiratory depression and circulatory collapse. After occupational exposure, the main symptoms and signs include loss of appetite, dryness of the mouth, suppression of sweating, a metallic taste in the mouth, and the garlic odor of the breath, sweat, and urine. Acute toxicity from inhalation results in the relatively nonspecific symptoms of nausea, sweating, and loss of sleep in some and drowsiness in others. Kidney damage and fatty degeneration of the liver have been noted in severe cases. In two fatal cases, cyanosis and garlic breath were prominent before coma and death, and fatty degeneration and edema were noted in both cases.

Chronic Toxicity (or Exposure)

Animal

Acute oral parenteral tellurium intoxication in animals results in restlessness, tremor, diminished reflexes, paralysis, convulsions, somnolence, coma, and death. Hematuria was prompt and occurred in all animals. Exposure of weanling rats to a diet containing elemental tellurium results in a peripheral neuropathy characterized by segmental demyelination and minimal axonal degeneration. It is noteworthy that functional recovery occurred despite continual administration of tellurium. One of the earliest ultrastructural abnormalities in tellurium neuropathy is an increased number of cytoplasmic lipid droplets in myelinating Schwann cells. The earliest biochemical abnormality observed in tellurium neuropathy is an inhibition of cholesterol synthesis at the squalene epoxidase step. This leads to an accumulation of squalene within the nerve.

After exposure to 3300 ppm tellurium in the diet for 5 months, rats were markedly impaired in their ability to learn a sequence of behavioral tasks. The administration of 500–3000 ppm tellurium through the diet to pregnant rats resulted in a high incidence of hydrocephalic offspring. Neonatal rats exposed to tellurium via the mother's milk from the day of birth until killing at 7, 14, 21, or 28 days of age developed Schwann cell and myelin degeneration in the sciatic nerves at each age studied. In the central nervous system (CNS), hypomyelination of the optic nerves was demonstrated at 14, 21, and 28 days of age.

The tissue response to tellurium and tellurium dioxide particulates retained in rat lungs following endotracheal introduction was studied. Rats were administered single endotracheal injections of tellurium, tellurium dioxide, or sodium chloride, and were sacrificed 180 days later. Micropathological investigations revealed no difference in the lung tissue of the control and treated rats, except for those resulting from expected defensive mechanisms against any foreign material in the lungs. Black deposits were observed in the lung tissue of treated rats, indicating an inability of the clearance mechanisms of the lungs to remove all of the injected particulates within the 180 day study period. No evidence of a fibrotic tissue response was observed. Many internal organs of the rats treated with tellurium and tellurium dioxide had a bluish tint, however, this discoloration was not accompanied by specific lesions. The 180 day study period was an insufficient time period to draw any conclusions regarding the absence of tumorigenic potential of these compounds.

The peroxidation related effects of tellurium on the brain were studied in rats. Rats were given drinking water containing tellurium tetrachloride at a concentration of $100\,mg\,l^{-1}$ and were killed after 7, 21, or 35 days of exposure. Blood, liver, kidney, and brain samples were analyzed for tellurium. Exposed rats accumulated relatively high concentrations of tellurium. Blood had the highest tellurium concentrations, with an increasing trend according to the exposure period. Liver also showed a rapid increase, while the kidneys and brain had a continuous accumulation. Appreciable neurochemical effects were seen after the brain content exceeded $2\,nmol\,g^{-1}$. Succinic dehydrogenase activity was

above the control range after 21 days, while creatine-kinase activity decreased or remained stable. Brain glutathione content was above the control range at 35 days, possibly as a result of attempts to counteract peroxidative effects associated with mitochondrial damage. The initially low uptake of tellurium in the brain may have been due to a blood–brain barrier. Once incorporated into the nervous system, accumulation apparently occurred because of the long half-life of tellurium.

The developmental toxicity of tellurium was evaluated in rats and rabbits by means of standard segment II-type studies. Groups of pregnant rats were fed a diet containing 0, 30, 300, 3000, or 15 000 ppm of tellurium on days 6 through 15 of gestation (microscopic detection of sperm in a smear of vaginal contents considered as day 0), and artificially inseminated rabbits were fed a diet containing 0, 17.5, 175, 1750, and 5250 ppm of tellurium during days 6 through 18 of gestation (day of insemination considered as day 0). Signs of maternal toxicity were observed during the treatment period in a statistically significant and dose-related manner at dietary concentrations of 300 ppm and greater in rats and 1750 ppm and greater in rabbits. Exposure of these pregnant rats and rabbits to tellurium had no effect upon reproduction as measured by pregnancy rate, litter size, dead or resorbed implantations, or fetal sex ratio. Both skeletal (primarily skeletal maturational delays) and soft tissue malformations (primarily hydrocephalus) were noted in the offspring of pregnant rats exposed to the highest levels (3000 and 15 000 ppm) of tellurium. Rabbit fetuses of the highest dosage group (5250 ppm) had a slightly elevated evidence of skeletal delays and nonspecific abnormalities. Since maternal toxicity was observed at dosages that did not affect the developing conceptus, there were no indications of unique developmental susceptibility upon exposure of pregnant rats or rabbits to tellurium.

Human

Chronic exposure may lead to garlic breath, metallic taste, decreased sweating, dry mouth, fatigue, lassitude, anorexia, and nausea.

Clinical Management

Vitamin C (ascorbic acid) reduces the characteristic garlic breath; however, it may also adversely affect the kidneys when an excess amount of tellurium is present. BAL (British antilewisite; 2,3-dimercaptopropanol) is contraindicated since it enhances the toxicity of tellurium. There are no available treatments for poisoning.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value, 8 h time-weighted average (TWA), for tellurium and its compounds is $0.1\,mg\,m^{-3}$. The (US) Occupational Safety and Health Administration (OSHA) permissible exposure limit, 8 h TWA, is $0.1\,mg\,m^{-3}$ for tellurium and compounds (as tellurium). The (US) National Institute for Occupational Safety and Health (NIOSH) recommended exposure level, averaged over a 10 h work day, is $0.1\,mg\,m^{-3}$ for tellurium compounds (as tellurium) except tellurium hexafluoride and bismuth telluride, and the NIOSH immediately dangerous to life or health (IDLH) value is $25\,mg\,m^{-3}$ (as tellurium).

See also: Metals; Selenium.

Further Reading

Dart RC (2004) *Medical Toxicology*, 3rd edn. Baltimore, MD: Williams and Wilkens.

Goyer RA, Klaassen CD, and Waalkes MP (1995) *Metal Toxicology*. San Diego, CA: Academic Press.

Taylor AC (1996) Biochemistry of tellurium. *Biological Trace Element Research* 55: 231–239.

Relevant Website

http://www.intox.org – The International Programme on Chemical Safety (IPCS).

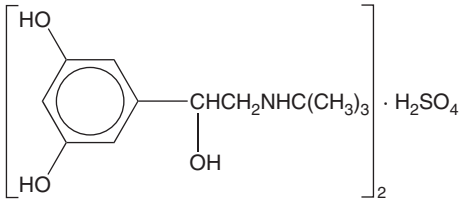
Teratology *See* Developmental Toxicology.

Teratology Testing *See* Toxicity Testing, Developmental.

Terbutaline

Henry A Spiller

- Chemical Abstracts Service Registry Number: CAS 23031-25-6
- Synonym: Brethine
- Chemical/Pharmaceutical/Other Class: A selective β_1 agonist
- Chemical Formula: $C_{12}H_{19}NO_3$
- Chemical Structure:



Uses

Terbutaline is used as a bronchodilator and for the prevention of premature labor. Unlabeled use includes treatment of hyperkalemia.

Exposure Routes and Pathways

Ingestion is the most common route of accidental and intentional exposure to terbutaline. Inappropriate overuse of the inhalation aerosol may also occur. Terbutaline is available as an inhalation aerosol, as tablets (2.5 and 5 mg), and as a solution for subcutaneous injection (1 mg ml^{-1}).

Toxicokinetics

Taken orally, terbutaline is poorly and incompletely absorbed from the gastrointestinal tract, with ~47% of the unchanged drug found in the feces. Administered subcutaneously, it is well absorbed with peak serum levels in 20 min. The bioavailability and biotransformation of terbutaline depend greatly on route of administration. With oral dosing there is significant first-pass biotransformation by sulfate and glucuronide conjugation in the liver and gut wall, with only 15% of absorbed terbutaline available as unchanged drug. With inhalation and parenteral dosing, the majority of the drug is available as unchanged terbutaline. The volume of distribution is 1.47 l kg^{-1}. The percentage of protein binding is 15%. With oral dosing terbutaline is eliminated primarily as sulfate (70%) and glucuronide (30%) conjugates. Approximately 10–15% is cleared in the urine as unchanged drug. With parenteral and inhalation exposure, the majority of the drug is cleared in the urine as unchanged terbutaline (68% and 60%, respectively). The elimination half-life is 12–20 h.

Mechanism of Toxicity

The primary mechanism of terbutaline is the stimulation of adenylcyclase, which catalyzes cyclic adenosine monophosphate (AMP) from adenosine triphosphate (ATP). In the liver, buildup of cyclic AMP stimulates glycogenolysis and an increase in serum glucose. In skeletal muscle, this process results in increased lactate production. Direct stimulus of sodium/potassium ATPase in skeletal muscle produces a shift of potassium from the extracellular space to the intracellular space. Relaxation of smooth muscle produces a dilation of the vasculature supplying skeletal muscle, which results in a drop in diastolic and mean arterial pressure (MAP). Tachycardia occurs as a reflex to the drop in MAP or as a result of β_1 stimulus. β_1-Adrenergic receptors in the locus ceruleus also regulate norepinephrine-induced inhibitory effects, resulting in agitation, restlessness, and tremor.

Acute and Short-Term Toxicity (or Exposure)

Animal

Clinical effects of agitation, tremors, and tachycardia may occur. No specific information on a minimal toxic dose was available.

Human

The toxic events of terbutaline overdose follow its β_1-adrenergic agonist activity. The effects of terbutaline overdose are usually mild and benign; however, they can be prolonged. Cardiovascular effects are usually limited to a sinus tachycardia and widened pulse pressure. Although there may be a drop in diastolic pressure, the systolic pressure is maintained by increased cardiac output from the tachycardia. Evidence of myocardial ischemia after terbutaline overdose has been infrequently reported. Transient hypokalemia may occur, caused by a shift of extracellular potassium to the intracellular space. A transient metabolic acidosis can be seen due to increased lactate production. Restlessness, agitation, and tremors are common in terbutaline overdose.

Chronic Toxicity (or Exposure)

Animal

Terbutaline is used in veterinary practice for the management of bronchoconstriction. Toxic effects

are commonly related to beta stimulation (e.g., tachycardia, hypertension).

Human

Toxic effects are an extension of terbutaline's pharmacologic activity. Common symptoms include hypertension, tachycardia, arrhythmias, central nervous system stimulation, gastrointestinal effects, and transient electrolyte changes (e.g., hypokalemia).

In Vitro Toxicity Data

Studies in rat alveolar type II cells have demonstrated that terbutaline stimulates sodium influx as well as potassium and chloride release via cAMP ;accumulation.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Terbutaline overdoses rarely require treatment beyond gastrointestinal decontamination. Activated charcoal effectively binds terbutaline. The hypokalemia produced reflects a transient shift in potassium location rather than a true deficit of potassium. Therefore, only rarely is there a need for external replacement therapy. A conservative approach to the tachycardia is recommended. In the rare event of complications, intravenous propranolol rapidly and effectively reverses the symptoms of terbutaline poisoning.

Environmental Fate

No information is currently available on breakdown in soil, groundwater, or surface water.

See also: Potassium.

Further Reading

Blake PG and Ryan F (1989) Rhabdomyolysis and acute renal failure after terbutaline overdose. *Nephron* 53: 76–77.

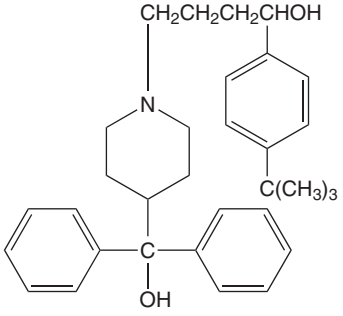
Heath A and Hulten BA (1987) Terbutaline concentrations in self poisoning: A case report. *Human Toxicology* 6: 525–526.

Terfenadine

Elizabeth J Scharman

This article is a revision of the previous print edition article by Carla M Goetz, volume 3, pp. 218–219, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 50679-08-8
- Synonyms: α-[4-(1,1-Dimethylethyl)phenyl]-4-(hydroxydiphenylmethyl)-1-piperidinebutalol; Seldane® (former brand name in United States); Teldane®; Teldanex®
- Chemical/Pharmaceutical/Other Class: An H-1 receptor antagonist; Piperidine derivative
- Chemical Formula: $C_{32}H_{41}NO_2$
- Chemical Structure:



Uses

Terfenadine is indicated for the symptomatic relief of seasonal allergic rhinitis. The Food and Drug Administration removed terfenadine from the US market in 1998.

Exposure Routes and Pathways

Ingestion is the route of both accidental and intentional exposures to terfenadine.

Toxicokinetics

Approximately 70% of an oral dose of terfenadine is absorbed rapidly with peak plasma concentrations occurring in 2 h. The onset of action is 1–2 h, is maximal in 3–4 h, and lasts over 12 h. The first-pass metabolism of terfenadine is 99%. Terfenadine is metabolized by the cytochrome P450 IIIA4 (CYP3A4) microsomal enzyme system to an acid metabolite that is active and a dealkylated metabolite that is inactive. *In vitro*, the acid metabolite has demonstrated ~30% of the H-1 blocking activity of the parent compound. The volume of distribution of terfenadine is undetermined; high concentrations are found in the liver, lung, and gastrointestinal tract. Terfenadine is 97% protein bound and penetrates the

blood–brain barrier poorly. Approximately 60% and 40% of an oral dose of the drug is excreted in the feces and urine, respectively, principally as metabolites. The half-life of terfenadine is 8.5 h. The elimination of the acid metabolite is biphasic with an initial half-life of 3.5 h and a terminal half-life of 6 h.

Mechanism of Toxicity

Terfenadine binds to peripheral H-1 receptors. Receptor affinity for muscarinic, α, and β-adrenergic receptors is low. Poor penetration of terfenadine across the blood–brain barrier limits central nervous system effects. Therefore, terfenadine is classified as 'nonsedating' and lacks anticholinergic side effects. However, accumulation of the parent drug, terfenadine, results in prolongation of the QT interval by blocking the delayed rectifier potassium current in the heart. Prolongation of the QT interval can lead to torsade de pointes and death.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute toxicity in dogs has been reported. Vomiting and lethargy are often reported. Central nervous system excitation leading to seizures, ataxia, agitation, and hyperthermia are possible. In one case, tachycardia and premature ventricular contractions were documented.

Human

Cardiac effects seen include prolongation of the QT interval, arrhythmias (e.g., torsades de pointes, ventri tachycardia, ventricular fibrillation), and cardiac arrest. Symptoms seen in patients with torsades de pointes include dizziness, syncope, palpitations, and sudden death. Seizures have occurred following overdose.

Chronic Toxicity (or Exposure)

Animal

Carcinogenicity studies in mice and rats over >18 months at doses of 150 mg kg^{-1} day^{-1} were negative.

Human

Accumulation of the parent drug and resultant QT prolongation may occur following a overdose, a drug interaction that limits metabolism of terfenadine (e.g., concomitant administration with erythromycin or other macrolide antibiotic or with the azole derivatives ketoconazole or itraconazole), or significant hepatic dysfunction that limits metabolism of terfenadine. Patients with preexisting cardiac disease or those with electrolyte abnormalities are also at increased risk for cardiac toxicity.

In Vitro Toxicity Data

Mutagenicity studies using the Ames *Salmonella* and mouse micronucleus assays have been negative.

Clinical Management

Terfenadine is adsorbed by activated charcoal and charcoal may be considered for substantial recent ingestions. There is no antidote for terfenadine overdose. Terfenadine therapy should be discontinued and standard supportive therapies should be utilized as clinically necessary. Close electrocardiographic monitoring should be instituted for a minimum of 24 h. Torsades de pointes may be treated with electrical cardioversion if the patient is hemodynamically unstable. Otherwise, magnesium, isoproterenol, and/or atrial overdrive pacing may be used to manage this arrhythmia.

See also: Cytochrome P-450.

Further Reading

June RA and Nasr I (1997) Torsades de pointes with terfenadine ingestion. *American Journal of Emergency Medicine* 15: 542–543.

Monahan BP, Ferguson CL, and Killeavy ES (1990) Torsades de pointes occurring in association with terfenadine use. *Journal of the American Medical Association* 264: 2788–2790.

Terrrestrial Ecotoxicology *See* Ecotoxicology, Terrestrial.

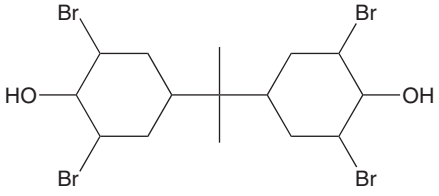
Terrorism *See* Bio Warfare and Terrorism: Toxins and Other Mid-Spectrum Agents.

Testosterone *See* Androgens.

Tetrabromobisphenol A

Paul Jones, Katie Coady, and John Newsted

- Representative Chemicals: 2,2′,6,6′-Tetrabromobisphenol A; 3,3′,5,5′-Tetrabromobisphenol A
- Chemical Abstracts Service Registry Number: CAS 79-94-7
- Synonyms: 2,2′,6,6′-Tetrabromo-4,4′-isopropylidenediphenol; 2,2-Bis(3,5-dibromo-4-hydroxyphenyl) propane; 4,4′-Isopropylidene bis(2, 6-dibromophenol); Saytex 111; Saytex RB-100; Bromdian; FG 2000, Fire Guard 2000; Firemaster BP4A; 4,4′-(1-Methylethylidene) bis(2,6-dibromo)phenol; Tetrabromodian; Tetrabromodiphenyl propane
- Chemical/Pharmaceutical/Other Class: Brominated phenolic
- Chemical Formula: $C_{15}H_{12}Br_4O_2$
- Chemical Structure:



Uses

Tetrabromobisphenol A (TBBPA) is primarily used as a reactive flame retardant in epoxy resin circuit boards. Both hydroxyl groups on TBBPA can be reacted with epichlorohydrin under basic conditions to form the diglycidyl ether, which is widely used in epoxy resin formulations. TBBPA is also used in polycarbonate and ether polyester resins and is used as a chemical intermediate for the synthesis of: tetrabromobisphenol A allyl ether, -bis(2-hydroxyethyl ether), -carbonate oligomer, and -diglycidyl ether. TBBA is also used as a flame retardant in plastics, paper, and textiles, and as a plasticizer in adhesives and coatings.

Exposure Routes and Pathways

Occupational exposure to TBBPA can occur through inhalation of dusts and dermal contact at workplaces where TBBPA is produced or used. Monitoring data indicate that the general population may also be exposed to TBBPA via inhalation of ambient air and dermal contact. Other possible exposure routes include ingestion of drinking water that has been stored in polycarbonate containers and from the consumption of fish and shellfish.

Toxicokinetics

In a study with rats, TBBPA was readily absorbed from the gastrointestinal tract, metabolized in the liver and excreted via the bile to the gut. Of the dose administered to the rats, ~90% was excreted in the feces as parent TBBPA. Three glucoronide conjugates of TBBPA were identified in the feces but only accounted for a small amount of the administered dose. Urine was a minor route for excretion of TBBPA. The half-life of TBBPA in rats was estimated to be less than 3 days with the longest half-lives in fat and testes. The shortest half-lives were in liver and kidneys. In a study of occupationally exposed Swedish workers, the half-life for elimination from the serum was 2.2 days indicating a rapid elimination from the body.

Mechanism of Toxicity

Studies of the effects of TBBPA on the function of biological membranes showed that it resulted in hemolysis of human erythrocytes and the uncoupling of oxidative phosphorylation in rat mitochondria. In addition, TBBPA exposure resulted in the inhibition of calcium accumulation in isolated mitochondria that was associated with an increase in potassium release and latent ATPase activity. These studies suggest that the primary activity of TBBPA *in vitro* is to change the permeability of biological membranes disrupting normal ion transport and respiration of cells. TBBPA has also been shown to weakly induce liver microsomal enzymes *in vitro*. In the E-Screen assay, TBBPA expressed weak receptor-mediated estrogenic activity with an estrogenic potency ~5–6 orders of magnitude lower than that of the native ligand, 17β-estradiol. TBBPA has been shown to bind to human transthyretin *in vitro* with a 10 times greater potency than thyroxin, the natural ligand. However, in a study with pregnant rats, TBBPA did

not bind to transthyretin and did not alter thyroid hormone concentration in the exposed animals. The differences between *in vitro* and *in vivo* studies may have been due to toxicokinetic factors that altered the effective concentrations at the site of action. In female rats, an intragastric dose of 250 mg kg^{-1} for 28 days resulted in the alteration of several serum enzymes including several indicators of porphyrogenic action. These results suggest that TBBPA is capable of disturbing heme metabolism in rats.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute toxicity of TBBPA in traditional test animals is low. The oral LD_{50} for rat and mouse is >5 and 10 g kg^{-1} body weight and the dermal LD_{50} in rabbits is >2.0 g kg^{-1} body weight. In rats, the inhalation LC_{50} of TBBPA dust is >15.5 mg l^{-1}. In dermal tests, TBBPA was not irritating and gave no sensitization reaction. Upon dermal exposure on abraded skin of up to 2500 mg kg^{-1}, a slight erythema was seen in rabbits. Application of up to 100 mg of TBBPA in the eyes of rabbits did not result in corneal damage, iris irritation, or conjunctival discharge indicating that this compound is not an eye irritant. In rats fed up to 1000 mg kg^{-1} of TBBPA in the diet for 28 days, no effects were observed on mortality, body weight, and feed consumption, and no gross pathological lesions, or histopathological changes were seen. In a 90 day oral study with rats, exposure to 1000 mg kg^{-1} did not induce adverse effects on body weight, and no changes in hematology, clinical chemistry, urinalysis, or histopathology were observed. In an oral, 90 day study with mice, a dietary dose of 700 mg kg^{-1} did not cause any adverse effects while 2200 mg kg^{-1} resulted in decreased body weight, increased spleen weight and reduced concentration of red blood cells, serum proteins and serum triglycerides.

Chronic Toxicity (or Exposure)

Animal

TBBPA administered to rats via gavage on gestation days 0–19 produced no signs of toxicity including no abnormalities in the offspring. TBBPA, orally administered on gestation days 6–15, was more toxic to the conceptus than to the dams. The embryo/fetal no-effect level was 2.5 mg kg^{-1} day^{-1} while the maternal no effect-level was >25 mg kg^{-1} day^{-1}. At $\geqslant 10$ mg kg^{-1} day^{-1}, dose-dependent effects on conceptuses were observed and included fetal malformation, reduced fetal body weight, and delayed skeletal ossification. In a study with Firemaster BP4-A, concentrations up to 10 g kg^{-1} in rats exposed on gestation days 6–15 were not toxic to the dams or conceptus. No carcinogenicity studies have been reported to date.

Human

No skin irritation or sensitization has been observed in humans exposed to TBBPA. No epidemiological or other data are available on effects of TBBPA in humans. To date, no carcinogenicity or long-term studies have been reported.

In Vitro Toxicity Data

TBBPA tested negative for mutagenicity in the Ames assay using five *Salmonella typhimurium* strains (TA1535, TA1537, TA97, TA98, and TA100) with and without metabolic activation. TBBPA caused an effect on induction of intragenic recombination in two *in vitro* mammalian cell assays. TBBPA reduced CD25 (IL-2 receptor-α chain), an inducible receptor chain essential for proliferation of activated T cells in an *in vitro* immunotoxicity assay. However, observed immunosuppression of T cell proliferation was thought to be mediated through the Ah receptor.

Environmental Fate

TBBPA is expected to adsorb to suspended solids and sediment based on an estimated K_{oc} value of 56 000. Volatilization from water surfaces is not expected, based upon an estimated Henry's law constant of 7.0×10^{-11} atm m^3 mol^{-1}. Bioconcentration factor values ranging from 20 to 3200 suggest the potential for bioconcentration is moderate to high in aquatic organisms. In fish, a half-life of less than one day has been observed while in oysters it was less than 5 days. If released to air, TBBPA is expected to exist solely in the particulate phase (based on an estimated vapor pressure of 1.8×10^{-11} mmHg at 25°C) and may be removed from the air by wet and dry deposition. TBBPA is expected to be immobile in soil based on its estimated K_{oc}. Volatilization of TBBPA from moist and dry soil surfaces is not expected to be an important fate process given its Henry's law constant. Biodegradation of TBBPA in three different soils under anaerobic conditions resulted in 44–91% of the parent material with only 0.03–0.35% of the compound being recovered as carbon dioxide. Thus, under anaerobic conditions TBBPA is expected to undergo rapid primary degradation and slow mineralization in soils. A similar biodegradation process

was also observed in a sediment/water microbial test system where mineralization was slow. Photodegradation of TBBPA in water is seasonally dependent with half-lives of 10.2 days (spring), 6.6 days (summer), 25.9 days (autumn), and 80.7 days (winter). Cloud cover increased the times by a factor of 2. The main photodegradation product of TBBPA in the presence or absence of hydroxyl radicals was 2,4,6-tribromophenol. TBBPA has persistence half-life values in the range 44–179 days in soil, 48–84 days in water, and 1–9 days in air.

Exposure Standards and Guidelines

TBBPA is listed in the Environmental Protection Agency Toxic Substances Control Act under Section 8(b) and as a result all manufacturers, importers and processors of TBBPA are required to report all health and safety studies that they have conducted. As particulates not otherwise regulated, the Occupational Safety and Health Administration permissible exposure level time-weighted average (TWA) is $15\,mg\,m^{-3}$. As particulates not otherwise specified, the American Conference of Governmental Industrial Hygienists threshold limit value TWA is $10\,mg\,m^{-3}$.

See also: Bisphenol A; Chlorophenols.

Further Reading

Darnerud PO (2003) Toxic effects of brominated flame retardants in man and wildlife. *Environment International* 29: 841–853.

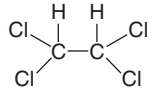
Hakk H and Letcher R (2003) Metabolism and toxicokinetics and fate of brominated flame retardants – a review. *Environment International* 29: 801–828.

Tetracholordibenzo-*p*-Dioxin, 2,3,7,8- *See* TCDD (2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin).

Tetrachloroethane

Robert Kapp

- Related Compounds: Carbon tetrachloride (CAS 56-23-5); Perchloroethylene (CAS 127-18-4); Trichloroethylene (CAS 79-01-6); Trichloroethane (CAS 79-00-5); Pentachlorophenol (CAS 87-86-5)
- Chemical Abstracts Service Registry Number: CAS 79-34-5
- Synonyms: 1,1-Dichloro-2,2-dichloroethane; Acetosal; Acetylene tetrachloride; Bonoform; Cellon; Dichloro-2, 2-dichloroethane; Ethane, 1,1,2,2-tetrachloro-; TCE (ambiguous); Tetrachloroethane; Tetrachloroethane (VAN); Tetrachlorure d'acetylene (French); Westron; s-Tetrachloroethane
- Chemical/Pharmaceutical/Other Class: Chlorinated aliphatic solvent; Haloalkane
- Chemical Formula: $C_2H_2Cl_4$
- Chemical Structure:



Uses

1,1,2,2-Tetrachloroethane has been used primarily as an intermediate in the synthesis of other chlorinated hydrocarbons such as trichloroethylene, tetrachloroethylene and 1,2,-dichloroethylene. It was also used as a solvent in cleaning and degreasing metals, in paint removers, in photographic films, as an extractant for oils and fats, and in pesticides. Recently, however, the production of 1,1,2,2-tetrachloroethane as an end product has significantly decreased due to replacement with less toxic solvents.

Exposure Routes and Pathways

A primary pathway for tetrachloroethane exposure is by inhalation since tetrachloroethane can be found at low levels in both indoor and outdoor air. The material has not been reported in food or soil; however, in rare instances, tetrachloroethane has been found in public water supplies. Since production of tetrachloroethane has stopped, exposure of most workers would be limited. Minimal exposure could occur from breathing in vapors or touching the material during accidental spills in the workplace.

Toxicokinetics

1,1,2,2-Tetrachloroethane is well absorbed from the gastrointestinal and respiratory tracts. It is also absorbed through the skin upon dermal exposure.

When introduced via the oral or inhalation routes, tetrachloroethane is metabolized primarily to trichloroethanol, trichloroacetic acids that are subsequently broken down to glyoxylic acid, oxalic acid and carbon dioxide and are excreted chiefly as metabolites in the breath and urine. A small amount is expired in the breath as carbon dioxide and as the parent compound.

Mechanism of Toxicity

Generally, 1,1,2,2-tetrachloroethane is considered the most toxic of the common chlorinated hydrocarbons. It is a small lipophilic molecule, well-absorbed and distributed throughout tissue compartments by passive diffusion processes. Its metabolic fate involves both oxidative and reductive reactions, which are related to the mechanisms by which halocarbons are activated to proximate toxins. The presence of the terminal dichloromethyl moiety can convey toxicity because these moieties are hydroxylated to reactive acyl intermediates that bind to proteins and exert toxic effects. Both dichloro- and trichloroacetic acids are known to cause proliferation of peroxisomes, which could elicit a hepatotoxic response. On the other hand, studies investigating the reductive metabolism of 1,1,2,2-tetrachloroethane found a cytochrome P450-mediated reaction, which included lipid peroxidation and dehalogenation of 1,1,2,2-tetrachloroethane. The principal pathway of degradation involves hydrolytic cleavage of the carbon-chlorine bonds and oxidation to dichloroacetaldehyde hydrate, dichloroacetic acid and eventually glyoxylic acid. This glyoxylic acid is then metabolized to oxalic acid, glycine, formic acid, and carbon dioxide. The hepatic and carcinogenic effects of 1,1,2,2-tetrachloroethane may result from the oxidative and reductive pathways that produce direct- or indirect-acting toxins.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute toxicity of 1,1,2,2-tetrachloroethane in experimental animals is slight to moderate. This material is a skin, eye, and respiratory irritant. The oral LD_{50} has been reported from 250 to 1000 mg kg^{-1} in the rat. Exposure to 1000 ppm for 4–6 h caused death in rats. Dermal and ocular irritations were reported in rabbits exposed to 580 ppm of 1,1,2,2-tetrachloroethane. There are limited short-term data available; however, single low-level inhalation exposure effects have been reported to include hepatic congestion, fatty degeneration, histological changes, alterations in levels of enzymes and elevated DNA synthesis.

Human

Based upon the limited data from acute and subchronic studies, the liver appears to be the most sensitive target organ.

Chronic Toxicity (or Exposure)

Animal

Chronic exposure to 1,1,2,2-tetrachloroethane has resulted in increased incidence of liver tumors in mice after 78 weeks. Tumor increases are limited to one species and other data are incomplete and suggest an epigenetic mechanism. The Gene-Tox data collectively indicate that 1,1,2,2-tetrachloroethane is a weak genetic toxin. Reproductive toxicity studies revealed atrophy of the seminal vesicles and decreased spermatogenesis after 10 days' exposure to 2 ppm 1,1,2,2-tetrachloroethane; however, there was no effect on the mating index of the males after 38 days of similar exposure to the material. Similarly, males rats exposed to 2 ppm 1,1,2,2-tetrachloroethane for 4 h per day over a 9 month period showed no effects on any of the mating indices normally examined nor were there any abnormalities in the offspring.

Human

A group of army workers exposed to 1,1,2,2-tetrachloroethane in a gaseous form showed a slight increase in the incidence of death due to genital cancers, leukemia, and lymphomas when compared to nonexposed workers. The data in this study were suspect since the specific exposure levels were not measured and since the increases were small and there were other confounding factors. Because of these factors, the authors concluded that the data were inconclusive as to whether or not 1,1,2,2-tetrachloroethane causes cancer.

The Environmental Protection Agency has classified 1,1,2,2-tetrachloroethane as group C (possible human carcinogen: limited evidence of carcinogenicity in animals in the absence of human data). The International Agency for Research on Cancer classifies the material as group 3 (not classifiable as to carcinogenicity to humans). The National Institute for Occupational Safety and Health classifies 1,1,2,2-tetrachloroethane as Ca (potential occupational carcinogen, with no further categorization). The American Conference of Governmental Industrial Hygienists (ACGIH) classifies the material as A3 (confirmed animal carcinogen with unknown

relevance to humans); while the Federal Republic of Germany Maximum Concentration Values in the Workplace (MAK) classifies the material as 3B (substance for which *in vitro* test or animal studies have yielded evidence of carcinogenic effects that is not sufficient for classification of the substance in one of the other categories). 1,1,2,2-Tetrachloroethane is listed in Schedule 2 of the COSHH (Control of Substances Hazardous to Health Regulations). Its use in the United Kingdom is banned for diffusive applications such as surface or fabric cleaning except for R&D and analysis.

Clinical Management

Upon ocular exposure, the eye should be generously washed with tap water. Refer for medical attention. In case of dermal exposure, contaminated clothing should be removed and the skin should be rinsed with tap water. 1,1,2,2-Tetrachloroethane ingestion should be referred for medical attention. Vomiting should not be induced. Upon inhalation, the victim should be removed to fresh air and given artificial respiration if indicated. The affected individual should be referred for medical attention.

Environmental Fate

1,1,2,2-Tetrachloroethane has the potential to leach to groundwater. In surface water, it volatilizes with a half-life of ~6 h. Hydrolysis also occurs. Adsorption to sediment and bioconcentration in aquatic organisms is not significant.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration permissible exposure limit is 5 ppm (time-weighted average (TWA)) on skin while the ACGIH threshold limit value is 1 ppm (TWA) on skin.

Miscellaneous

1,1,2,2-Tetrachloroethane is a volatile, synthetic, colorless to pale-yellow liquid with a pungent, chloroform-like odor.

See also: Trichloroethane; Trichloroethylene.

Further Reading

Reid JB (2001) Saturated halogenated aliphatic hydrocarbons two and four carbons. In: Bingham E, Cohrssen B and Powell CH (eds.) *Patty's Toxicology*, 5th edn., vol. 5, pp. 136–142. New York: Wiley.

US Department of Health and Human Services (1996) *Toxicological Profile for 1,1,2,2-Tetrachloroethane*. Atlanta, GA: Public Health Service, Agency for Toxic Substances and Disease Registry.

Tetrachloroethylene

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 3, pp. 220–221, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 127-18-4
- Synonyms: Tetrachloroethene; 1,1,2,2-Tetrachloroethylene; Perchloroethene; Perchloroethylene
- Chemical/Pharmaceutical/Other Class: Chlorinated olefinic hydrocarbon
- Chemical Formula: C_2HCl_3

Uses

Perchloroethylene (PERC) is manufactured by direct chlorination of ethylene or a petroleum hydrocarbon stream. It has been used extensively in the dry cleaning industry for metal cleaning and degreasing, for processing and finishing textiles, as an extraction solvent, in chemical processing, as heat exchange fluid, as a grain fumigant, for fluorocarbon manufacturing processes, and in typewriter correction fluid. PERC is a clear liquid of high volatility. Its vapor is almost six times as dense as air and has a chloroform-like odor detectable from 5 to 50 ppm in air.

Exposure Routes and Pathways

Major human exposure has been in the dry cleaning industry and in industries employing degreasing procedures. In addition, inhalation of contaminated urban air (especially near point sources such as dry cleaners), drinking contaminated water from contaminated aquifers, and drinking water distributed in pipelines with vinyl liners may offer additional exposure opportunities.

Toxicokinetics

Comparison of the urinary trichloro-compound levels with tetrachloroethylene in the environment revealed that while the metabolite levels increased essentially parallel to PERC concentrations up to 100 ppm, leveling off was apparent in the metabolite excretion when the exposure to PERC was more intense (e.g., more than 100 ppm), indicating that the capacity of humans to metabolize this chlorinated hydrocarbon is rather limited. A tentative calculation indicated that at the end of an 8 h shift with exposure to tetrachloroethylene at 50 ppm (time-weighted average, TWA), 38% of the PERC absorbed through the lung would be exhaled unchanged, $<2\%$ would be metabolized to be excreted in the urine, while the rest would remain mostly in the fat stores of the body to be eliminated later.

Absorption typically takes place through inhalation of the volatile solvent but may also take place through dermal exposure and ingestion of contaminated drinking water. During PERC exposure, urinary metabolite levels of trichloroethanol, total trichloro compounds, and trichloroacetic acid increased until the atmospheric concentration of the solvent reached 50–100 ppm. Little increase in these metabolites occurred at higher solvent concentrations indicating a saturation of metabolic capability.

Metabolism is saturable and relatively slow with only a small percentage of the administered dose excreted as metabolites, the major one being trichloroacetic acid. Following exposure to PERC, trichloroacetic acid, and trichloroethanol have been found in the urine of humans and animals. Additionally, oxalic acid, dichloroacetic acid, and ethylene glycol have been reported in the urine of exposed animals. Other reported biotransformation products include inorganic chlorine and *trans*-1,2-dichloroethylene in expired air.

Once in the bloodstream, PERC tends to distribute to body fat. In human tissue at autopsy, ratios of fat to liver concentrations are greater than 6:1. An autopsy after a fatal PERC exposure revealed an eightfold greater concentration in the brain compared with blood. PERC reached near steady-state levels in the blood of human volunteers within 2 h of continuous exposure.

The respiratory half-life for elimination of PERC has been estimated at 65–70 h and is a result of the very slow elimination of PERC from fat stores. The half-life of elimination of trichloro metabolites of PERC is estimated as being 144 h. This long half-life of elimination has serious implications with regard to the accumulation of PERC during chronic or multiple exposure situations.

Mechanism of Toxicity

PERC is metabolized to trichloroacetic acid and other trichloro metabolites in the liver. Trichloroacetic acid has been shown to produce peroxisome proliferation in mice. This may have implications for the apparent increase in liver tumors in mice. PERC also has been shown to distribute rapidly to the central nervous system (CNS) and is known to have an affinity for the lipophilic cellular membranes in the brain.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} values in mice are reported to be between 6 and 8.5 g kg^{-1} body weight and between 2.4 and 13 for rats. The inhalation LC_{50} value for rats during a 6 h inhalation exposure is reported to be 4100 and 2978 ppm for mice.

Human

PERC defats the skin leading to dermatitis. It is irritating to the eyes, skin, and mucous membranes. Excessive exposure can produce CNS effects including depression, dizziness, disorientation, seizures, headache, vertigo, and unconsciousness. Psychoses, hallucinations, and distorted perceptions have been reported from inhalation exposures. Concentrations producing unconsciousness have also produced proteinuria and hematuria. Liver and kidney damage has been noted in cases of acute exposure to PERC which is also considered to be a mucous membrane and upper respiratory system irritant at levels approaching 100 ppm in the atmosphere. Ingestion of PERC may result in nausea, vomiting, and bloody stools, while inhalation of other compounds in this class has been noted to sensitize the myocardium to catecholamines. Acute inhalation exposure has been reported to cause CNS depression, alcohol intolerance, liver necrosis, kidney injury, malaise, headache, dizziness, fatigue, tinnitus, visual field reduction, sensory disturbances, lightheadedness, sweating, a staggering gait, inebriation, mental dullness, and death by anesthesia. Cardiac arrhythmias, peripheral neuropathies, proteinuria, hematuria, and oliguric renal failure have also been associated with PERC exposure.

Direct eye contact may cause pain, lacrimation, and burning. Dermal exposure can cause dermatitis, erythema, burns, and vesiculation.

At 50 ppm, the apparent odor threshold for PERC, and at 100 ppm few physiological effects are noted after 8 h of exposure. At 200 ppm definite odor is

perceived and faint to moderate eye irritation and minimal lightheadedness are noted. At 400 ppm a strong unpleasant odor is perceived along with definite eye irritation, slight nasal irritation, and definite incoordination after 2 h of exposure. At 600 ppm, it has a very strong but tolerable unpleasant odor and causes definite eye and nasal irritation, dizziness, loss of inhibitions after only 10 min of exposure. At 100 ppm the odor is very intense and irritating with marked irritation of the eyes and respiratory tract and considerable dizziness after a 2 min exposure and at 1500 ppm, the odor is almost intolerable with gagging, irritation almost intolerable to eyes and nose with complete incoordination within minutes to unconsciousness within 30 min.

Chronic Toxicity (or Exposure)

Animal

PERC produces leukemia in rats. Although PERC is known to induce peroxisome proliferation in mouse liver, this did not correlate well with tumor formation in the liver. Fetotoxicity and developmental abnormalities have been seen in animal experiments.

Mice receiving single doses of PERC did not show chromosomal aberrations in bone marrow cells nor did a positive mutagenic response result from the host-mediated assay using Salmonella strains in mice. Renal tubular effects have been noted in mice and dogs treated orally with PERC and both liver and kidney changes have been noted in rats inhaling PERC.

As a result of National Toxicology Program (NTP) bioassays, PERC has been reported to produce hepatocellular carcinomas in B6C3F1 mice of both sexes when administered by gavage. An NTP inhalation study also showed hepatocellular carcinomas in B6C3F1 mice and renal cell adenomas and adenocarcinomas and mononuclear cell leukemias and renal tubular cell neoplasms in Fisher 344 rats.

In cats and dogs, PERC increased the vulnerability of the ventricles to epinephrine-induced extrasystoles, bigeminal rhythms and tachycardia. PERC is considered an animal carcinogen

Human

Studies of chronic exposure of those working in dry cleaning plants have reported some CNS effects, some liver function abnormalities, renal dysfunction, and some definite central and peripheral neurotoxicity. Other effects from chronic exposure to PERC include cardiac arrhythmias, reduced color perception, impaired memory, peripheral neuropathy, impaired vision, confusion, disorientation, fatigue, personality changes, and agitation.

Exposure to PERC has been reported to elevate risks of esophageal cancer, non-Hodgkin's lymphoma and cervical cancer in several epidemiological studies and PERC has been classified by the International Agency for Research on Cancer (IARC) as a probable human carcinogen. Dry cleaners chronically exposed to PERC have shown early signs of renal damage and dysfunction. Chronic exposure to PERC may cause arrhythmias, defatting dermatitis, impaired memory, numbness in the extremities, peripheral neuropathy, and impaired vision. Chronic occupational exposure has resulted in hepatitis, confusion, disorientation, muscle cramps, fatigue, and agitation. Some epidemiological studies suggest an increased incidence of liver, esophageal, and urinary tract tumors, and leukemia in humans but the data is inadequate to come to any firm conclusions. Other studies of occupationally exposed workers suggest that there are increased cancer risks for lung, cervix, skin, liver, esophagus, urinary tract, and for leukemia. IARC has classified PERC as a probable human carcinogen based on positive findings in animals and suggestive, although inconclusive, findings in humans.

Scleroderma, an autoimmune disease involving the vascular system, has been associated with exposure to chlorinated ethylene compounds related to PERC, but the reports are not definitive.

In Vitro Toxicity Data

Few positive results have been noted for *in vitro* mutagenicity assays but one test using L5178Y mouse lymphoma cells demonstrated a positive response. Attempted cell transformation using the BALB/3T3 mouse cell line failed to produce a positive response as did an Ames test using TA98, TA100, TA1535, and TA1537. Chromosomal abnormalities have been reported in the circulating lymphocytes of some exposed workers but conflicting results are obvious in the literature. In some studies, factory workers have also failed to show effects of exposure on chromosomal aberrations and sister chromatid exchanges (SCEs).

Clinical Management

Those exposed to PERC regularly should be monitored for kidney and liver function. Current exposure can be monitored by analysis of exhaled PERC. For acute ingestion, emesis is not recommended because of the potential for CNS depression. Gastric lavage should be considered if the quantity of PERC ingested is life threatening but should be performed within 1 h of ingestion. Activated charcoal may be considered and endotracheal intubation and ventilatory

assistance with supplemental oxygen should be considered if CNS depression of the respiratory system is noted. Monitor level of consciousness, EKG, adequacy of respirations and oxygen saturation as well as renal and hepatic function tests. Careful EKG monitoring may aid in early detection of arrhythmias.

For inhalation exposures, move the patient to an uncontaminated atmosphere and administer oxygen as indicated. Insure a patent airway. Treat bronchospasm with inhaled $\beta 2$ agonists and oral or parenteral corticosteroids. Again monitor the level of consciousness, EKG, oxygen saturation, liver, and renal functions carefully. Cardiac sensitization has occurred with other compounds in this class so EKG monitoring should be carried out carefully. Epinephrine or other β-adrenergic agents should be immediately available should arrhythmias occur.

Environmental Fate

When released into the environment, PERC exists as a vapor and will be degraded by photochemically produced hydroxy radicals and the half-life for this reaction is estimated to be ~ 96 days. Since PERC only absorbs UV light weakly, direct photodegradation is not thought to be an important pathway. If released into the soil, PERC is quite mobile and is frequently found in groundwater. Volatilization from dry soil and water are thought to be important pathways of dispersion into the environment. Biodegradation in soil under aerobic and anerobic conditions is thought to proceed slowly. Anerobic biodegradation of PERC produces mainly trichloroethylene but traces of dichloroethylenes and vinyl chloride may also be found.

Other Hazards

Adolescents and others have used PERC to attain an inhalation 'high' by 'huffing' the fumes in a paper bag saturated with PERC from typewriter correction fluids. A clinical observation described as 'degreasers flush' has been repeatedly noted in those exposed to chlorinated solvents in combination with alcohol consumption. Thermal decomposition of PERC results in the production of hydrogen chloride gas and phosgene. Smoking or welding in a PERC-contaminated environment will produce these toxic gases which could result in life-threatening pulmonary edema.

Exposure Standards and Guidelines

- Federal drinking water standard (Environmental Protection Agency 11/93): $5\ \mu g\ l^{-1}$.
- Maine drinking water standard: $3\ \mu g\ l^{-1}$.
- Occupational Safety and Health Administration (OSHA) permissible exposure limit, (8 h TWA): 100 ppm.
- OSHA short-term exposure limit: 15 min 200 ppm.
- American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value, (8 h TWA): 100 ppm.
- Biological exposure index (BEI – ACGIH) and end of shift: 5 ppm in exhaled air.

The National Institute for Occupational Safety and Health recommends that PERC be regulated as a potential human carcinogen with lowest possible exposure level.

The IARC has classified PERC as being probably carcinogenic to man (2A) based on sufficient evidence in animals but limited evidence in humans.

The ACGIH classifies PERC as an animal carcinogen (A3).

Miscellaneous

PERC is a colorless liquid with an ether-like odor. It boils at $\sim 121^{\circ}C$ and has a liquid density of ~ 1.6. It is lipid-soluble with a distribution between octanol/water of 3.4 and has a solubility of 0.015 g $(100\ ml)^{-1}$ in water. Its vapor density is 5.7 and, consequently, it settles in low areas when released in quantity into the environment. PERC quickly desensitizes the olfactory nervous system but can be recognized in air at ~ 4.7 ppm but is generally thought to have an odor threshold of ~ 50 ppm. A worker may be exposed to high concentrations of PERC without smelling it.

See also: Peroxisome Proliferators; Pollution, Water.

Further Reading

Beliles RP (2002) Concordance across species in the reproductive and developmental toxicity of tetrachloroethylene. *Toxicology and Industrial Health* 18(2): 91–106.

Relevant Websites

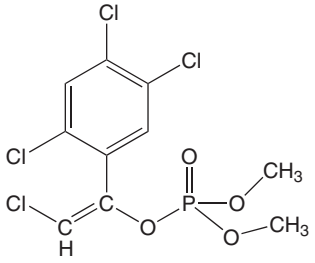
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Tetrachloroethylene.

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

Tetrachlorvinphos

Subramanya Karanth

- Chemical Abstracts Service Registry Number: CAS 22248-79-9
- Synonyms: Gardona; Stirophos; Rabon; Rabond
- Chemical/Pharmaceutical/Other Class: Organophosphate (vinyl phosphate) insecticide
- Chemical Formula: $C_{10}H_9Cl_4O_4P$
- Chemical Structure:



Uses

Tetrachlorvinphos is commonly used as a feed additive to control flies in livestock and as dusts, sprays, dips, and collar ingredient to control ticks and fleas on domestic pets. It is extensively used in poultry. In horses formulations are commonly used as 'feed-through' larvicide. In addition, tetrachlorvinphos is also used in the control of nuisance and public health pests.

Exposure Routes and Pathways

Dermal absorption and inhalation of dusts are the common routes of exposure for tetrachlorvinphos.

Toxicokinetics

Tetrachlorvinphos is readily absorbed through the gastrointestinal tract following oral exposure. Major metabolites following oral exposure in rats and dogs include desmethyl tetrachlorvinphos, 2,4,5-trichlorophenylethandiol glucuronide, and 2,4,5-trichloromandelic acid. Metabolism and excretion of radioactive tetrachlorvinphos in rats is rapid and majority of radioactivity appears between 0 and 24 h of exposure in urine and feces.

Mechanism of Toxicity

Like other organophosphorus insecticides, tetrachlorvinphos exerts toxicity by inhibiting the enzyme acetylcholinesterase (AChE). AChE inhibition results in accumulation of the neurotransmitter acetylcholine in the cholinergic synapse leading to overstimulation of postsynaptic receptors and cholinergic toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute toxicity studies in laboratory animals have shown that tetrachlorvinphos is one of the least toxic organophosphorus insecticides with an oral LD_{50} of $>2000\,mg\,kg^{-1}$ in laboratory animals. General signs of acute poisoning include salivation, diarrhea, urination, lacrimation, sweating, blurred vision, miosis, bradycardia, increased bronchial secretions, emesis, headache, dizziness, anxiety, lethargy, seizures, and depression of respiratory and cardiovascular centers.

Human

Like other organophosphorus insecticides, tetrachlorvinphos can cause cholinesterase inhibition in humans. Tetrachlorvinphos is classified as a skin sensitizer.

Chronic Toxicity (or Exposure)

Animal

Carcinogenicity studies in rats have shown that high doses of tetrachlorvinphos produce adrenal, thyroid, and hepatocellular carcinoma. Experiments in hens have shown that tetrachlorvinphos does not cause delayed neurotoxicity.

Human

Tetrachlorvinphos is classified as a possible human carcinogen. It is not registered for use on any food or feed crop. Human dietary exposure is mainly secondary through livestock uses. Industrial and agricultural workers who are involved in handling and applying tetrachlorvinphos are at a higher risk of exposure.

In Vitro Toxicity Data

In vitro studies have shown that tetrachlorvinphos is not mutagenic in bacteria and is a weak inducer of chromosomal aberrations in human lymphocytes. Studies with plasma and erythrocyte cholinesterases from different species have revealed that the

nontarget enzyme butyrylcholinesterase can be remarkably more sensitive to tetrachlorvinphos than the target enzyme acetylcholinesterase.

Clinical Management

General decontamination procedures should be followed immediately in case of tetrachlorvinphos poisoning. For skin decontamination, the contaminated area should be washed with water using soap and shampoo. If eyes are contaminated, they should be flushed with plenty of water repeatedly for 10–15 min. Contaminated clothing should be removed and a clear airway ensured. In case of ingestion, oral secretions should be removed and gastrointestinal decontamination started. Activated charcoal ($1\,g\,kg^{-1}$, $\sim 5\,ml\,g^{-1}$) may be used if the poisoning is detected within 60 min. Atropine treatment should be initiated immediately to counteract muscarinic effects. Atropine (adults and children >12 years: 2–4 mg; children <12 years: 0.05–0.1 mg) treatment should be repeated every 15 min until oral and bronchial secretions are controlled and atropinization is achieved. The duration and dosage of atropine treatment should be slowly reduced as the condition of the patient improves. Pralidoxime should be administered slowly at the recommended dosage (adults and children >12 years: 1–2 g; children <12 years: 20–50 mg by intravenous infusion in 100 ml saline at $\sim 0.2\,g\,min^{-1}$). This dosage can be repeated at every 1–2 h intervals initially and at 10–12 h intervals later depending on the condition of the patient.

Environmental Fate

Tetrachlorvinphos is nonpersistent in the environment. Based on the current use pattern, risks of contamination of ground or surface water by tetrachlorvinphos are minimal.

Ecotoxicology

Studies in birds indicate that tetrachlorvinphos is practically nontoxic to birds while it is highly toxic to fish and other aquatic organisms. It is also considered to pose minimal risk to wildlife.

See also: Acetylcholine; Cholinesterase Inhibition; Neurotoxicity; Organophosphates; Veterinary Toxicology.

Further Reading

Karanth S and Pope C (2003) *In vitro* inhibition of blood cholinesterase activities from horse, cow and rat by tetrachlorvinphos. *International Journal of Toxicology* 22: 429–433.

Vinggaard AM, Hnida C, Breinholt V, and Larsen JC (2000) Screening of selected pesticides for inhibition of CYP19 aromatase activity *in vitro*. *Toxicology In Vitro* 14: 227–234.

Relevant Website

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

Tetrahydrofuran

Sree L Jasti

This article is a revision of the previous print edition article by Edward Kerfoot, volume 3, pp. 222–224, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 109-99-9
- Synonyms: Cyclotetramethylene oxide; Diethylene oxide; THF; Tetramethylene oxide
- Chemical/Pharmaceutical/Other Class: Substituted epoxide
- Chemical Formula: C_4H_8O
- Chemical Structure:

Uses

Tetrahydrofuran is a solvent used in natural and synthetic polymers and resins such as polyvinyl chloride and vinylidene chloride copolymers. It is also used in the manufacture of lacquers, glues, paints, and inks.

Exposure Routes and Pathways

Industrial exposures to tetrahydrofuran are most likely to occur by inhalation with skin and eye contact possible. Accidental ingestion is also possible. Based on physical and chemical properties, production, use patterns, and environmental monitoring levels in the low ppb range, the environmental exposure potential is expected to be low.

Toxicokinetics

When healthy volunteers were exposed by inhalation to 100 or 400 ppm tetrahydrofuran in air, the percentage of expired tetrahydrofuran was 25–35%. The elimination half-life of tetrahydrofuran was 30 min in individuals exposed to 200 ppm for 3 h. Some tetrahydrofuran is absorbed in the nasal cavity due to its solubility and inspiratory flow rate. Tetrahydrofuran uptake in the nasal tissue is dependent on its reaction with tissue substrates. Some tetrahydrofuran can be metabolized in the nasal cavity. Tetrahydrofuran blood concentrations were higher at 1 h postexposure than immediately after cessation of exposure. *In vitro* studies indicated that tetrahydrofuran was first hydroxylated by microsomal enzymes. High concentrations (10^{-2} mol l^{-1}) of tetrahydrofuran inhibited the *in vitro* activity of rat hepatic cytochrome P450 by 80%. Tetrahydrofuran has been noted to enhance the toxic action of a number of compounds and stimulate the rapid absorption of reactive metabolites. Some of the tetrahydrofuran is excreted in the exhaled breath, while the various metabolites of tetrahydrofuran are excreted in the urine.

Additional metabolism studies have shed some light on a proposed pathway for tetrahydrofuran conversion to CO_2. *In vitro* metabolism studies with hepatic microsomes from rats, mice or humans identified γ-hydroxybutyric acid as a metabolite, a compound which is a potential intermediate in the formation of succinic acid. *In vitro* data demonstrated that liver macrodomes in mice have a greater inherent capacity to metabolize tetrahydrofuran than human or rat macrodomes; however, no data are available to confirm this *in vivo*. *In vivo* studies identify CO_2 as the major terminal metabolite. It has been found that in both rats and mice the metabolic pathway is increasingly saturated at high doses although there is some indication of species differences; however, experimental losses of CO_2 in the rat study make it difficult to interpret the data. Based on the *in vitro* and *in vivo* metabolism data, tetrahydrofuran undergoes oxidative metabolism to γ-butyrolactone, which is further metabolized to γ-hydroxybutyric acid, and then to the endogenous compound, succinic acid. Succinic acid, in its ionized form (succinate), undergoes a series of reactions through the citric acid cycle leading to the release of CO_2. Recent *in vivo* studies in mice have also provided evidence of P450 induction, that is, both ethoxyresorufin-*O*-deethylase activity and pentoxyresorufin-*O*-dependentylase activity, suggesting that tetrahydrofuran may be metabolized by CYP 1A/2B isoforms. Tetrahydrofuran is readily absorbed through multiple routes in animals, is systematically distributed and rapidly metabolized and excreted, suggesting that tetrahydrofuran does not bioaccumulate.

No physiologically based pharmacokinetic (PBPK) models are available for tetrahydrofuran in animals. Based on human volunteer studies, a PBPK model for tetrahydrofuran was developed which predicts rapid elimination of tetrahydrofuran from the body. The human PBPK model predicts that repeated inhalation exposure of 200 ppm would yield end of the work shift levels of tetrahydrofuran of 5.1 ppm in breath, 57 mol l^{-1} in the blood, and 100 mol in the urine.

Mechanism of Toxicity

Irritation of the upper respiratory tract is attributed to the solubility of tetrahydrofuran in the mucous membranes causing irritation of the sensory nerve endings. The direct action of tetrahydrofuran on the skin and eyes is the result of irritation of these tissues. Carcinogenic responses in male rat kidney and female mouse liver are through nongenotoxic mechanisms. Tetrhahydrofuran enhances tumor formation in male rat kidneys and female mouse liver via induction of cell proliferation. The induced cell proliferation, in the female mice liver was associated with an increased cytochrome P450 content. Increased cell proliferation in male rat kidney was coupled with α-2U-globulin accumulation in the renal cortex, indicating a mechanism for tumor formation, which is rodent specific and may not be relevant for human health risk assessment.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} in rats for tetrahydrofuran is 2.3 ml kg^{-1}. A single 4 h inhalation study of tetrahydrofuran in rabbits at 100–12 000 ppm produced a transient dose-related decrease of tracheal ciliary activity. Concentrations of tetrahydrofuran $\geqslant$25 000 ppm produced anesthesia. Tetrahydrofuran was irritating to rabbit skin when applied topically in solutions exceeding a 20% concentration. No lasting acute adverse effects on neurological endpoints were seen with THF exposures of 0, 500, 2500, or 5000 ppm for 6 h in rats except for sedation. The no-observed-effect level (NOEL) for the acute neurotoxicity in rats was 500 ppm.

Human

Exposure to tetrahydrofuran has been reported to cause irritation of the skin, eyes, and respiratory

tract. Individuals exposed to high concentrations of tetrahydrofuran have complained of nausea, dizziness, tinnitus, headache, and central nervous system (CNS) depression. Narcosis has been observed in humans exposed to tetrahydrofuran at ~25 000 ppm. The probable oral lethal dose in humans is estimated to be between 50 and 500 mg kg^{-1}.

Chronic Toxicity (or Exposure)

Animal

Male rats exposed to 5000 ppm tetrahydrofuran for 12 weeks at 4 h day^{-1} showed signs of systemic intoxication, skin and respiratory tract irritation, liver function disturbance, and abnormalities in glucose metabolism. Some rats exhibited slight respiratory tract irritation at 200 ppm. In another study, male rats exposed to 200, 1000, or 2000 ppm for 18 weeks at 6 h day^{-1} demonstrated an increase in muscle acetylcholinesterase activity in a concentration-dependent manner. Rats exposed to 200 ppm tetrahydrofuran, 4 h day^{-1}, 5 days week^{-1}, exhibited damage to the nasal and tracheal epithelium. At 1000 ppm tetrahydrofuran, severe damage to the same structures was observed. No lasting adverse effects on neurological endpoints, except for sedation, were seen with tetrahydrofuran exposures in rats after subchronic exposures of 0, 500, 1500, or 3000 ppm, 6 h day^{-1}, 5 days week^{-1}. The NOEL for subchronic neurotoxicity in rats was 500 ppm.

Rats and mice were exposed for 6 h day^{-1}, 7 days week^{-1} on gestation days 6–19 for rats and 6–17 for mice at 600, 1800, or 5000 ppm tetrahydrofuran. Pregnant mice that inhaled 5000 ppm tetrahydrofuran died, while those exposed to 1800 ppm were sedated. Some treatment-related effects were reduced fetal body weight and reduced ossification of the sternebrae. The maternal no-observed-adverse-effect level (NOAEL) for both species was 1800 ppm; the NOAEL for developmental toxicity was 1800 ppm in rats and 600 ppm in mice. A two-generation drinking water reproductive study in rats at 0, 1000, 3000, and 9000 ppm resulted in a NOAEL for fertility and reproductive performance of 9000 ppm. A NOAEL of 3000 ppm was also established for general systemic toxicity of parental generations, $F_1$and F_2 litters and developmental toxicity. Developmental effects were reduced pup growth and delayed eye opening.

The National Toxicology Program (NTP) completed prechronic oral gavage and inhalation studies of tetrahydrofuran in male and female Fischer 344 rats and B6C3F1 mice. No chronic gavage study was performed. However, NTP completed an inhalation bioassay with tetrahydrofuran in male and female Fischer 344 rats and B6C3F1 mice, which resulted in a significantly increased incidence of hepatocellular neoplasms in female mice and a positive trend for increased incidence of renal tubule epithelial adenoma or carcinoma (combined) in male rats.

Male and female Fischer 344 rats exposed for 2 years to inhalation concentrations of 200, 600, and 1800 ppm tetrahydrofuran showed marginally increased incidences of renal tubule epithelial adenoma in male rats at the mid- and high- concentration exposures. The combined occurrence of renal tubule epithelial adenoma and carcinoma exhibited a positive trend. The incidence of combined tumors in mid and high dose males exceeded the historical control range. No tumors were observed in female rats. NTP concluded that tetrahydrofuran showed some evidence of carcinogenic activity in male rats but no evidence in female rats. The mode of action for the kidney tumors observed in male rats is via a nongenotoxic mechanism with associated increases in cell proliferation and evidence that supports a role of α-2U-globulin.

Male and female B6C3F1 mice exposed for 2 years to inhalation concentrations of 200, 600, and 1800 ppm tetrahydrofuran showed increased incidences of hepatocellular neoplasms (adenoma and carcinoma) in high dose females (85%) that were significantly greater than chamber controls (34%) and exceeded the historical control range (3–54%). The incidences of hepatocellular neoplasms in male mice (low dose 62%, mid-dose 60%, and high dose 36%) were not significantly different than chamber controls (70%). The historical control range in males is 11–60%. The lower incidence of combined neoplasms in high dose males was attributed to their lower survival rate. NTP concluded that tetrahydrofuran exhibits no evidence of carcinogenicity in male mice but showed clear evidence of carcinogenic activity in female mice. The mode of action for the liver tumor formation is via a nongenotoxic mechanism with evidence of an associated cell proliferation and cytochrome P450 induction.

Human

No information could be found on the effects of chronic exposure of tetrahydrofuran in humans.

In Vitro Toxicity Data

Tetrahydrofuran was not mutagenic in *Salmonella typhimurium* TA100 at 50 µl per plate and it failed to induce sex-linked recessive lethals in *Drosophila melanogaster* by ingestion or injection. In cultured

Chinese hamster ovary cells, there was no indication of induction of chromosomal aberrations or sister chromatid exchanges. The weight of evidence from several studies indicates that tetrahydrofuran is nongenotoxic.

Clinical Management

Those exposed to tetrahydrofuran by inhalation should be monitored for respiratory tract irritation, bronchitis, or pneumonitis. Humidified supplemental 100% oxygen should be administered. Following ingestion, milk or water should be used to dilute the tetrahydrofuran in the stomach. A charcoal slurry with saline cathartic should be administered. Gastric lavage may be indicated. Treatment of CNS depression is symptomatic. Renal and hepatic function should be monitored. Exposed eyes should be irrigated with copious amounts of water for at least 15 min. If irritation, pain, swelling, lacrimation, or photophobia persists, the patient should be seen in a healthcare facility. After dermal exposure, the affected skin should be washed thoroughly with soap and water. If irritation persists, a healthcare facility should be contacted.

Environmental Fate

Tetrahydrofuran is a liquid at room temperature and boils at 66°C. The fugacity model predicts that tetrahydrofuran will be found in the environment where it is released. Photodegradation by hydroxyl radicals in air is estimated to be rapid and the hydroxyl radical reaction half-life is estimated at 7.3 h. Tetrahydrofuran released to water could partition to the water compartment and readily biodegrade, but not hydrolyze. Tetrahydrofuran has a very low bioaccumulation potential as evidenced by its low octanol/water partition coefficient.

Ecotoxicology

Tetrahydrofuran is essentially nontoxic to aquatic organisms. The 96 h LC_{50} value in static acute fish (*Pimephales promelas*) was 2160 mg l^{-1}; 24 h LC_{50} in *Daphnia magna* was 5930 mg l^{-1}; and the 8 days no-observed-effect concentration (NOEC) in algae, *Schenedesmus quandricauda* was 3700 mg l^{-1}. An NOEC of 216 mg l^{-1} was established in a fish early life stage test with the Fathead minnow.

Other Hazards

Tetrahydrofuran is a flammable liquid with a flash point of 6°F and explosive limits ranging from 2% (lower) to 11.8% (upper). It is incompatible with strong oxidizers and lithium-aluminum alloys. Peroxides may accumulate upon prolonged storage in the air.

Exposure Standards and Guidelines

Occupational exposure limits generally range between 50 and 600 ppm internationally and are expressed as an 8 h time-weighted average (TWA), with 200 ppm being most commonly used. The US Occupational Safety and Health Administration, the American Conference of Governmental Industrial Hygienists, and the National Institute for Occupational Safety and Health (NIOSH) have established an 8 h threshold limit value (TLV)TWA of 200 ppm and a 15 min TLV short-term exposure limit of 250 ppm based on irritation and narcosis. NIOSH lists a concentration of 20 000 ppm tetrahydrofuran as immediately dangerous to life and health.

See also: Respiratory Tract; Sensory Organs.

Further Reading

Chhabra RS, Elwell MR, Chou B, Miller RA, and Renne RA (1990) Subchronic toxicity of tetrahydrofuran vapors in rats and mice. *Fundamental and Applied Toxicology* 14: 338–345.

Mast TJ, Weigel RJ, Westerberg RB, Schwetz BA, and Morrissey RE (1992) Evaluation of the potential for developmental toxicity in rats and mice following inhalation exposure to tetrahydrofuran. *Fundamental and Applied Toxicology* 18: 255–265.

Tetranitromethane

Ruth Custance and Cathy Villaroman

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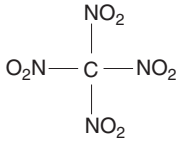
- Chemical Abstracts Service Registry Number: CAS 509-14-8
- Synonyms: TNM; Tetan
- Chemical/Pharmaceutical/Other Class: Aliphatic nitro compounds
- Chemical Formula: CN_4O_8
- Chemical Structure:



Uses

Tetranitromethane is used as an oxidizer in rocket propellants, as a diesel fuel additive, and as an explosive. It is used as a biochemical agent to nitrate tyrosine proteins. It is also used as an organic reagent for detecting the presence of double bonds, and as a mild nitrating reagent, reacting with tyrosine residues in proteins and peptides.

Exposure Routes and Pathways

Tetranitromethane is an oily liquid with a vapor pressure less than that of water. It occurs as an impurity in 2,4,6-trinitrotoluene (TNT). The primary routes of potential human exposure are inhalation and dermal contact. Historically, exposure to tetranitromethane presumably occurred during the manufacture and use of TNT.

Toxicokinetics

No data exist regarding the absorption of tetranitromethane; however, based on toxicity reported in humans and animals, it is clear that it is readily absorbed by the oral route and through inhalation. Rats administered single oral doses exhibited dose-related methemoglobinemia at 90 min, suggesting that the metabolism of tetranitromethane results in the formation of nitrites. Methemoglobinemia was not observed following intravenous or inhalation exposures, suggesting that the blood effects seen in oral studies resulted from nitrase reduction in the gut. No data are available regarding the distribution of absorbed tetranitromethane. No elimination data are available for this compound.

Mechanism of Toxicity

The mechanism of toxicity for tetranitromethane is not known. Methemoglobinemia formation reported following oral administration may be a result of reduction of tetranitromethane in the gut. Nasal lesions observed in lifetime inhalation studies of rats and mice were attributed to the significant irritating properties of the material.

Acute and Short-Term Toxicity (or Exposure)

Animal

Tetranitromethane is a strong irritant of the eyes and respiratory tract in animals. Tetranitromethane is highly toxic to mice and rats by the oral and inhalation routes. In rats with 4 h exposure, an inhalation LC_{50} of 17.5 ppm and an oral LD_{50} of 130 mg kg^{-1} have been reported. The corresponding values in mice are 54.4 ppm and 375 mg kg^{-1}. Effects of overexposure in animals include eye and respiratory irritation, pulmonary edema, lung injury, bronchopneumonia, liver and kidney injury, and in cats, methemoglobinemia. The treatment also affected the body weight of rats and mice and survival of the male animals in the high exposure group.

Human

Tetranitromethane is a strong irritant of the eyes and mucous membranes, which can subsequently cause runny nose, tearing, burning, and redness of the eyes. Other symptoms from acute exposure include coughing, difficult breathing, chest pain, dizziness, and mild skin burns. Workers exposed to the heated tetranitromethane have complained of irritation of the eyes and respiratory tract.

Chronic Toxicity (or Exposure)

Animal

Rats exposed to tetranitromethane at a concentration of 6.4 ppm for 6 h day^{-1}, 5 days week^{-1} for 6 months died; autopsy revealed lung damage. In other lifetime inhalation studies, tetranitromethane caused nasal lesions indicative of chronic irritation of the nasal cavity in rats and mice. In addition, in a National Toxicology Program inhalation bioassay,

tetranitromethane caused increased incidences of alveolar and bronchiolar neoplasms in rats and mice and lung carcinoma in rats.

Human

Chronic exposure to TNT may damage the liver and kidneys with repeated exposure potentially causing low blood cell count (anemia) and nervous system damage. Other symptoms from long-term exposures include headache, drowsiness, chest pain, and respiratory distress. Salivation, shortness of breath, coughing, and pulmonary edema have also been reported. Exposure to high levels can interfere with the ability of the blood to carry oxygen, causing headaches, fatigue, dizziness, and a blue color to the skin and lips (methemoglobinemia). Deaths due to methemoglobinemia and respiratory failure have been reported following exposure to crude TNT.

No adequate human studies have shown a relationship between exposure to tetranitromethane and human carcinogenicity. However, based on sufficient evidence of carcinogenicity in experimental animals, tetranitromethane was classified as possibly carcinogenic to humans (group 2B).

In Vitro Toxicity Data

Tetranitromethane was positive when tested with and without metabolic activation in *Salmonella typhimurium*. In addition, sister chromatid exchanges were induced in cultured Chinese hamster ovary cells when tested without or with a metabolic activation system and chromosome aberrations were also induced. Tetranitromethane also induced DNA single-strand breaks in an *in vitro* assay using primary rat hepatocytes.

Clinical Management

If contact with the liquid occurs, affected areas should be flushed thoroughly with water for at least 15 min. The victim should be observed for burns or resulting irritation. In case of inhalation, the victim should be moved to fresh air, an airway established, and respiration maintained as necessary. The patient should be monitored for irritation and pulmonary edema. If ingestion occurs, emesis should be induced if the victim is conscious. Gastric lavage may be indicated if the victim is unconscious or convulsing. Treatment for methemoglobinemia and/or monitoring for possible liver and kidney injury may be required.

Other Hazards

Tetranitromethane is an oxidizer that may react with a wide variety of materials including organics, brass, zinc, cotton, sodium, pyridine, toluene, aluminum, and finely powdered metals. It is considered heat, friction, and shock sensitive. It may also decompose or react with other chemicals violently.

Exposure Standards and Guidelines

The occupational exposure standards and guidelines for tetranitromethane include the following:

- American Conference of Governmental Industrial Hygienists threshold limit value of 5 ppb ($40\ \mu g\ m^{-3}$).
- US National Institute for Occupational Safety and Health recommended exposure level of 1 ppm as a 10 h time-weighted average, and an immediately dangerous to life and health value of 4 ppm.
- US Occupational Safety and Health Administration permissible exposure limit of 1 ppm ($8\ mg\ m^{-3}$).

Miscellaneous

Tetranitromethane is a colorless to pale yellow, oily liquid with a pungent odor. It is an oxidizer that is highly explosive in the presence of impurities. Tetranitromethane is the primary volatile contaminant of TNT, comprising up to 0.12% of the crude material.

See also: Respiratory Tract.

Further Reading

NTP (1990) National Toxicology Program. Technical Report Series No. 386. Toxicology and Carcinogenesis Studies of Tetranitromethane (CAS 509-14-8) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). NIH Publication No. 90-2841. 207 pp. National Toxicology Program, Research Triangle Park, NC, and Bethesda, MD.

Relevant Websites

http://chem.sis.nlm.nih.gov

http://www.osha.gov – Occupational Safety and Health Administration (OSHA). *Occupational Safety and Health Guideline for Tetranitromethane*. September 1996.

http://ehp.niehs.nih.gov – Tetranitromethane: Tenth Report on Carcinogens.

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

Tetrodotoxin

Elizabeth J Scharman

- Chemical Abstracts Service Registry Number: CAS 4368-28-9
- Synonyms: TTX; Tarichatoxin; Tetrodontoxin; Fugu poison; Maculotoxin (MTX); Spheroidine
- Chemical Formula: $C_{11}H_{17}N_3O_8$

Background Information

Tetrodotoxin is a nonprotein, water-soluble, heat-stable neurotoxin found in fish from the order Tetraodontiformes whose suborders include the Tetrodontoidei (pufferfish and porcupine fish) and Moloidei (sunfish). Tetrodotoxin is thought to be identical to tarichatoxin found in selected North American and Japanese newts (e.g., California newt *Tarichatorosa*), salamanders of the family Salamandridae, Central American frogs (genus *Atelopus*), the goby (*Gobius criniger*), some shellfish (e.g., trumpet shell, *Babylonia japonica*), starfish (*Astropecten polyacanthus*), some species of ribbonworm, the flatworm, crab *Atergatus floridus*, horseshoe crab, some species of red calcareous alga, and the Australian blue-ringed octopus.

Exposure Routes and Pathways

Exposure occurs through ingestion of flesh, viscera (e.g., liver, gonads), or skin containing tetrodotoxin. The viscera contain the highest concentration.

Toxicokinetics

Tetrodotoxin is readily absorbed from the gastrointestinal tract. Effects can occur within 10 min to 4 h. The toxin can also be absorbed through the skin.

Mechanism of Toxicity

Tetrodotoxin is believed to be synthesized by a bacterial or dinoflagellate species. Tetrodotoxin blocks axonal transmission by lowering the conductance of sodium at nodes of Ranvier. It is a selective sodium channel blocker that can block nerve and muscle conduction; action potentials are blocked while resting membrane potentials and resting membrane resistance are not affected. Tetrodotoxin does not affect the presynaptic release of acetylcholine or acetylcholine's effects on the neuromuscular junction. Vomiting occurs because the toxin can act directly at or near the chemoreceptor trigger zone. Respiratory depression is caused by either a specific action of tetrodotoxin on the brain's respiratory center or because paralysis of respiratory nerves and muscles occurs.

Acute and Short-Term Toxicity (or Exposure)

Human

A dose of 1–2 mg of purified tetrodotoxin can be lethal; however, because the concentration of tetrodotoxin varies greatly among species, a toxic quantity of pufferfish or other tetrodoxin containing species is not well defined. Paresthesia of the lips and tongue begins shortly after ingestion. Facial and extremity paresthesias and numbness follow. Diaphoresis, hypersalivation, dysphagia, vomiting, diarrhea, and abdominal pain occur early in the course of toxicity as do lightheadedness, dizziness, headache, ataxia, and weakness. Weakness develops first in the hands and arms and then in the legs. Hypoventilation and speech difficulties occur followed by ascending flaccid paralysis with respiratory depression. If ventilation is maintained, victims may remain conscious even though they are paralyzed. Hypotension, dysrhythmias, and seizures may develop. Death occurs within 4–6 h; usually from respiratory muscle paralysis. The prognosis is stated to be good if the patient survives the first 24 h.

In Vitro Toxicity Data

Tetrodotoxin is an important research tool because of its unique voltage gated sodium channels blocking properties. Applications include assessment of pain, basic physiology of nerve generation and organization, understanding of hearing, to bladder pain.

Clinical Management

No antidote is available. Tetrodotoxin is adsorbed by activated charcoal. Treatment is symptomatic and supportive with special attention to airway management and cardiac support.

See also: Marine Organisms; Shellfish Poisoning, Paralytic.

Further Reading

CDC (1996) Tetrodotoxin poisoning associated with eating puffer fish transported from Japan–California. *Morbidity and Mortality Weekly Report* 45: 389–391.

King BR, Hamilton RJ, and Kassutto Z (2000) 'Tail of newt': An unusual ingestion. *Pediatric Emergency Care* 16: 268–269.

Noguchi T and Ebesu JSM (2001) Puffer poisoning: Epidemiology and treatment. *Journal of Toxicology: Toxin Reviews* 20: 1–10.

Texas City Disaster

Paramasivam Srinivasan

The Texas City Disaster is generally considered to be one of the most significant industrial accidents in US history. The following provides a brief summary of the event.

Incident Narrative

The clear spring morning of April 16, 1947, was a morning that many citizens of Texas City thought was the end of the world. The French Liberty ship, SS *Grandcamp*, bearing a cargo of ammonium nitrate fertilizer (over 2300 tons) destined for war-torn Europe, caught fire in the Texas City harbor. The bright orange color that came out of the black smoke caught the attention of passers by. Many people, including children, gathered to watch firefighters putting out the fire. The crowd did not have any idea that potentially explosive materials were stored in the ship.

There was neither a full-scale emergency response plan nor a tug boat to tow the dangerously burning *Grandcamp* away from the port. A little after 9.00 a.m., the Texas City Disaster (as it is popularly known) occurred as the ship *Grandcamp* exploded. A great column of smoke shot up to an estimated 2000 ft, followed in ~10 s, by another even more violent shockwave. Within minutes of the second blast, the Monsanto Chemical Plant was in flames that resulted from broken lines and shattered containers. Entire buildings collapsed and people were trapped inside. Fire spread to the refineries that made up the Texas City industrial complex.

Another catastrophic event happened when a miniature tidal wave (resulted due to the bay water being thrown away by the explosion) swept everything in its path of 150 ft inland and over the docks. Fear mounted throughout the night of April 16, 1947, as the another firefighter the High Flyer, loaded with ammonium nitrate and sulfur, burning all day long, exploded at early morning 1.10 a.m. on April 17, 1947. The fire from High Flyer destroyed a concrete warehouse and a grain elevator and triggered even more fires.

The losses from the disaster were unprecedented. Nearly 600 deaths in a town of ~16 000 was a terrible toll. Not a single family could be found that did not suffer a death, an injury, or severe property damage.

Ammonium Nitrate Characteristics

The chemical compound ammonium nitrate, the nitrate of ammonium with chemical formula NH_4NO_3 is commonly used in agriculture as a high-nitrogen fertilizer. It is a crystalline powder, varying in color from almost white to brown. As a strong oxidizing agent, it has applications as a component of explosives. Ammonium nitrate decomposes into gases including oxygen when heated (nonexplosive reaction); however, ammonium nitrate can be induced to decompose explosively by detonation.

Ammonium Nitrate Involved in Texas City Explosion

The ammonium nitrate involved in the Texas City explosion was brown in color and in small pellets or grains about the size of medium grains of sand. It was packed in six-ply moisture-proof paper bags, two of which were impregnated with some material, apparently an asphaltic compound.

Causes for the Explosion

During the time frame of occurrence of this explosion, little was known regarding the hazards of ammonium nitrate to anyone handling or storing this commodity. The false security engendered in the handling of ammonium nitrate, which was such a major factor in this disaster, was caused by the improper labeling of the paper bags. No instruction was printed on the bags concerning the handling of the material nor was it labeled as being a hazardous chemical. The storage of ammonium nitrate pending

shipment either by ship or railroad had not received the attention it deserved.

Selected web information related to the Texas City Disaster event says whether or not the fire originated from smoking. Smoking in piers or on docks must be considered as a common source of ignition and always be prohibited regardless of the cargo being handled. The use of open lights in these same areas should carry the same restriction as smoking regulations.

Health and Safety Measures

The lessons learned from the Texas City Disaster event from health and safety viewpoint are:

- Anyone dealing with or handling ammonium nitrate should be fully advised of the hazardous nature of the chemical and fully instructed about the proper methods of storage and handling. Proper labeling of the containers is of utmost importance.
- Material should be stored only in masonry or fireproof sprinkled buildings on skids or pallets on concrete floors with at least 1 ft clearance from walls.
- Storage should preferably be in separate fire divisions from highly combustible commodities or well segregated from not so highly combustible commodities such as sulfur, flour, sugar, compressed cotton, and charcoal.
- Intimate contact with metals such as cadmium, zinc, copper, tin, and lead must be avoided.
- A minimum clearance of 5 ft should be maintained between ammonium nitrate and other chemicals.
- Any ship with hazardous material such as ammonium nitrate as cargo entering a port must notify the port facility who in turn should notify the chief of the fire department immediately.
- Fire departments combating ammonium nitrate fires should use only water in large quantities (applied gently so as not to scatter the material) as an extinguishing agent.
- Fire in ammonium nitrate usually generates large quantities of oxides of nitrogen gases which are extremely toxic and therefore all personnel entering the fire area must wear masks approved for use in such locations.

See also: Ammonium Nitrate; Cadmium; Copper; Lead; Tin; Zinc.

Further Reading

Stephens HW (1997) *The Texas City Disaster, 1947*, 1st edn. Austin, TX: University of Texas Press.

Relevant Websites

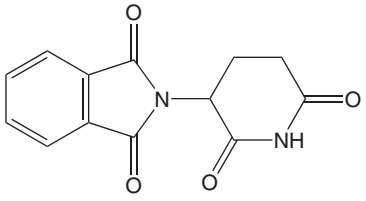
http://www.local1259iaff.org – The Texas City Disaster. April 16, 1947.

http://www.tsha.utexas.edu – Handbook of Texas Online. Texas City Disaster.

Thalidomide

S Rutherfoord Rose

- Chemical Abstracts Service Registry Number: CAS 50-35-1
- Synonyms: K-17; 2-Phthalimidoglutarimide; *N*-Phthalylglutamic acid imide; *N*-(2,6-Dioxo-3-piperdyl)-phthalimide; Talimol®; Sedalis®; Kevadon®; Distavil®; Thalomid®; NSC-66847
- Chemical/Pharmaceutical/Other Class: Thalidomide is a piperidinedione derivative
- Chemical Formula: $C_{13}H_{10}N_2O_4$
- Chemical Structure:



Uses

Thalidomide was formerly used as a sedative/hypnotic. It was approved in 1998 by the US Food and Drug Administration for use as an immunosuppressant in the treatment of erythema nodosum leprosum. It is also used in the treatment of graft-versus-host disease (Orphan drug status in the United States), macular degeneration, oral ulcers in AIDS patients, and reflex sympathetic dystrophy associated with chronic pain syndromes.

Background Information

Thalidomide was first marketed as a sedative/hypnotic agent in Germany in 1956 and in the United Kingdom 2 years later. It was subsequently withdrawn from the market in 1961 after more 12 000 reports of fetal abnormalities, particularly phocomelias.

Exposure Routes and Pathways

Exposures have only been reported by ingestion.

Toxicokinetics

All available data are derived from therapeutic dosing. Peak plasma levels occur 2–6 h following oral doses. Bioavailability in animals varies from 67% to 93%. It is a nonpolar compound that is extensively bound to plasma proteins and has a volume of distribution of ~120 l in healthy adults. There are conflicting data on whether thalidomide undergoes hepatic metabolism. Less than 1% of a dose is excreted unchanged in the urine, suggesting that elimination is largely nonrenal. The serum elimination half-life is ~8 or 9 h after a single oral dose.

Mechanism of Toxicity

Thalidomide has significant teratogenic effects in humans, and it also affects the central and peripheral nervous systems through unknown mechanisms. Evidence of a toxic arene oxide metabolite is unsubstantiated. Thalidomide likely inhibits neutrophil chemotaxis and monocyte phagocytosis, inhibits free radical formation, and alters the ratio of helper and suppressor T-cells. Reduced formation of tissue necrosis factor-α may be at least partially responsible for the antiinflammatory effects of thalidomide.

Acute and Short-Term Toxicity (or Exposure)

Human

In addition to phocomelias, other teratogenic effects include eye and ear abnormalities, esophageal and duodenal atresias, and defects in internal organs such as the heart and kidneys. Congenital defects of the kidneys and nervous system may persist throughout life. Very large doses taken with ethanol have been associated with transient hypotension. Bradycardia has been rarely reported with therapeutic use. Acute toxicity appears infrequent.

Chronic Toxicity (or Exposure)

Animal

Teratogenic effects of thalidomide are well described in several animal models. Pregnant cats have tolerated doses of $500\,mg\,kg^{-1}\,day^{-1}$ of thalidomide with no fetal toxicity evident in offspring. Many rat strains have had no teratogenic effects seen at doses of $4000\,mg\,kg^{-1}\,day^{-1}$ during pregnancy.

Human

Adverse reactions include dose-related peripheral neuropathy (primarily sensory), nausea, vomiting, constipation, dry mouth, headache, and erythematous rashes. Dose-related central nervous system depression is relatively common. Thalidomide is contraindicated in pregnancy and in women of childbearing age.

In Vitro Toxicity Data

Ames *Salmonella*, *Drosophila*, and male mouse sperm morphology assays of thalidomide have been negative; mutagenicity tests using *Allium cepa* and *Vicia faba* have been positive.

Clinical Management

Patients with thalidomide overdose should receive supportive care with attention to airway maintenance. There are no antidotes and no data to support measures to enhance elimination of thalidomide. Hypotension should be treated if needed with intravenous fluids, positioning, and pressors as needed.

See also: Neurotoxicity.

Further Reading

Clark TE, Edom N, Larson J, *et al.* (2001) Thalomid(R) (thalidomide) capsules. A review of the first 18 months of spontaneous postmarketing adverse event surveillance, including off-label prescribing (review). *Drug Safety* 24: 87–117.

Lenz W (1988) A short history of thalidomide embryopathy. *Teratology* 38: 203–215.

Mellin GW and Katzenstein M (1962) The saga of thalidomide. Neuropathy to embryopathy, with case reports on congenital abnormalities. *New England Journal of Medicine* 267: 1184–1193. (see also pp. 1238–1244).

Nasca MR, Micalli G, Cheigh NH, *et al.* (2003) Dermatologic and nondermatologic uses of thalidomide. *Annals of Pharmacotherapy* 37(9): 1307–1320.

Tseng S, Pak G, Washenik K, *et al.* (1996) Rediscovering thalidomide: A review of its mechanism of action, side effects, and potential uses. *Journal of the American Academy of Dermatology* 35: 969–979.

Relevant Website

http://www.nlm.nih.gov – US National Library of Medicine. Thalidomide: Potential Benefits and Risks. Current Bibliographies in Medicine 97-4. August 1997.

Thallium

Shayne C Gad

- Selected Compounds: Thallium nitrate ($TlNO_3$); Thallium sulfate (Tl_2SO_4)
- Chemical Abstracts Service Registry Number: CAS 7440-28-0
- Synonym: Ramor
- Chemical/Pharmaceutical/Other Class: Metal
- Chemical Formulas: Tl^+; Tl^{3+}

Uses

Thallium is a by-product of iron, cadmium, and zinc refining. It is used in metal alloys, imitation jewelry, optical lenses, artists' pigments, semiconductors, ceramics, and X-ray detection devices. It has limited use as a catalyst in organic chemistry. In the past, thallium (chiefly thallium sulfate) was used as a rodenticide and insecticide. Its use as a rodenticide was outlawed in 1965 due to its severe toxicity (a source of accidental and suicidal human exposures). Medicinally, it has been used as a depilatory and in the treatment of venereal disease, skin fungal infections, and tuberculosis.

Background Information

It was discovered in 1861, and occurs in the earth's crust at 0.7 ppm. Thallium is a heavy metallic element that exists in the environment mainly combined with other elements (primarily oxygen, sulfur, and the halogens) in inorganic compounds.

Exposure Routes and Pathways

Industrial poisoning from thallium is a special risk in the manufacture of fused halides for the production of lenses and windows. Humans may be exposed to thallium by ingestion, inhalation, or dermal absorption. However, the general population is exposed most frequently by ingestion of thallium-containing foods, especially homegrown fruits and green vegetables. Thallium is a waste product of coal combustion and the manufacturing of cement, and inhalation of contaminated air near emission sources or in the workplace may also contribute to thallium exposure of some individuals.

Toxicokinetics

Thallium and thallium salts are readily absorbed by virtually all routes, with gastrointestinal exposure being the most common route to produce toxicity. Thallium also crosses the placenta freely. Thallium enters cells by a unique process governed by its similarity in charge and ionic radius to potassium. Unlike potassium, however, once thallium enters the cells, it is released slowly. It can concentrate in the liver and kidneys. Since it is soluble at physiological pH, it does not form complexes with bone. Most thallium is excreted in the urine, but it is excreted slowly and can be detected months after exposure.

Mechanism of Toxicity

Thallium's mechanism of toxicity is related to its ability to interfere with potassium ion functions. Thallium interferes with energy production at essential steps in glycolysis, the Kreb's cycle, and oxidative phosphorylation. Other effects include inhibition of sodium–potassium–adenosine triphosphatase and binding to sulfhydryl groups.

Acute and Short-Term Toxicity (or Exposure)

Animal

Among animal species, the toxicity of thallium acetates, nitrates, and sulfates varies. In rats, the LD_{50} ranges from 15 to 30 mg kg^{-1}. The oxide is slightly less toxic (LD_{50}, 70 mg kg^{-1}).

Human

Unlike exposure to most metal salts, gastrointestinal symptoms of thallium toxicity are relatively minor, and constipation is more characteristic than diarrhea. The major manifestations of toxicity consist of a rapidly progressive, ascending, extremely painful sensory neuropathy and alopecia. Other potential symptoms of overexposure are nausea, diarrhea, abdominal pain, and vomiting; seizure, tremor and psychosis. Thallium is one of the most toxic of all metals. It is a cumulative poison with an estimated lethal dose of 8–20 mg kg^{-1} in humans. It is difficult to predict the out come of thallium poisoning. With high exposure, death results very soon.

Hair loss throughout the body is common and begins a little over a week after exposure. Gastrointestinal symptoms include abdominal pain and

bleeding and ulceration of the colon. Neurological signs appear within a few days of exposure.

Thallium crosses the placental barrier and can be active in the last trimester of pregnancy. Loss of hair, and nail deformation are noted in exposed newborns. Loss of vision plus the other signs of thallium poisoning have been related to industrial exposures.

Chronic Toxicity (or Exposure)

Animal

Thallium has not been shown to be carcinogenic, although rats that were chemically exposed developed papillomas and exhibited inflammatory proliferation in the forestomach. Thallium causes malformation in chicks; however, teratological studies in animals produced ambiguous results.

Human

Regardless of the entry route, the major symptoms of thallium poisoning are gastrointestinal stress, neurological problems, and hair loss. Pain develops, fingers become numb, motor weakness is noted, and lower limbs may become paralyzed. The eyes become inflamed and retrobulbar neuritis with some loss of central vision follows. Intraocular hemorrhage, formation of cataracts, and optic nerve atrophy can occur.

Myocardial damage with EKG changes can result, and hypotension followed by hypertension can occur. Although thallium can concentrate in the kidneys, renal damage occurs in some cases (it is not generally extensive).

Clinical Management

For acute exposure, ipecac should be administered and lavage performed. The use of single- or multiple-dose activated charcoal is supported by *in vitro* binding experiments and some animal data, and charcoal hemoperfusion may be a useful adjunct. Forced potassium diuresis appears to be harmful. Hemodialysis is also recommended with potassium administration. Since calcium metabolism is disturbed, supplementary calcium is indicated. The use of traditional metal chelators such as dimercaprol (British antilewisite) and penicillamine is not supported by the available evidence. In fact, the use of penicillamine may lead to redistribution of thallium into the central nervous system. Multiple animal studies have found evidence of enhanced elimination and improved survival with Prussian blue; however, despite the fact that many humans have been treated with Prussian blue, the data presented are insufficient to judge its true efficacy. Despite this, one publication notes that Prussian blue's safety profile is superior to that of other proposed therapies, and that it should be considered the drug of choice in acute thallium poisoning.

Environmental Fate

Thallium is quite stable in the environment, since it is neither transformed nor biodegraded. Thallium is bioaccumulated and biomagnified.

Compounds of thallium are generally soluble in water and the element is found primarily as the monovalent ion (Tl^+). Thallium tends to be sorbed to soils and sediments, and to bioconcentrate in aquatic plants, invertebrates, and fish. Terrestrial plants can also absorb thallium from soil. Thallium may be bioconcentrated by organisms from water. The (US) Environmental Protection Agency has identified several 'National Priorities List' sites polluted by thallium.

Ecotoxicology

Environmental concerns are growing, mostly because thallium is a waste product of coal combustion and the manufacturing of cement. Thallium poisoning has been observed in many wildlife populations of the Great Lakes basin. Major releases of thallium to the environment are from processes such as coal-burning and smelting, in which thallium is a trace contaminant of the raw materials, rather than from facilities producing or using thallium compounds.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value, 8 h time-weighted average, for thallium (elemental and soluble compounds) is 0.1 mg m^{-3} with a skin exposure warning.

See also: Metals; Sensory Organs.

Further Reading

Goyer RA, Klaassen CD, and Waalkes MP (1995) *Metal Toxicology.* San Diego, CA: Academic Press.

Hoffman RS (2003) Thallium toxicity and the role of prussian blue in therapy. *Toxicology Review* 22: 29–40.

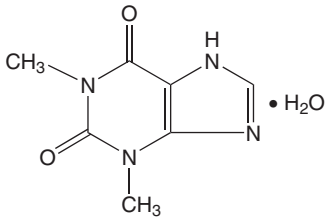
Wierzbicka M, Szarek-Lukaszewska G, and Grodzinska K (2004) Highly toxic thallium in plants from the vicinity of Olkusz (Poland). *Ecotoxicology and Environmental Safety* 59: 84–88.

Xiao T, Guha J, Boyle D, *et al.* (2004) Naturally occurring thallium: A hidden geoenvironmental health hazard? *Environment International* 30: 501–507.

Theophylline

Henry A Spiller

- Chemical Abstracts Service Registry Number: CAS 58-55-9
- Synonyms: 1,3-Dimethylxanthine; Anhydrous theophylline; Elixophyllin SR; Somophyllin; Theophyl; Theolair; Slo-Bid; Slo-phyllin; Theodur. Aminophylline is the ethylenediamine salt of theophylline
- Chemical/Pharmaceutical/Other Class: A naturally occurring methylxanthine derivative structurally related to caffeine
- Chemical Formula: $C_7H_8N_4O_2$
- Chemical Structure:



Uses

Theophylline is used as a bronchodilator in the treatment of asthma and reversible bronchospasm associated with chronic bronchitis and emphysema. Unlabeled use includes treatment of sleep apnea in neonates.

Background Information

Theophylline is a potent methylxanthine. Methylxanthines are widely distributed in the plant world in plants such as *Thea sinensis*, *Coffea Arabica* (coffee), and *Theobroma cacao* (coco, chocolate).

Exposure Routes and Pathways

Ingestion of sustained-release products is the most common route of both accidental and intentional exposure to theophylline. Theophylline is available in oral and intravenous dosage forms. Aminophylline is available in oral, rectal, and intravenous dosage forms.

Toxicokinetics

In therapeutic oral dosing, theophylline is well absorbed, producing peak serum levels in 2 h. However, overdose with the commonly available sustained-release formulations produces a delayed absorption pattern, with peak levels as late as 16 h postingestion. The matrices of these sustained-release formulations may agglutinate, with the potential to form pharmacobezors, further altering and delaying the absorption phase. In adults and children, theophylline is metabolized in the liver by oxidation and *N*-demethylation, producing 3-methybcanthine, 1,3-dimethyluric acid, and 1-methyluric acid. In premature neonates, minimal biotransformation occurs, with the main metabolite being caffeine. The average volume of distribution is $0.45\,l\,kg^{-1}$. Protein binding is 40%. The elimination half-life varies by age. The average half-lives by age are as follows: adults, 6 or 7 h; children 6 months to 13 years, 3.5–4 h; children less than 6 months, 7 h; and neonates, 20 h.

Mechanism of Toxicity

The mechanism of action is multifactorial. Suggested theories of action include increased cellular cyclic adenosine monophosphate levels via inhibition of phosphodiesterase, increased turnover of monoamines in the central nervous system, inhibition of prostaglandins, and antagonism of adenosine receptors. Theophylline causes a release of endogenous catecholamines. There is a positive inotropic and dose-dependent chronotropic response. Hypokalemia, hypercalcemia, and hyperglycemia are caused by a mechanism regulated by the beta-adrenergic system. Methylxanthines are weak diuretics by inhibition of renal tubular sodium resorption. Antagonism of adenosine receptors may play a role in the seizures seen with theophylline.

Acute and Short-Term Toxicity (or Exposure)

Animal

Toxicity would be expected from ingestion of pharmaceutical sources. Methylxanthines are not commonly used in animals. Limited information on toxicity exists. Tachyarrhythmias, hypotension, and seizures have been seen.

Human

Theophylline has a narrow therapeutic index, with 12–25% of overdose patients developing serious or life-threatening symptoms. Age >60 years and chronic use are risk factors for increased morbidity and mortality.

In acute overdose, peak serum levels $>100\,\mu g\,ml^{-1}$ may be predictive of arrhythmias and seizures. The use of sustained-release formulations and the presence of pharmacobezors in the gut may make it difficult to determine peak serum levels. Sinus tachycardia is the most common cardiac sign of theophylline toxicity. Ventricular and supraventricular tachycardia, ectopic beats, hypotension, and cardiac arrest may occur. Metabolic acidosis, hypokalemia, hypercalcemia, and hyperglycemia may be seen. Tremulousness and agitation frequently occur. Intractable seizures may occur in severe intoxications, probably secondary to adenosine receptor antagonism in the brain. Onset of seizures is a poor prognostic indicator. Persistent vomiting is commonly seen and may interfere with attempts at therapy.

Chronic Toxicity (or Exposure)

Animal

Rats and mice fed theophylline daily over 2 years found no evidence of carcinogenic activity at doses up to $75\,mg\,kg^{-1}$.

Human

In chronic overdose, peak serum levels $>40\,\mu g\,ml^{-1}$ are suggestive of increased risk of serious toxicity. The first sign of chronic theophylline toxicity may be development of seizures. Sinus tachycardia is another common cardiac finding in theophylline poisoned patients. Ventricular and supraventricular tachycardia, ectopic beats, hypotension, and cardiac arrest may occur. Metabolic acidosis, hypokalemia, hypercalcemia, and hyperglycemia may be seen. Onset of seizures is a poor prognostic indicator.

In Vitro Toxicity Data

Mutagenicity studies using sister-chromatid exchange and *Allium cepa* models were positive; studies using the Ames *Salmonella* assay have been negative.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Activated charcoal effectively adsorbs theophylline and should be employed in both acute and chronic overdoses. Multiple-dose activated charcoal (MDAC) has been shown to significantly increase drug clearance and reduce serum theophylline levels. If persistent vomiting interferes with the administration of MDAC, antiemetics and ranitidine may be effective. Ventricular dysrhythmias may respond to lidocaine. Tachyarrhythmias as well as ventricular dysrhythmias unresponsive to lidocaine may respond to beta blockers or verapamil. Beta blockers may control dysrhythmias, as well as reverse hypotension and hypokalemia. However, beta-blockers should be used with caution in persons with a history of asthma. Seizures should be treated with benzodiazepines or phenobarbital. Intractable seizures may require midazolam or pentobarbital. Phenytoin is ineffective in theophylline-induced seizures. Extracorporeal removal (ECR) may improve outcome if instituted before the onset of life-threatening symptoms. Hemoperfusion with a charcoal cartridge has been used on conjunction with hemodialysis to further enhance drug extraction when available. ECR should be considered in acute overdose patients with levels $>100\,\mu g\,ml^{-1}$, patients older than 60 years with levels $>50\,\mu g\,ml^{-1}$, and chronic overdose patients with levels $>40\,\mu g\,ml^{-1}$. Due to the routine use of sustained-release theophylline preparations, early serum level measurements may not be representative of the peak level. Repeated assessment of theophylline blood levels is required.

Environmental Fate

Screening tests for biodegradability indicate that theophylline may be biodegradable in soil and water. The adsorption of theophylline to suspended solids and sediments in water and to soil should be unimportant. The estimated bioconcentration factor indicates that bioconcentration of theophylline in aquatic organisms should not be important.

See also: Caffeine.

Further Reading

Emerman CL, Devlin C, and Connors AF (1990) Risk of toxicity in patients with elevated theophylline levels. *Annals of Emergency Medicine* 19: 643–648.

Olson KR, Benowitz NL, and Woo OF (1985) Theophylline overdose: Acute single ingestion versus chronic repeated overmedication. *American Journal of Emergency Medicine* 3: 386–394.

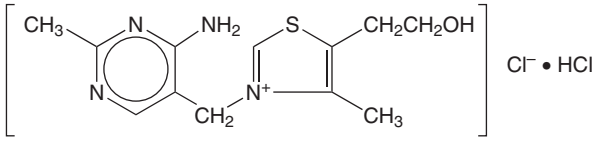
Olson KR, Kearney TE, and Dyer JE (1993) Seizures associated with poisoning and drug overdose. *American Journal of Emergency Medicine* 11: 565–568.

Thiamine

Diana Ku

This article is a revision of the previous print edition article by Denise L Kurta, volume 3, p. 230, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 67-03-8
- Synonyms: Vitamin B_1; Aneurine hydrochloride; Thiamine hydrochloride; Thiadoxine; Thiamin; Vitamin B_1 hydrochloride
- Chemical/Pharmaceutical/Other Class: Water-soluble vitamin
- Chemical Formula: $C_{12}H_{17}ClN_4OS$
- Chemical Structure:



Uses

Thiamine is a nutritional supplement used during periods of deficiency known as beriberi and its manifestations such as Wernicke–Korsakoff syndrome. Thiamine needs increase during diseases of the small intestine, malabsorption, congenital metabolic dysfunction, liver disease, alcoholism, and during pregnancy and lactation. Supplementation of thiamine for treatment of Alzheimer's disease, congestive heart failure, and cataracts has been investigated; however, evidence is unclear as to its benefits at this time.

Background Information

In 1912, Cashmir Funk isolated thiamine from rice husks and coined the term 'vitamine' because they were required for life ('vita') and because thiamine contained nitrogen ('amine'). The original term 'vitamine' was changed to 'vitamin' when scientists identified and purified all the vitamins and discovered that they did not all contain the element nitrogen.

Exposure Routes and Pathways

Routes of exposure are oral, intravenous, and intramuscular. Dietary sources include cereal grains, the hull of rice, yeast, peas, beans, pork, and beef.

Toxicokinetics

Thiamine is readily absorbed from the gastrointestinal tract mainly in the duodenum. It is hepatically metabolized and widely distributed to almost all body tissues. Thiamine is renally excreted almost entirely as metabolites. Excess thiamine (beyond the daily body need) is excreted unchanged and as metabolites in the urine.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute toxicity not expected.

Human

Acute toxic effects are not expected even with doses of 50–100 times the recommended daily allowance; however, hypersensitivity reactions have been reported.

Chronic Toxicity (or Exposure)

Animal

It would be unlikely for animals to be given chronic thiamine overdoses. Very large parenteral doses of thiamine have produced neuromuscular and ganglionic blockage in animal studies.

Human

Chronic large doses of more than $3\,g\,day^{-1}$ may cause headache, irritability, insomnia, weakness, tremors, ulcers, and tachycardia.

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

Acute ingestions seldom require treatment. Chronic excessive use should be discontinued and any toxic effects treated symptomatically.

See also: Dietary Supplements.

Further Reading

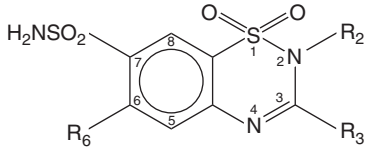
Rodriguez-Martin JL, Qizilbash N, and Lopez-Arrieta JM (2001) Thiamine for Alzheimer's disease. *Cochrane Database of Systematic Reviews* 2: CD001498.

Thomson AD (2000) Mechanisms of vitamin deficiency in chronic alcohol misusers and the development of the Wernicke–Korsakoff syndrome. *Alcohol & Alcoholism* 35(Suppl 1): 2–7.

Thiazide Diuretics

Elizabeth J Scharman

- Chemical Abstracts Service Registry Numbers:
  - Bendroflumethiazide: CAS 73-48-3
  - Benzthiazide: CAS 91-33-8
  - Chlorothiazide: CAS 58-94-6
  - Chlorthalidone (phthalimidine derivative; similar to thiazides in structure and pharmacology): CAS 77-36-1
  - Hydrochlorothiazide: CAS 58-93-5
  - Hydroflumethiazide: CAS 135-09-1
  - Indapamide (an indoline; similar to thiazides in structure and pharmacology): CAS 26807-65-8
  - Methyclothiazide: CAS 135-07-9
  - Metolazone (quinazoline derivative; similar to thiazides in structure and pharmacology): CAS 17560-51-9
  - Polythiazide: CAS 346-18-9
  - Quinethazone (quinazoline derivative; similar to thiazides in structure and pharmacology): CAS 73-49-4
  - Trichlormethiazide: CAS 133-67-5
- Synonyms:
  - Bendroflumethiazide: Bendrofluazide; Benzydroflumethiazide; Naturetin®
  - Benzthiazide: Benzothiazide; Exna®
  - Chlorothiazide: 6-Chloro-7-sulfamoyl-2*H*-1,2,4-benzothiadiazine 1,1-dioxide; Diuril®
  - Chlorthalidone: Chlorphthalidolone; Hygroton®
  - Hydrochlorothiazide: HCTZ; 3,4-Dihydrochlorothiazide; Esidrex®; Oretic®
  - Hydroflumethiazide: Trifluoromethylhydrothiazide; Dihydroflumethiazide; Diucardin®
  - Indapamine: *N*-(3-Sulfamyl-4-chlorobenzamido)-2-methylindoline; Lozol®
  - Methyclothiazide: 6-Chloro-3-chloromethyl-2-methyl-7-sulfamyl-3,4-dihydro-1,2,4-benzothiadiazine-1,1-dioxide; Enduron®
  - Metolazone: 2-Methyl-3-*o*-tolyl-6-sulfamyl-7-chloro-1,2,3,4-tetrahydro-4-quinazolinone; Mykrox® (rapid and complete absorption); Zaroxolyn® (slow and incomplete absorption)
  - Polythiazide: 6-Chloro-3,4-dihydro-2-methyl-7-sulphamoyl-3-(2,2,2-trifluoroethylthiomethyl)-2*H*-benzo-1,2,4-thiadiazine-1,1-dioxide; Renese®
  - Quinethazone: 7-Chloro-2-ethyl-6-sulfamoyl-1,2,3,4-tetrahydro-4-quinazolinone; Hydromox®
  - Trichlormethiazide: 3-Dichloromethylhydrochlorothiazide; Hydrotrichlorothiazide; Metahydrin®; Naqua®
- Chemical/Pharmaceutical/Other Class: Diuretic; Antihypertensive
- Chemical Formulas:
  - Bendroflumethiazide: $C_{15}H_{14}F_3N_3O_4S_2$
  - Benzthiazide: $C_{15}H_{14}ClN_3O_4S_3$
  - Chlorothiazide: $C_7H_6ClN_3O_4S_2$
  - Chlorthalidone: $C_{14}H_{11}ClN_2O_4S$
  - Hydrochlorothiazide: $C_7H_8ClN_3O_4S_2$
  - Hydroflumethiazide: $C_8H_8F_3N_3O_4S_2$
  - Indapamine: $C_{16}H_{16}ClN_3O_3S$
  - Methyclothiazide: $C_9H_{11}Cl_2N_3O_4S_2$
  - Metolazone: $C_{16}H_{16}ClN_3O_3S$
  - Polythiazide: $C_{11}H_{13}ClF_3N_3O_4S_3$
  - Quinethazone: $C_{10}H_{12}ClN_3O_3S$
  - Trichlormethiazide: $C_8H_8Cl_3N_3O_4S_2$
- Chemical Structure:



Uses

Thiazide diuretics are used in the management of edema, the management of hypertension, the treatment of nephrogenic diabetes insipidus, and the prophylaxis of renal calculus formation.

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposures to the thiazide diuretics. Thiazides and related diuretics are available in oral dosage forms. Chlorothiazide is also available in a parenteral dosage form.

Toxicokinetics

Thiazides are absorbed in varying degrees from the gastrointestinal tract. Bendroflumethiazide is ~100% absorbed, benzthiazide 25%, chlorothiazide 10–21%, chlorthalidone 65%, hydrochlorothiazide 65–75%, hydroflumethiazide 50%, indapamide 93%, and metolazone 40–65%; absorption data are unavailable for the other drugs in this class. Plasma levels do not correlate with diuretic effects. The onset of diuretic action occurs at 2 h with the exception of metolazone whose onset is 1 h, indapamide 1–2 h, and

chlorthalidone 2–3 h. Peak diuretic effects occur within 2 h for indapamide and metolazone, 2–6 h for chlorthalidone, 4–6 h for benzthiazide and hydrochlorothiazide, 4 h for bendroflumethiazide, chlorothiazide, and hydroflumethiazide, and 6 h for methyclothiazide, polythiazide, quienthazone, and trichlormethiazide. Following intravenous administration of chlorothiazide, the onset of action occurs in 15 min with a peak effect occurring in 30 min. The diuretic duration of action is 2 h for chlorothiazide (intravenous), 6–12 h for bendroflumethiazide, chlorothiazide (oral), and hydrochlorothiazide, 12–18 h for benzthiazide, 24–72 h for chlorthalidone, 12–24 h for hydroflumethiazide, 24 h for methyclothiazide and trichlormethiazide, 12–24 h for metolazone, 24–48 h for polythiazide, and 18–24 h for quinethazone. The antihypertensive effects of these agents may not appear for 3–4 days with maximum effect being delayed for 3–4 weeks. The volume of distribution for chlorothiazide is $0.2\,l\,kg^{-1}$, chlorthalidone $3.9\,l\,kg^{-1}$, and hydrochlorothiazide $0.83\,l\,kg^{-1}$. Thiazides cross the placenta and are excreted into breast milk. Bendroflumethiazide is 94% protein bound, chlorothiazide 20–80% protein bound, chlorthalidone 75% protein bound, hydrochlorothiazide 64% protein bound, hydroflumethiazide 74% protein bound, indapamine 79% protein bound, metolazone 95% protein bound, and polythiazide 84% protein bound. Thiazides are primarily excreted unchanged in urine. The half-life of bendroflumethiazide is 3 h, chlorothiazide 1.5 h, chlothalidone 44 h, hydrochlorothiazide 2.5 h, hydroflumethiazide 2–17 h (biphasic), indapamide 14–18 h, metolazone (Mykrox®) 8–14 h, polythiazide 25.7 h, and trichlormethiazide 2.3 h. The antihypertensive effect of these agents may persist for a week after therapy is discontinued.

Mechanism of Toxicity

Thiazides and the related diuretics inhibit the transport of sodium in the early distal tubules, which results in the enhanced elimination of sodium, chloride, and water. Potassium and sodium bicarbonate elimination is also enhanced; calcium excretion is decreased, uric acid is retained. Glomerular filtration rate is decreased. The antihypertensive effects may be the result of direct arteriolar dilation but the full mechanism has not been identified.

Acute and Short-Term Toxicity (or Exposure)

Animal

Chlorothiazide is used therapeutically in dogs and cattle. Hydrochlorothizide is used therapeutically in dogs, cats, and cattle. Toxic effects are similar to those seen in humans.

Human

Determination of toxicity is based on observation as there is no milligram per kilogram toxic dose established. Ingestion of amounts exceeding maximum daily doses has been tolerated in children. Overdose may result in diuresis with accompanying fluid and electrolyte loss, lethargy, and coma. Clinical effects seen, which are secondary to the fluid and electrolyte loss, include hypotension, tachycardia, contraction alkalosis, muscle weakness, headache, and dysrhythmias.

Chronic Toxicity (or Exposure)

Animal

Syrian golden hamsters fed hydrochlorothiazide up to $4\,mg\,kg^{-1}\,day^{-1}$ for 6 months developed increased cholesterol and trigyceride levels. Dogs given doses up to 200 mg hydrochlorothiazide daily for 9 months all developed enlarged parathyroid glands.

Human

Side effects that may occur include hypokalemia, hypomagnesemia, hyponatremia, hyperglycemia, hyperuricemia or gout, anorexia, orthostatic hypotension, anorexia, nausea, and photosensitivity.

In Vitro Toxicity Data

Mutagenicity studies using Ames *Salmonalla* assays have been negative; studies of sister chromatid exchange and mouse lymphoma line assays have been positive.

Clinical Management

Most cases of unintentional thiazide overdoses can be managed safely at home as serious effects are not expected. Thiazides and related agents are adsorbed by activated charcoal and it may be used for substantial recent exposures. Because cathartics can also cause fluid and electrolyte losses, their use should be avoided. Fluid status, electrolytes, and EKG should be monitored. Standard supportive therapies with attention to replacement of fluid and electrolyte losses should be utilized as clinically necessary. No antidote is available. Drug levels are not readily available and are not helpful in assessing toxicity.

See also: Kidney.

Further Reading

Farge D, Turner MW, and Roy DR (1986) Dyazide-induced reversible acute renal failure associated with intracellular crystal deposition. *American Journal of Kidney Diseases* 8: 445–449.

Klein MD (1987) Noncardiogenic pulmonary edema following hydrochlorothiazide ingestion. *Annals of Emergency Medicine* 16: 113–115.

Thioacetamide

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 62-55-5
- Synonyms: Acetothioamide; Ethanethioamide; Thiacetamide; TAA
- Chemical Formula: C_2H_5NS

Uses

Thioacetamide is used as a replacement for gaseous hydrogen sulfide in qualitative analysis.

Exposure Routes and Pathways

Inhalation, dermal, oral, and ocular exposures are possible. Occupational exposure to thioacetamide may occur through inhalation and dermal contact with this compound at workplaces.

Toxicokinetics

Thioacetamide is readily absorbed through the skin. The highest levels of radioactivity were observed in the liver following oral administration of [^{3}H]thioacetamide (in diet) to male rats. Approximately 80% of [^{35}S]thioacetamide was excreted in the urine of rats within 24 h of intravenous administration.

Mechanism of Toxicity

Thioacetamide acts as an indirect hepatotoxin and causes parenchymal cell necrosis. It can be metabolized *in vivo* to acetamide, which itself is carcinogenic. Acetamide is then hydrolyzed to acetate. Thioacetamide-induced liver necrosis has been explained by a scheme that includes the metabolic conversion of thioacetamide to its S-oxide, followed by the further metabolism of thioacetamide S-oxide to a reactive intermediate that can either bind to liver macromolecules or be further degraded to acetamide and polar products. Examples of thioacetamide's biochemical effects in the liver include glucose-6-phosphate dehydrogenase being induced within days after rats are treated with thioacetamide, and the level of urea product is decreased as are the activities of hepatic carbamyl phosphate synthetase, ornithine transcarbamylase, and arginase. Thus, thioacetamide can produce marked disturbances in the urea cycle in the liver. Further, thioacetamide administered to rats leads to functional disturbances in mitochondria isolated from livers after 24 h, and the maximum respiratory activity of the mitochondria is also depressed, mitochondrial Ca^{2+} content is significantly increased, and the Ca^{2+} transport behavior of the hepatic mitochondria is altered. The results are indicative of structural alterations of the inner mitochondrial membranes. The potential role of thioacetamide in the initiation phase of carcinogenesis may be associated with an increase in nucleoside triphosphate activity in cell nuclear envelopes with a corresponding increase in RNA transport activity. Alterations in the transport phenomenon of nuclear RNA sequences are considered an early response to carcinogens.

Acute and Short-Term Toxicity (or Exposure)

Animal

Rat oral $LD_{50} = 301\,mg\,kg^{-1}$; mouse intraperitoneal $LD_{50} = 300\,mg\,kg^{-1}$; death is delayed after dosing, even with massive doses. Hepatic necrosis and cirrhosis is observed with toxic doses.

Human

Inhalation may cause irritation of the respiratory tract characterized by rhinitis, tracheitis, and pulmonary edema. High concentrations may result in central nervous system depression and death from respiratory paralysis. Skin contact may cause irritation and ocular contact may be associated with palpebral edema, keratitis, and corneal defects. Ingestion may cause nausea, vomiting, headache, convulsions, and unconsciousness. Several people developed degenerative changes in their livers from drinking the juice of

oranges that had been immersed for 2–5 s in thioacetamide to prevent growth of molds.

Chronic Toxicity (or Exposure)

Animal

There is sufficient evidence of carcinogenicity in animals. Repeated dietary administration has produced liver cell tumors in mice and bile duct and liver tumors in rats. Cirrhosis has also been observed in both rats and mice. Thioacetamide is a developmental toxin.

Human

Prolonged exposure by inhalation may result in headache, irritability, nausea, and vomiting. Repeated contact with skin may cause dermatitis and prolonged ocular contact may cause conjunctivitis. Falls within group 2B (possibly carcinogenic to humans) according to the International Agency for Research on Cancer; however, no data are available in humans. Thioacetamide is among the group of Reasonably Anticipated to be Human Carcinogens; according to the US National Toxicology Program's 10th Report on Carcinogens. An oral thioacetamide-induced model of rat cholangiocarcinoma (CCA) has been developed that recapitulates the histologic progression of human CCA. CCA is a lethal disease, afflicting many thousands the world over. Human CCA develops through a multistep progression model, preceded by the onset of dysplasia in the cholangiolar ductal epithelium. The thioacetamide animal model is useful because its multistep process leading to cancer in the biliary tree will enable the study of genetic changes in human CCA and may serve as a powerful preclinical platform for therapeutic and chemoprevention strategies.

In Vitro Toxicity Data

Thioacetamide induced an increase in sex-linked recessive mutations in *Drosophila*. It was nonmutagenic in the *Salmonella*/Ames mutagenicity assay, and in the *Escherichia coli* recombination assay. Protein synthesis in mouse hepatoma (MH-134), but not in L-929 cells, was enhanced by adding thioacetamide.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Gastric decontamination may be accomplished by lavage or emesis. Sodium bicarbonate solution should be used to reduce acidity. The use of amyl nitrite by inhalation for 15–30 s of every minute may be indicated in severe poisonings.

Environmental Fate

Thioacetamide's production and use as a substitute for hydrogen sulfide in the laboratory may result in its release to the environment through various waste streams. If released to air, thioacetamide's estimated vapor pressure indicates it will exist solely as a vapor in the ambient atmosphere. Vapor-phase thioacetamide 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 18 h. Thioacetamide was not biodegraded by activated sludge after 5 days, and therefore may be resistant to biodegradation in the environment. Hydrolysis is not expected since amides hydrolyze very slowly at environmental conditions. An estimated bioconcentration factor for thioacetamide suggests the potential for bioconcentration in aquatic organisms is low.

See also: International Agency for Research on Cancer; Liver.

Further Reading

Arni P (1989) Review on the genotoxic activity of thioacetamide. *Mutation Research* 221: 153–162.

International Agency for Research on Cancer (IARC) (1982) IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. *Chemicals, Industrial Processes and Industries Associated with Cancer in Humans*. Supplement 4, 292 pp. Lyon, France: IARC.

Yeh CN, Maitra A, Lee KF, Jan YY, and Chen MF (2004) Thioacetamide-induced intestinal-type cholangiocarcinoma in rat: An animal model recapitulating the multistage progression of human cholangiocarcinoma. *Carcinogenesis* 25: 631–636.

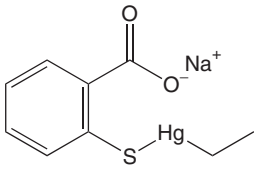
Relevant Website

http://www.cdc.gov – US National Institute for Occupation Safety and Health (NIOSH). Thioacetamide (International Chemical Safety Cards).

Thiomerosal

Arezoo Campbell

- Chemical Abstracts Service Registry Number: CAS 54-64-8
- Synonyms: Sodium ethylmercury thiosalicylate; Merthiolate; Thimerosal
- Chemical Formula: $C_9H_9HgNaO_2S$
- Chemical Structure:



Uses

Thiomerosal is used as a preservative in vaccines and ophthalmic solutions.

Background Information

Thiomerosal (sodium ethylmercury thiosalicylate) is used as a preservative in vaccines to protect from bacterial and fungal contamination. There is currently a controversy as to whether the use of this organic ethylmercury compound is an etiological factor in autism. It is estimated that children treated with these mercury-containing vaccines are exposed to levels of the metal far beyond those considered safe by the US Food and Drug Administration standards. By the age of 2, children can receive as much as 237 mg of mercury through vaccination. This has led to a 2002 class action lawsuit against the manufacturer of thiomerosal by parents with autistic children or children who have not yet developed any symptoms but were exposed to high concentrations of mercury contained in the preservative. Recent studies discount a causal relation between exposure to thiomerosal-containing vaccines and autism. The risk of autism is not increased in children treated with vaccines containing the preservative compared to children who were vaccinated with thiomerosal-free formulations. Furthermore, in Denmark, the use of the preservative was discontinued after 1992. After removal of thiomerosal, there was a continued rise in new cases of autism. Even with these new emerging data, the potential link between autism and mercury poisoning remains inconclusive.

Exposure Routes and Pathways

The most common route of entry is by intravenous injection since the compound is used as a preservative in vaccines.

Toxicokinetics

Thiomerosal is metabolized to ethylmercury and thiosalicylate. Toxicologists have assumed that ethylmercury poisoning is similar to the toxicity of methylmercury. However, ethylmercury cannot bypass the blood–brain barrier as easily as methylmercury. The entry of methylmercury into the brain relies on an active transport system. Ethylmercury on the other hand is a larger molecule and cannot use this system. Furthermore, it is more rapidly decomposed. Because of these limitations, when the same dose of both mercurial compounds is administered, the concentrations of methylmercury are greater in the brain when compared to ethylmercury. Due to the limited entry of the latter into the brain, this compound is more likely to cause damage to the spinal cord, myocardium and skeletal muscle.

Mechanism of Toxicity

Not much is known about the toxic effects of ethylmercury and most toxicologists have assumed that the toxic changes would be similar to that caused by methylmercury. These alterations in turn are very complex and depend on duration of exposure, dose, and the age of the individual. Mercury salts have a strong affinity for sulfhydryl groups and this is likely to play a role in effecting their neurotoxicity. Some *in vitro* studies indicate that oxidative stress leading to lipid peroxidation and DNA damage may also underlie the mechanism of toxicity.

Acute Toxicity (or Exposure)

Animal

There are not many studies addressing the potential toxicity of ethylmercury in animal models. Exposure of rats to ethylmercury results in patchy damage to the granule cells in the cerebellum while the Purkinje cells are generally spared.

Human

In a case report of four patients who were exposed to ethyl mercury, toxicity was seen in the brain, spinal motor neurons, peripheral nerves, skeletal muscles, and myocardium. Several case studies of accidental occupational exposure have also been documented. The most common signs of ethylmercury toxicity are paraesthesia, dysarthria, and constriction of the visual field. However, none of the symptoms of ethylmercury toxicity are specific and death is a common outcome if exposure levels are high.

Chronic Toxicity (or Exposure)

Human

Accidental exposure to massive doses of methylmercury occurred in Japan and Iraq. The former was due to contaminated seafood while the latter was due to contaminated grain. In Japan, neurotoxicity was found in humans after extended periods of fish consumption. Slow onset of symptoms resulted in a high incidence of severe, largely irreversible damage to the central nervous system. Postmortem analysis of the brain of individuals exposed to high levels of the mercury compound showed neuronal cell loss and an increase in glial cell numbers in the cortex. The cerebellar granule cells were also damaged.

In Vitro Toxicity Data

Treatment of cell cultures containing both neuronal and glial cells derived from fetal rat brain with low concentrations of both organic and inorganic mercury compounds leads to cell death. In cell cultures derived from a more mature fetal stage, the organic form of mercury was more toxic and showed specific neuronal toxicity. Below the cytotoxic concentration of mercury ($>1\,\mu mol\,l^{-1}$), pronounced gliosis was observed. In a human fetal hepatic cell line, exposure to low concentrations of inorganic mercury led to lipid peroxidation and single-strand breaks in the DNA. This suggests that oxidative stress may play a role in mercury-induced cytotoxicity.

Clinical Management

Diagnosis of mercury intoxication can be confirmed by measuring levels of the metal in serum. The toxic effects are largely irreversible. In severe cases, death is the major outcome.

See also: Dimethylmercury; Methylmercury.

Further Reading

Magos L (2001) Review on the toxicity of ethylmercury, including its presence as a preservative in biological and pharmaceutical products. *Journal of Applied Toxicology* 21: 1–5.

Nelson KB and Bauman ML (2003) Thiomerosal and autism? *Pediatrics* 111: 674–679.

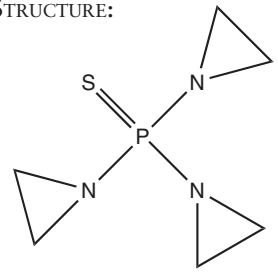
Relevant Website

http://www.fda.gov – The FDA's Center for Biologics Evaluation and Research, search for Thiomerosal.

Thiotepa

Marcia D Howard

- Chemical Abstracts Service Registry Number: CAS 52-24-4
- Synonyms: Triethylene thiophosphoramide; *N*,*N*′,*N*″-Triethylenethiophosphamide; *N*,*N*′,*N*″-Triethylenethiophosphoramide; *N*,*N*′,*N*″-Tri-1,2-ethanediylphosphorothioic triamide; *N*,*N*′,*N*″-Tri-1,2-ethanediylthiophosphoramide; *N*,*N*′,*N*″-Triethylenephosphorothioic triamide; *N*,*N*′,*N*″-Triethylenethiophosphortriamide; 1,1′,1″-Phosphinothioylidynetrisaziridine; Girostan; Ledertepa
- Chemical/Pharmaceutical/Other Class: Aziridine; Alkylating antineoplastic agent, Insect sterilant
- Chemical Formula: $C_6H_{12}N_3PS$
- Chemical Structure:



Uses

Thiotepa is used in the treatment of bladder, ovarian, and breast cancer as well as a component of experimental high-dose chemotherapy regimens. Thiotepa is also used as an insect sterilant.

Exposure Routes and Pathways

Thiotepa may be absorbed through inhalation, ingestion, dermal contact, or eye contact. However, the general public is not likely to be exposed due to its limited use in cancer therapy.

Toxicokinetics

Absorption of thiotepa from the gastrointestinal tract is incomplete while absorption through serous membranes (e.g., pleura and bladder) and from intramuscular injection sites is variable. Distribution of thiotepa occurs rapidly and extensively to tissues. Thiotepa is rapidly converted to its primary metabolite, triethylenephosphoramide (TEPA) by hepatic mixed function oxygenases. TEPA becomes the predominant form of thiotepa within 5 min of

administration of the drug. The plasma half-life of thiotepa is 1–3 h while TEPA has a half-life of 3–24 h. Thiotepa is excreted in urine (60% within 72 h). Less than 10% of the drug or its primary metabolite (TEPA) appears in the urine with the remainder of the drug either metabolized, interacting with biological molecules or undergoing spontaneous chemical degradation. Toxicokinetics are similar for adults and children when conventional doses ($80\,mg\,m^{-2}$) are administered. Protein binding (as determined by ultrafiltration) is reported to be less than 40% under physiological conditions.

Mechanism of Toxicity

Thiotepa is a phase nonspecific polyfunctional alkylating agent (i.e., more than one reactive ethylenimine group). It is chemically and pharmacologically related to nitrogen mustards. Thiotepa (and TEPA) form DNA crosslinks that lead to a reactive metabolite. The aziridine ring opens after protonation of the ring nitrogen. As an alkylating agent, thiotepa interferes with DNA replication and RNA transcription, ultimately leading to the disruption of nucleic acid function. Alkylation causes breaks in DNA and cross-linking of the twin strands. One of the principle bond disruptions occurs when the N-7 position of guanine is alkylated, severing the link between the purine base and sugar, which liberates an alkylated guanine.

Acute and Short-Term Toxicity (or Exposure)

Animal

The reported LD_{50} values in rats are $8\,mg\,kg^{-1}$ (intraperitoneal); 9–$15\,mg\,kg^{-1}$ (intravenous, iv); $2.3\,mg\,kg^{-1}$ (oral), and $7.8\,mg\,kg^{-1}$ (subcutaneous, sc). Reported LD_{50} values are $11\,mg\,kg^{-1}$ (intraperitoneal, ip); $14.5\,mg\,kg^{-1}$ (iv); $38\,mg\,kg^{-1}$ (oral), and $16.5\,mg\,kg^{-1}$ (sc) for mice.

Human

Direct vesicant effects, which can occur with active alkylating agents, can damage tissue at the site of injection as well as cause systemic toxicity. Toxic side effects of thiotepa include nausea, vomiting, fever, anorexia, headache, neutropenia, thrombocytopenia, and variable anemia. Although allergic reactions are rare, hives and skin rashes are occasionally noted. Other side effects include myelosuppression and to a lesser extent, mucositis. Cardiac dysrhythmias and hypotension as well as pulmonary edema or pneumonitis may also occur. Toxicity is generally dose-related and occurs particularly in rapidly growing tissues such as bone marrow, GI tract, and gonadal tissue. Target organs are bone marrow, kidneys, liver, heart, lungs, spleen, blood systems, eyes, gastrointestinal tract, and reproductive systems. It is contraindicated during the first trimester of pregnancy.

Chronic Toxicity (or Exposure)

Animal

Thiotepa is believed to be carcinogenic in both male and female rats and mice. Malignant tumors developed in rats treated weekly with $1\,mg\,kg^{-1}$ body weight (iv). The minimum ip teratogenic dose (TD) in pregnant mice is $1\,mg\,kg^{-1}$ body weight.

Human

Thiotepa is a carcinogen in humans (group 1). Chronic exposure to thiotepa may cause skin depigmentation and allergic reactions as well as effects seen with acute exposure.

Clinical Management

Poisoned patients should be treated for the symptoms of the poisoning and not the drug itself. For dermal exposures, the skin should be immediately flushed with water and all contaminated clothing isolated. Affected skin areas should be thoroughly but gently washed with soap and water. Contact should also be made with a hospital or poison control center (even if the victim has no visible symptoms) and the victim immediately transported to the hospital after washing the affected areas.

For ocular exposure to the compound, the victim should be checked for contact lenses and if present, should be removed. The eyes should be flushed with copious amounts of water or normal saline for 20–30 min while the hospital or poison control center is notified. No ointments, oils, or medications should be instilled into the victim's eyes without specific instructions from a physician.

If the chemical is ingested, first aid will depend on the victim's state of consciousness. If the exposed person is conscious and not convulsing, one or two glasses of water should be given to dilute the chemical and the poison control center or hospital should be immediately called. Generally, it is not recommended that vomiting be induced outside of the care of a medical doctor due to the possibility of aspiration of the chemical into the lungs. However, if the victim is conscious, not convulsing, and medical care is not readily available, induction of vomiting should

be considered due to the high toxicity of the chemical. The victim should be immediately taken to the hospital. If the victim is convulsing or is unconscious, nothing should be administered by mouth. It should be ensured that the victim's airway is open; the victim should be made to lie on his or her side with the head lower than the body and should be immediately transported to the hospital. However, vomiting should not be induced. Bone marrow toxicity with overdosage may be limited by blood transfusion.

See also: Nitrogen Mustard.

Further Reading

Calabresi P and Chabner BA (2001) Chemotherapy of neoplastic diseases. In: Hardman JG and Limbird LE (eds.) *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 10th edn., pp. 1381–1459. New York: McGraw Hill.

Relevant Websites

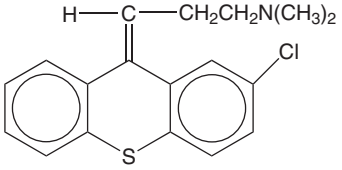
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Thiotepa.
http://www.cancer.org – American Cancer Society.

Thiothixene *See* Thioxanthenes.

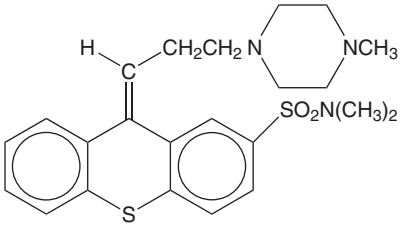
Thioxanthenes

Douglas J Borys

- Representative Compounds: Chlorprothixene; Thiothixene; Flupenthixol; Zuclopenthixol
- Chemical Abstracts Service Registry Numbers: Chlorprothixene (CAS 113-59-7); Thiothixene (CAS 5591-45-7); Flupenthixol (CAS 2413-38-9); Zuclopenthixol (CAS 53772-83-1)
- Synonyms:
  - Chlorprothixene: 2-Chloro-9-[3-(dimethylamino)propylidene]-thioxanthene; Paxyl; Taractan
  - Thiothixene: Thioxanthene-2-sulfonamide; *N*,*N*-Dimethyl-9-[3-(4-methylpiperazin-1-yl)propylidene]; Navane
  - Flupenthixol: 2-[4-[3-(*E*,*Z*)-2-(Trifluoromethyl)-9*H*-thioxanthen-9-ylidene]propyl]piperazin-1-yl]-ethanol dihydrochloride; Depixol
  - Zuclopenthixol: (*Z*)-4[3-(2-Chloro-9*H*-thioxan-9-ylidene)propyl]-1-piperazine ethanol; Clopixol
- Chemical/Pharmaceutical/Other Class: Neuroleptic agent; Antipsychotic; Major tranquilizer
- Chemical Structures:



Chlorprothixene



Thiothixene

Uses

Thioxanthenes are used in the treatment of psychosis, including schizophrenia, senile psychosis, pathological jealousy, and borderline personality disorder. Other uses include the treatment of pain, postoperative neuralgia, sedation, anxiety neurosis, childhood behavior problems, and depression. The maximum therapeutic daily oral dose for chlorprothixene, flupenthixol, and thiothixene is 600, 224, and 60 mg respectively; the maximum intramuscular dose of each is 200 mg day^{-1}, 100 mg weekly, and 30 mg day^{-1}, respectively. Some thioxanthenes and thioxanthenones have shown signs in mice and *in vitro* assays of possible human therapeutic potential against tumors, and some thioxanthenes have been shown to have cytotoxic and antimicrobial activities.

Background Information

In 2002, the American Association of Poison Control Centers' Toxic Exposure Surveillance System reported

5224 human exposures to phenothiazines, thioxanthenes, and other neuroleptic medications. Of those exposures 3691 were in adults and 808 in children. Unintentional and intentional exposures accounted for 43.7% and 47.8% of the exposures, respectively. There were 417 (8.0%) adverse drug reactions reported.

Exposure Routes and Pathways

Thioxanthenes are available in injectable and oral dosage forms. The primary exposure pathway is intentional ingestion by adults; accidental ingestion by small children also does occur.

Toxicokinetics

Thioxanthenes are readily but incompletely absorbed due to first-pass metabolism in the gut wall. Oral bioavailability ranges from 40% to 50%. Peak absorption occurs in 1 or 2 h. Thioxanthenes are extensively metabolized in the liver through glucuronic acid conjugation, *N*-dealkylation, and sulfoxidation. Thioxanthenes are widely distributed throughout the body, including the central nervous system (CNS). They are highly protein bound (>99%), with a volume of distribution ranging from 11 to 23 l kg^{-1}. The main metabolites are excreted in both the urine and feces. There is some enterohepatic circulation. The elimination half-life ranges from 8 to 12 h. The thioxanthenes and their metabolites can be excreted through breast milk.

Mechanism of Toxicity

Thioxanthenes work primarily by blocking postsynaptic dopamine-mediated neurotransmission by binding to dopamine (DA-1 and DA-2) receptors. In addition to significant antidopaminergic action, the thioxanthenes also possess weak anticholinergic and serotonergic blockade, moderate α-adrenergic blockade, quinidine-like effects, and depress the release of most hypothalamic and hypophyseal hormones. Thioxanthenes may also inhibit presynaptic dopamine auto receptors.

Acute and Short-Term Toxicity (or Exposure)

Animal

Sublethal doses produce ataxia and respiratory paralysis, while lethal doses produce convulsions. The oral LD_{50} data are in the range of 400 mg kg^{-1} or greater for mice and rats administered thioxanthenes.

Human

Clinical signs of toxicity frequently reported include extrapyramidal effects, sedation, coma, and rarely seizures, acute renal insufficiency, hypotension, and cardiac arrhythmias. Other adverse reactions following therapeutic use include dysphoria, photosensitivity, anorexia, nausea, vomiting, constipation, diarrhea, and dyspepsia. The extrapyramidal reactions induced by thioxanthenes will result in increased motor activity of the head, face, and neck. Neuroleptic malignant syndrome has been reported after therapeutic use and acute intoxication. The most commonly reported dystonic reactions include akathisias, stiff neck, stiff or protruding tongue, and tremor. Anticholinergic effects, including dry mouth, blurred vision, and tachycardia, may occur. Cardiac effects include prolonged Q–T interval and mild hypotension. Hypokalemia has also been noted. Patients receiving thiothixene should avoid undue exposure to sunlight. These drugs have been implicated in the etiology of acquired hemophilia.

Leukopenic and thrombocytopenic effects of thioxanthenes may result in an increased incidence of microbial infection, delayed healing, and gingival bleeding. When a thioxanthene is used concomitantly with other CNS depressants, caution should be taken to avoid overdosage. Prior administration of thioxanthenes may decrease the pressor response to phenylephrine because of the α-adrenergic blocking action of thioxanthenes. Hypersensitivity reactions, including rash, pruritus, urticaria, photosensitivity, and rarely anaphylaxis, have been reported in patients receiving thiothixene.

Chronic Toxicity (or Exposure)

Human

Tardive dyskinesias (TDs) are involuntary movements of the tongue, lips, face, trunk, and extremities that occur in patients treated with long-term dopaminergic antagonist medications. TDs can be differentiated from acute movement disorders that commonly occur in the same patient groups; the acute movement disorders resulting from exposure to dopamine antagonists are commonly termed extrapyramidal syndromes.

In Vitro Toxicity Data

Efflux-related multidrug resistance (MDR) is a significant means by which bacteria can evade the effects of selected antimicrobial agents. Two geometric stereoisomers of flupentixol, with intrinsic antimicrobial

activity, were studied using strains of *Staphylococcus aureus* possessing unique efflux-related MDR phenotypes, and the results suggest that the mechanism by which thioxanthenes inhibit efflux by proton motive force-dependent pumps may involve an interaction with the pump itself and, to a lesser extent, a reduction in the transmembrane potential.

Clinical Management

Treatment consists of gastric decontamination, hydration, and aggressive supportive care; all basic and advanced life-support measures should be implemented. Gastric decontamination should be performed. Syrup of ipecac is contraindicated. Lavage may be performed and activated charcoal administered, if within 60 min of an acute ingestion. Thioxanthenes are readily absorbed by activated charcoal. Dystonic reactions respond to intravenous benztropine or diphenhydramine. Oral therapy with diphenhydramine or benztropine should be continued for 2 days to prevent recurrence of the dystonic reaction. For patients suffering from neuroleptic malignant syndrome treatment consists of dantrolene sodium, diphenhydramine, and oral bromocriptine in conjunction with cooling and other supportive measures. Arrhythmias should be treated with lidocaine or phenytoin. Diazepam is the drug of choice for seizures while phenytoin is the drug of choice to prevent recurrence. Fluid challenge alone will frequently correct hypotension. Hemodialysis and hemoperfusion have not been shown to be effective.

See also: Tricyclic Antidepressants.

Further Reading

Azuine MA, Tokuda H, Takayasu J, *et al.* (2004) Cancer chemopreventive effect of phenothiazines and related tri-heterocyclic analogues in the 12-*O*-tetradecanoylphorbol-13-acetate promoted Epstein–Barr virus early antigen activation and the mouse skin two-stage carcinogenesis models. *Pharmacology Research* 49: 161–169.

Ginestet D (2002) L'essor de la psychopharmacologie et son impact sur la psychiatrie (The development of psychopharmacology and its impact on the psychiatry). *Annales Médico-psychologiques, revue psychiatrique* 160: 767–771.

Grohmann R, Engel R, Hippius H, and Ruther E (2000) Adverse effects of the comedication of neuroleptics and antidepressants. *European Neuropsychopharmacology* 10(Suppl. 3): 112–113.

Kaatz GW, Moudgal VV, Seo SM, and Kristiansen JE (2003) Phenothiazines and thioxanthenes inhibit multidrug efflux pump activity in *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* 47: 719–726.

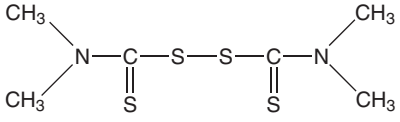
Ravn JF (1970) The history of thioxanthenes. In: Ayd FJ Jr. and Blackwell B (eds.) *Discoveries in Biological Psychiatry*, pp. 180–193. Philadelphia: J.B: Lippincott Company.

Stewart AJ, Manson LM, Dasani H, *et al.* (2000) Acquired haemophilia in recipients of depot thioxanthenes. *Haemophilia* 6: 709–712.

Thiram

Mona Thiruchelvam

- Chemical Abstracts Service Registry Number: CAS 137-26-8
- Synonyms: Tetramethylthiuram disulfide; Bis(dimethyldithiocarbanoyl)disulfide; Arasan; Fermide; Fernacol; Vancide
- Chemical/Pharmaceutical/Other Class: Dimethyl dithiocarbamate
- Chemical Formula: $C_6H_{12}N_2S_4$
- Chemical Structure:



Uses

Thiram is used as a seed protectant and to protect fruit, vegetable, ornamental, and turf crops from a variety of fungal diseases. It is also used as an animal repellent to protect fruit trees and ornamentals from damage by rabbits, rodents, and deer. Thiram has been used in the treatment of human scabies, as a sunscreen and as a bactericide applied directly to the skin or incorporated into soap. Thiram is available as dust, flowable, wettable powder, water-dispersible granules, and water suspension formulations and in mixtures with other fungicides. It has other applications including use as an accelerator and vulcanizing agent for synthetic and natural rubber, an activator in plastics manufacturing and as a chemosterilant in plastic film dry-wound dressing. It is also used as a wood preservative and lubricant oil additive.

Exposure Routes and Pathways

Thiram is a broad-spectrum fungicide and is found in most home-garden formulations whereby day-to-day human exposure can occur. Its use in various industries leads to multiple avenues for occupational exposures. The highest exposures occur in workers utilizing and/or manufacturing this compound. Exposure to thiram can occur via inhalation, ingestion, and eye or skin contact.

Toxicokinetics

Thiram is absorbed via the skin, mucous membranes, respiratory, and gastrointestinal tracts. Thiram is rapidly absorbed from the gastrointestinal tract. Thiram and other dimethyldithiocarbamates are metabolized to diethyldithiocarbamic (DDC) acid, diethylamine, and carbon disulfide. DDC is rapidly absorbed by the gastrointestinal tract and further metabolized by hepatic enzymes. A portion of the acid is excreted unchanged or as glucuronide conjugate. Further metabolism can result in the formation of dimethylamine and carbon disulfide residues.

Mechanism of Toxicity

Thiram and other dithiocarbamates are metabolic poisons. The acute effects of thiram are very similar to that of carbon disulfide, supporting the notion that the common metabolite of this compound is responsible for its toxic effects. The exact mechanism of toxicity is still unclear, however it has been postulated that the intracellular action of thiram involves metabolites of carbon disulfide, causing microsome injury and cytochrome P450 disruption, leading to increased heme-oxygenase activity. The intracellular mechanism of toxicity of thiram may include inhibition of monoamine oxidase, altered vitamin B_6 and tryptophan metabolism, and cellular deprivation of zinc and copper. It induces accumulation of acetaldehyde in the bloodstream following ethanol or paraldehyde treatment. Thiram inhibits the *in vitro* conversion of dopamine to noradrenalin in cardiac and adrenal medulla cell preparations. It depresses some hepatic microsomal demethylation reactions, microsomal cytochrome P450 content and the synthesis of phospholipids. Thiram has also been shown to have moderate inhibitory action on decarboxylases and, in fish, on muscle acetylcholinesterases.

Thiram also leads to thyroid dysfunction. This effect is thought to be a result of metabolic release of sulfur in follicular cells, causing inhibition of tyrosine iodination and ultimately hormone synthesis. Thiram induces alcohol intolerance similar to that of ant-abuse (disulfiram) either through its ability to inhibit acetaldehyde dehydrogenase or through the formation of a quaternary compound with ethanol.

Acute and Short-Term Toxicity (or Exposure)

The acute toxicity of thiram is rather low both in humans and experimental animals. Thus acute poisoning is highly unlikely unless large amounts are ingested. Thiram is an irritant of the eyes, mucous membranes, and skin and can elicit signs of neurotoxicity with acute high exposures.

Animal

In general, thiram is not very toxic unless high levels of exposure occur. The oral LD_{50} in rats is $560\,mg\,kg^{-1}$, and the lowest lethal dermal dose in rabbits is $1\,g\,kg^{-1}$. In contact with the skin and eyes of exposed rabbits, thiram caused irritation. In rabbits and guinea pigs, this substance has been shown to cause skin sensitization. Rats, cats, and rabbits survived a 4 h exposure to thiram dust at concentrations that ranged from 500 to $6225\,mg\,m^{-3}$. Animals killed by single oral doses of thiram showed patchy demyelination in the central nervous system, initially in the cerebellum and medulla. Thiram ($300\,mg\,kg^{-1}$) elicited convulsions and calcification in the cerebellum, hypothalamus, and medulla oblongata in rats.

Animals sacrificed after a single oral dose showed hyperemia and focal ulcerations of the gastrointestinal tract. Single dermal applications of 1000–$2000\,mg\,kg^{-1}$ to rats and 500–$1000\,mg\,kg^{-1}$ to rabbits produced only slight skin irritation.

Human

Since the acute toxicity of thiram is relatively low as is with most dithiocarbamates, acute intoxication in humans is unlikely to occur unless large amounts are ingested. Thiram can be absorbed from the gastrointestinal tract, through the skin and by inhalation of dust and fine spray mist. Inhalation can irritate the nose and throat causing coughing and wheezing. High exposure can lead to headache, dizziness, confusion, fatigue, nausea, and vomiting. Contact with thiram can irritate and burn the skin and eyes. Thiram has been given a toxicity rating of 4 and the probably lethal dose for humans is 50–$500\,mg\,kg^{-1}$. Alcohol, regardless of the route of exposure to thiram, can increase thiram toxicity and contributes to most systemic poisonings.

Chronic Toxicity (or Exposure)

Animal

Chronic exposure to thiram produces toxicity to several different organ systems in addition to those affected following acute exposure.

In an 80 day feeding study in rats 5 mg kg^{-1} day^{-1} in males and 6 mg kg^{-1} in females were found to be the no-effect levels. Paralysis and atrophy of the hind legs of females was observed at 67 mg kg^{-1} day^{-1}. In a dietary study where male rats were fed thiram at doses of 30, 58, and 132 mg kg^{-1} day^{-1} for 13 weeks, a dose-dependent reduction in body weight and food consumption was observed. In an 80 week study, male rats were fed 5, 20, and 52 mg of thiram per kg per day, and females 6, 26, or 67 mg kg^{-1} day^{-1}, resulting in a dose-dependent decrease in body weight and food consumption in males starting at 5 mg kg^{-1} and in females starting at 26 mg kg^{-1}. There were no treatment-related mortalities and moderate to severe clinical signs of toxicity were observed only among the females in the highest dosage group. In a 1 year dietary study in dogs the no-effect level was found to be 4.0 mg kg^{-1} day^{-1}. In a 2 year feeding study in rats the no-effect level was found to be ~4.9 mg kg^{-1} day^{-1}. At 2500 ppm there was 100% mortality within 17 weeks. General weakness, ataxia, and occasional paralysis were observed at 300 and 1000 ppm but there was no treatment-related mortality. Thiram caused an increase in squamous epithelial metaplasia in the thyroid and fatty infiltration in males. There was a reduction in incidences of spontaneous nephritis in both sexes.

Thiram is classified as an equivocal tumorigen with no known carcinogenic effects. No clear carcinogenic effect was demonstrated in mice given the maximum tolerated doses in a 77 week feeding study.

Thiram was shown to be teratogenic in rats (400 mg kg^{-1}, p.o. on days 6–15 of gestation), in mice (250 mg kg^{-1}, p.o. on days 6–15 of gestation), and in hamsters (250 mg kg^{-1}, p.o. on days 7 or 8 of gestation). The pattern of fetal defects was not well defined, with many changes linked to retardation of growth. In hamsters the combined effects of thiram and the solvent dimethyl sulfoxide were possibly synergistic. In mice, simultaneous co-administration of L-cysteine tended to abolish the teratogenic effect of thiram.

Thiram was found to have adverse effects on reproduction and to be embryotoxic in mice, rats, and hamsters at high doses that are toxic to adults. In a three-generation dietary study in rats administered 100 mg kg^{-1}day^{-1}, no adverse effects on reproduction or fetal development were noted. In a single generation study in rats, thiram (50 mg kg^{-1} day^{-1}, from gestation day 16 to postpartum day 21), caused reduced pup growth and survival. These effects were prevented when the pups were transferred to untreated lactating dams. In an inhalation study in rats, thiram (3.8 mg m^{-3} of air for 6 h per day, 5 days per week for 4.5 months) caused reproductive defects: prolonged estrous cycles, decreased conception rates, decreased fertility and reduced fetal weights. In mice, thiram (132 mg kg^{-1} day^{-1}, p.o. for 13 weeks) caused male infertility and 96 mg kg^{-1} day^{-1} for 14 days delayed estrous cycles. These adverse effects were reversed when treatment ceased.

In another chronic study, eight out of 24 female rats fed 67 mg kg^{-1} day^{-1} for 80 weeks developed severe signs of neurotoxicity including ataxia and ascending paralysis; degeneration of axis-cylinders and presence of macrophages in the bundle of the sciatic nerve were observed.

Human

In humans, thiram is an eye, nose, and throat irritant, a central nervous system toxicant, and a skin sensitizer. Volunteers given daily thiram doses of 0.5 g day^{-1} for several weeks showed no adverse effects. One worker who treated seeds with thiram for a 10 h period, during which he was estimated to have received a substantial exposure, died 4 days later. Several members of a group of workers exposed to thiram during the planting of trees reported experiencing eye, nose, and throat irritation, headache, and skin problems.

Workers chronically exposed to thiram and concurrently ingesting alcohol have developed skin reactions without any systemic effects. In these cases, the skin becomes red, flushed, and itchy. In susceptible individuals, thiram can cause dermatitis even without concomitant alcohol ingestion, and sensitization of the skin has occurred on the hands, forearms, and feet of exposed individuals. The International Agency for Research on Cancer notes that studies from the USSR report thyroid gland enlargement, one case of thyroid cancer, and seven cases of thyroid abnormalities in a group of 105 workers exposed to thiram at unspecified concentrations for more than 3 years.

The use of thiram in the manufacture of many rubber and plastic products (e.g., shoes) and as a fungicide in recreational areas (e.g., golf courses and bowling greens) presents considerable opportunity for exposure of sensitive individuals to the

compound. Thiram is considered to be a borderline allergen, requiring several exposures to produce sensitization.

In Vitro Toxicity Data

In vitro systems have been developed to try and understand the mechanism of action of thiram alone and in the presence of other potentiating compounds. The genotoxic, cytotoxic, and neurotoxic effects of thiram have been studied using a variety of primary cultures as well as cell-lines. Lymphocytes exhibited sister chromatid exchanges and micronuclei with exposure to thiram. Thiram caused single-strand DNA breaks in testicular cells *in vitro*. Other studies indicate that thiram can be both clastogenic and mutagenic.

Clinical Management

Thiram 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, and vomiting induced immediately. If thiram dust is inhaled, the exposed individual should be moved 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, the eyes should be flushed with large amounts of water for at least 15 min.

Environmental Fate

Thiram is of low to moderate persistence. It is only slightly soluble in water ($30\,mg\,l^{-1}$) and has a strong tendency to adsorb to soil particles, and thus is not expected to contaminate groundwater. The soil half-life for thiram is reported to be 15 days. Thiram degrades more readily in acidic soils and in soils high in organic matter. Thiram has been shown to persist up to 2 months in sandy soil but disappeared within 1 week from compost soil. The major metabolites of thiram in soil are copper dimethyldithiocarbamates, dithiocarbamate, dimethylamine, and carbon disulfide. In soil, thiram can be degraded by microbial action or by hydrolysis under acidic conditions. In water, thiram is rapidly broken down by hydrolysis and photodegradation, especially under acidic conditions.

Ecotoxicology

Thiram is generally of low toxicity to most wildlife. It is moderately toxic to birds. The reported dietary LC_{50} of thiram in Japanese quail is greater than 5000 ppm.

Thiram is highly toxic to fish. The LC_{50} for the compound is $0.23\,mg\,l^{-1}$ in bluegill sunfish and $0.13\,mg\,l^{-1}$ in trout. Thiram does not bioconcentrate in aquatic organisms.

Exposure Standards and Guidelines

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

See also: Dithiocarbamates; Pesticides.

Further Reading

Extoxnet Extension Toxicology Network (1993) Thiram. Pesticide Management Education Program, Cornell University, Ithaca, NY.

US Environmental Protection Agency (October 1988) Pesticide Fact Sheet: Maneb. Office of Pesticides and Toxic Substances, Office of Pesticide Programs, US EPA, Washington, DC.

US Environmental Protection Agency (1992) Integrated Risk Information System – Thiram (CASRN 137-26-8). US EPA, Washington, DC.

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

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, International Program on Chemical Safety (1988) Thiram. Environmental Health Criteria No. 71. Geneva, Switzerland.

Thorium and Thorium Dioxide

Shayne C Gad

- Representative Compounds: Thorium dioxide (ThO_2); Thorium disulfide (ThS_2)
- Chemical Abstracts Service Registry Number: CAS 7440-29-1
- Chemical/Pharmaceutical/Other Class: Actinide metals
- Chemical Formula: Th^{4+}

Uses

Discovered in 1829, thorium is a naturally occurring radioactive metal with no stable isotopes. It is about as abundant as lead. Soil commonly contains an average of about six parts of thorium per million parts (ppm) of soil. Thorium occurs in the minerals thorite, thorianite, orangite, and yttrocrasite, and in monazite sand. Rocks in some underground mines may also contain thorium in a more concentrated form. After these rocks are mined, thorium is usually concentrated and changed into thorium dioxide or other chemical forms. Thorium-bearing rock that has had most of the thorium removed from it is called 'depleted' ore or tailings. Thorium is present in nuclear reactor fuels, and is used in the manufacture of incandescent gas-light mantles, welding electrodes and ceramics, as a hardener in magnesium alloys, and as a chemical catalyst. In addition, it is used in sun lamps, photoelectric cells, and in target materials for X-ray tubes. Thorium is present in fires and explosions caused by thorium metal powder and has been recovered as a by-product of uranium production.

Exposure Routes and Pathways

Ingestion of liquid, inhalation of dust or gas, and percutaneous absorption are the routes of exposure. Occupational exposure to thorium and thorium compounds may occur through handling of various thorium salts in the fabrication of thorium ingots, in handling thorium salts in various industrial uses, in the fume from welding with thoriated tungsten electrodes, in the casting and machining of thorium alloy parts, and from fires and explosions caused by thorium metal powder. As thorium is a naturally occurring background element, the general population may be exposed daily to thorium and thorium compounds through dermal and other contact.

Toxicokinetics

Thorium is poorly absorbed from both the lung and digestive tract, and 70% of the thorium reaching the blood is translocated to the bone, 4% to the liver, and 16% to all other organs and tissues of the body. Thorium accumulates in the liver, spleen, lymph nodes, and bone marrow, leading to long-term exposure with a diversity of cells. Thorium is retained the longest when it has entered the body in the form of an insoluble compound. Transferrin plays a major role in the transport and cellular uptake of thorium. Thorium can be displaced from transferrin by an excess of iron, but it is not known whether thorium and iron bind to the same sites on the transferrin molecule. Tissue distribution and retention are highly dependent upon dose and route. Most of the absorbed dose goes to the reticuloendothelial system, liver, spleen, and bone marrow. Thorium is excreted slowly and primarily via bile to feces. Thorium can also be eliminated via exhalation of radioactive thoron daughter gas.

Mechanism of Toxicity

Binding with bone and other glucoproteins and, in some cases, an interaction with zinc. Thorium oxide is radioactive. As noted above, thorium accumulates in the liver, spleen, lymph nodes, and bone marrow, leading to long-term exposure with a diversity of cells.

Acute and Short-Term Toxicity (or Exposure)

Animal

No deaths were reported in animals following inhalation exposure, and high exposure levels were necessary to produce death in animals following oral exposure, since gastrointestinal absorption is very poor.

Human

Acute exposure results in dermatitis. Thorium oxide has a TD_{Lo} of $1\,g\,kg^{-1}$.

Chronic Toxicity (or Exposure)

Animal

It is a carcinogen and developmental toxin. Animal studies have shown that breathing in thorium may result in lung damage. Other studies in animals

suggest drinking massive amounts of thorium can cause death from metal poisoning.

Human

Studies of thorium workers have shown that breathing thorium dust may cause an increased chance of developing lung disease and cancer of the lung or pancreas many years after being exposed.

Clinical Management

The removal of thorium from the body has been achieved by the use of chelating agents, for example, ethylenediaminetetraacetic acid and diethylenetriaminepentaacetic acid.

Environmental Fate

Thorium's usage may result in release of thorium compounds to the environment through various waste streams. As noted above, thorium is also found naturally. Thorium compounds are expected to exist in the particulate phase based on their low vapor pressures and may be removed from the air by wet and dry depositions.

See also: Lead.

Further Reading

Bingham E, Cohrssen B, and Powell CH (eds.) (2001) *Patty's Toxicology*, vol. 3, pp. 409–422. New York: Wiley.

Fisenne IM, Perry PM, Decker KM, and Keller HW (1987) The daily intake of uranium-234, uranium-235, uranium-238, and thorium-228, thorium-230, thorium-232, and radium-226, radium-228 by New York City residents, New York, USA. *Health Physics* 53: 357–364.

Stradling N, Phipps A, and Hodgson A (2001) Dosimetric models for thorium and practical problems in assessing intakes. *Radiation Protection Dosimetry* 97(2): 89–94.

Relevant Website

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

Three Mile Island

John Sorensen

Introduction

The accident at the Three Mile Island Unit 2 (TMI-2) nuclear power plant near Middletown, Pennsylvania, on Wednesday, March 28, 1979, was the most serious in a commercial nuclear power plant in the United States even though it led to no deaths or injuries to plant workers or members of adjacent communities. It did have major implications for the nuclear industry because it resulted in major changes in the regulatory requirements involving emergency response planning, reactor operator training, radiation monitoring, human factors engineering, radiation protection, and many other areas of nuclear power plant operations. It also caused the US Nuclear Regulatory Commission (NRC) to tighten and heighten its regulatory oversight.

The accident at TMI-2 was initiated at 4 AM by a minor malfunction, or transient, in the non-nuclear part of the reactor. The main feed-water pumps stopped running, caused by either a mechanical or electrical failure, which prevented the steam generators from removing heat. This minor event would evolve into a series of automated responses in the reactor's coolant system, and during all of this, the relief valve on top of a piece of equipment called 'the pressurizer' would become stuck in an open position. Misreading of the plant conditions by the operators over a 2 1/4 h period before the relief valve was closed and the turning off of an automatic emergency cooling system caused the reactor core to become partially uncovered and severely damaged. The major consequences of the accident would unfold over the next week and it would take a month to bring the reactor to a cold shutdown.

Emergency Response

During the first several days of the accident, communications between the NRC Incident Response Center in Bethesda, Maryland, and the site were problematic and making it extremely difficult for the NRC to obtain up-to-date information from the plant and utility. Communications were so poor that by Friday morning the NRC management personnel still did not have a clear understanding of conditions at the site. As a result, the NRC recommended an evacuation to the state on the basis of poor and incomplete information. A general evacuation was never officially ordered. Communications did not

improve until Harold Denton, designated the sole source of information, arrived at the TMI site and communicated directly with NRC headquarters, the Governor's office, and the White House.

The suggestion by the NRC of a possible large-scale evacuation out to 20 miles was quite different from the planning requirements imposed by the NRC and Pennsylvania before the accident. The 5 mile emergency plans were developed according to a Pennsylvania requirement for emergency planning within a 5 mile radius of nuclear power plants. At TMI-2, although the radiation releases were significantly lower than the design-basis accident, evacuation was being considered for distances much greater than 5 miles.

On mid-morning of Friday, March 30, the governor's press secretary told reporters that there was no need for evacuation and that people in a 10 mile vicinity of the plant remain inside for a while. The only official warning to the public to evacuate came at ~12:30 PM, on Friday, when then Governor Thornburgh advised pregnant women and preschool age children to leave the area within a 5mile radius of TMI until further notice. He also ordered schools to close. The advisory to pregnant women and preschool children was lifted on April 9.

On Saturday and Sunday, other NRC officials believed there was an imminent danger of an explosion of a hydrogen bubble that had formed within the reactor vessel, and the possibility of a large evacuation was again a major subject of discussion. By Monday, the hydrogen bubble had been substantially reduced. Harold Denton announced on Tuesday, April 3, that the bubble had been eliminated.

Human Exposure to Radiation

It is estimated that between March 28 and April 15, the collective dose (total population dose) resulting from the radioactivity released to the population living within a 50 mile radius of the plant was ~2000 person-rems. The estimated annual collective dose to this population from natural background radiation in this area is ~240 000 person-rems. Thus, the increment of radiation dose to persons living within a 50 mile radius due to the accident was somewhat less than 1% of the annual background level.

The maximum estimated radiation dose received by any one individual in the off-site general population (excluding the plant workers) during the accident was 70 millirems. Estimates are that the average dose to ~2 million people in the area was only about 1 millirem. To put this into context, exposure from a full set of chest X-rays is ~6 millirems. Compared to the natural radioactive background dose of ~100–125 millirems per year for the area, the average dose to a person living within 5 miles of the nuclear plant was calculated to be ~10% of annual background radiation and probably was less. The maximum dose to a person at the site boundary would have been less than 100 millirems.

On the basis of scientific knowledge, the radiation doses received by the general population as a result of exposure to the radioactivity released during the accident were so small that there will be no detectable additional cases of cancer, developmental abnormalities, or genetic ill-health as a consequence of the accident at TMI.

In the months following the accident, many questions were raised by members of the public and interest groups about possible adverse effects from radiation on human, animal, and plant life in the TMI area. Thousands of environmental samples of air, water, milk, vegetation, soil, and foodstuffs were collected by a number of groups monitoring the area. These samples showed that very low levels of radionuclide could be attributed to releases from the accident. However, comprehensive investigations and assessments have concluded that in spite of serious damage to the reactor, most of the radiation was contained and that the actual release had negligible effects on the physical health of individuals or the environment and no adverse effects could be directly correlated to the accident.

Public Response

The governor's warning was the only official warning issued by the government. People in the vicinity of the plan were bombarded by media coverage of the events. This coverage suggested that a major evacuation was imminent. As a result, many people decided to evacuate despite the limited recommendation by the governor. It is estimated that 144 000 people within a 15 mile radius evacuated. It is also estimated that some people in the 15–40 mile radius also evacuated. Major reasons for evacuating were concern over the hydrogen bubble or conflicting information. The main reasons for not evacuating were that people had to work or they were waiting for an official evacuation order.

Most people within the 15 mile radius evacuated on Friday, March 30. A much smaller number evacuated on Thursday, Saturday, and Sunday. Most people had returned to their homes by Thursday, April 5, well before the lifting of the governor's order.

Conclusion

Following the TMI accident President Carter formed a commission to investigate the accident and make recommendations about needed changes in the

nuclear power industry. Six months later the 12 member commission issued its findings, recommending fundamental changes in the organization, procedures, practices, as well as the nuclear industry. Radical changes in the way the industry was regulated ensued. These regulations still continue to evolve as the industry matures.

See also: Chernobyl; Cuyahoga River; Radiation Toxicology, Ionizing and Nonionizing.

Further Reading

Walker JS (2004) *Three Mile Island: A Nuclear Crisis in Historical Perspective*. Berkeley: University of California Press.

Relevant Website

http://www.nrc.gov – Fact Sheet on the Accident at Three Mile Island.

Threshold Limit Value *See* Occupational Exposure Limits.

Thyroid Extract

Greene Shepherd

This article is a revision of the previous print edition article by Bridget Flaherty, volume 3, pp. 236–237, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 8028-36-2
- Synonyms: Dry thyroid; Desiccated thyroid; Thyreoidin; Thyroid Strong, Thyroglobin
- Chemical/Pharmaceutical/Other Class: Natural hormone that provides a mixture of levothyroxine (T4) and liothyronine (T3)

Uses

Thyroid extract is used in the treatment of hypothyroidism, myxedema, and cretinism. It is also used as a diagnostic agent and for suppression of pituitary thyroid-stimulating hormone. Synthetic derivatives are preferred because of uniform potency.

Exposure Routes and Pathways

Ingestion is the route of exposure in both accidental and intentional exposures.

Toxicokinetics

Thyroid extract is partially absorbed from the gastrointestinal tract. Up to 79% of a therapeutic dose is absorbed. Approximately 99% is protein bound. Thyroid extract contains both levothyroxine (T4) and liothyronine (T3). T4 is deiodinated in the liver, kidney, and tissues to form active T3 and inactive T2. The half-life of T4 is 5.3–9.4 days. T3 has a half-life of 2.5 days.

Mechanism of Toxicity

Thyroid hormones are necessary for metabolism, growth, and development. The main effect of thyroid hormones is increased metabolic rate, increased oxygen consumption, and increased metabolism of carbohydrates. Because the mixture contains both T3 and T4, systemic toxicity will be evident within a few hours and may be quite prolonged. Synthetic products that contain only T4 can have a latent period of several days before the development of significant symptoms.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals that ingest thyroid extract are at risk for thyroid toxicity. Signs of toxicity include vomiting, diarrhea, tachycardia, tachypnea, a decreased level of consciousness, and restlessness. Animal preparations frequently have higher concentrations that human preparations do.

Human

Ingestion of small amounts of thyroid extract produces few symptoms, if any. Symptoms of thyroid toxicity include increased heart rate, nausea, vomiting, diarrhea, restlessness, and fever. The development of thyrotoxicosis with acute exposure is rare except in very large overdoses (grams of thyroid extract containing several milligrams of T3 and/or T4).

Chronic Toxicity (or Exposure)

Animal

Thyroid extract has been used in veterinary practice. Current practice involves synthetic levothyroxine.

Toxicity is related to excessive thyroid hormone (manifested as polyuria, polydipsia, nervousness, aggressiveness, tachycardia, hyperthermia).

Human

Chronic overdosing is more likely to cause thyrotoxicosis than an acute overdose. Thyrotoxicosis should be suspected in patients exhibiting tachycardia, cardiac arrhythmias, hypertension, tremors, and seizures. Coma and circulatory collapse can be seen in severe cases of thyrotoxicosis. This is especially dangerous in patients with cardiac conditions. Deaths have also occurred in healthy adults that have used thyroid extract to lose weight.

In Vitro Toxicity Data

Studies of *in vitro* and *in vivo* models of hyperthyroidism have documented substantial impact on rat liver function. Recent developments have suggested that these findings are likely due to induction of apoptosis via a mitochondria-mediated pathway or pathways.

Clinical Management

Basic and advanced life-support measures should be utilized as needed. Activated charcoal is an effective method of gastric decontamination for large ingestions that present soon after exposure. EKG and blood pressure monitoring should be utilized in severe cases. Measurements of T3 and T4 levels should be obtained frequently in large ingestions until levels have normalized. Propranolol (a nonselective beta antagonist) can be used to treat hypertension, tachycardia, and cardiac arrhythmias. Extracorporeal means of elimination are ineffective in most cases due to extensive protein binding.

See also: Levothyroxine; Liothyronine.

Further Reading

Bergeron GA, Goldsmith R, and Schiller NB (1988) Myocardial infarction, severe reversible ischemia, and shock following excess thyroid administration in a woman with normal coronary arteries. *Archives of Internal Medicine* 148: 1450–1453.

Golightly LK, Smolinske SC, and Kulig KW (1987) Clinical effects of accidental levothyroxine ingestion in children. *American Journal of Diseases of Children* 141: 1025–1027.

Gorman RL, Chamberlain JM, and Rose SR (1988) High anxiety-low toxicity: A massive T4 ingestion. *Pediatrics* 82: 666–669.

Ticks *See* Lyme Disease.

Times Beach

Pertti J Hakkinen

The Town of Times Beach, Missouri, USA, gained international attention in 1982 when the US Environmental Protection Agency (US EPA), acting upon recommendations from the US Centers for Disease Control (now the Centers for Disease Control and Prevention), closed down the town after discovering dangerous levels of dioxin. The roads to the town were blocked off, and the site was patrolled around-the-clock by security guards. The contamination occurred because the town and other local towns, businesses, farmers, and churches had, in the early 1970s, hired a waste oil recycler to spray dioxin-contaminated used oil on local roads and parking lots to control dust. The dioxin was an unwanted chemical by-product of certain manufacturing processes, and the recycler had mixed dioxin wastes from a chemical plant into the oil.

Times Beach was one of the most extensive cleanups in US EPA Superfund history. The cleanup effort officially began when, in 1983, the US EPA added the site to the first Superfund National Priorities List (NPL) for further investigation and long-term cleanup actions. After the site was listed, the US EPA permanently relocated >2000 people and tore down all of the homes and businesses.

Cleaning the Times Beach site was a massive estimated $200 million effort that included installation of a temporary incinerator to burn the contaminated soil, and the erection of a 15 ft high barrier around the incinerator to protect that area from regular flooding by the Meramec River. Contaminated soils were dug up, burned, and the resulting waste ash was buried on site. Cleanup of the site was completed by the end of 1997 by the US EPA and Syntex Agribusiness, the company that assumed responsibility for the site's cleanup. More than 265 000 tons of dioxin-contaminated soil from the site and 27

nearby areas that had been sprayed with dioxin-contaminated waste oil had been cleaned. The US EPA and the State of Missouri worked closely with Syntex during cleanup to ensure that the restoration made the site suitable for productive use. It is now a home to an extensive bird sanctuary and migratory bird waterways as a result of the cleanup. The migratory bird waterways were created by allowing some of the soil excavation pits to fill with rain water. Further, in 1999, a new 500-acre State park commemorating the famous US highway named Route 66 opened on the site.

See also: Dioxins.

Relevant Website

http://www.epa.gov – Details of 'Times Beach' and 'Superfund Successfully Responds in Times Beach' can be found in the US EPA website.

Tin

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 7440-31-5
- Synonym: Stannum
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formulas: Sn^{2+}; Sn^{4+}

Uses

Tin is found in many forms, including stannous oxide (SnO), triethyltin $(Sn(C_2H_3))_3$, and triphenyltin hydroxide $(Sn(C_6H_6)OH)$. Tin compounds have many uses, including protective coatings, tin plate, and cans. Alloys such as bronze, brass, and solders also contain tin. Minor uses include dyes, ceramics, flame retardants, and pigments. Stannous fluoride is often used in toothpaste to prevent cavities. Organotin compounds have been used as antifouling agents, as pesticides, and as stabilizers in plastics.

Background Information

Tin has a long, colorful history. This metal was discovered first in Thailand over 2000 years ago. Early craftsmen discovered that bronze – a noncorrosive metal that is extremely hard and strong enough to be used for spears, swords, arrows, and other especially important objects at that time – could be produced by smelting tin with copper. Tin is also the primary constituent of pewter. Long ago, people developed the belief that trace amounts of tin seemed to help prevent fatigue and depression, and that drinking out of tin cups could help combat these ailments. Tin toys and tin roofs have also enjoyed great popularity in the past.

Exposure Routes and Pathways

Inhalation, dermal contact, and ingestion are all potential pathways of exposure to the different forms of inorganic and organic tin one might encounter in the environment. Human exposure to tin is primarily by ingestion of food, especially canned food products. Occupational exposure to tin may be significant in some industrial environments. In industrial areas, tin is inhaled from polluted air. Organotin compounds, typically found mostly in water, can be absorbed dermally. Stannous fluoride can be swallowed from toothpaste. Elemental tin may be ingested with food. Large amounts of tin must be ingested before levels of absorbed tin are detectable.

Ambient environmental levels of tin are generally quite low, except in the vicinity of pollution sources. The Environmental Protection Agency has previously identified tin in just 11 of 1177 evaluated hazardous waste sites.

Toxicokinetics

Inorganic tin compounds are not easily absorbed from the gastrointestinal tract. Inhaled tin first resides in the lungs and then is transferred to the liver and kidneys. Absorbed compounds are carried by the red blood cells. Inorganic tin is mainly excreted in urine. Organic tin compounds are more easily absorbed from the gastrointestinal tract and skin, concentrated in the blood and urine, and excreted in the bile. Most of an administered dose is excreted within 48 h.

There is little information on the effect of tin on enzymes. Organic tin compounds can inhibit the hydrolysis of adenosine triphosphate, resulting in uncoupling of oxidative phosphorylation.

Mechanism of Toxicity

All organic tin compounds inhibit mitochondrial oxidative phosphorylation (hydrolysis of adenine triphosphate) and brain glucose oxidation and are toxic. Very little data are available on inorganic tin.

Acute and Short-Term Toxicity (or Exposure)

Animal

Organic tin can be corrosive. In general, however, tin is considered to have relatively low acute toxic effects.

Human

Organic tin compounds are classified as eye, skin, and respiratory irritants. Inhaled tin particles lead to a mild pneumoconiosis known as stannosis. Orally absorbed inorganic tin produces nonspecific symptoms, including nausea, vomiting, diarrhea, muscle twitching, and even paralysis. Organic tin compounds are much more toxic than inorganic tin compounds.

Chronic Toxicity (or Exposure)

Animal

Although tumorigenic in rat implant studies, there is no conclusive evidence that tin compounds are mutagenic, carcinogenic, or teratogenic. Absorbed tin concentrates in the kidneys, liver, and bone of experimental animals.

Human

Major target organs are the nervous system, respiratory system, gastrointestinal system, and kidneys. Tetraethyltin is converted to triethyltin, which is a potent skin irritant and neurotoxin. It produces depression with loss of memory and aggressive behavior. It also produces cerebral edema and en cephalopathy. Triphenyltin is an immunodepressant. Some organic tin compounds are unusually toxic to the central nervous system.

Clinical Management

Supportive measures must be taken; there is no specific antidote or chelating agent for tin. Administration of water (for dilution) after ingestion of a tin compound may be helpful. Emesis is not recommended.

Ecotoxicology

Tin is a naturally occurring element found in environmental media in inorganic compounds. Tin may be released to the environment from natural and anthropogenic sources. The most significant releases of tin are from burning of fossil fuels and industrial production and use of tin. Tin compounds are generally only sparingly soluble in water and are likely to partition to soils, sediments, and possibly to aquatic organisms. Photodegradation of organotins may occur at relatively slow rates. Organotin compounds may be significantly bioconcentrated by aquatic organisms. Tin has been historically used in antifouling paints and coatings for the bottom of boats, but this has been discontinued due to its extreme toxicity to marine organisms.

A bioconcentration factor (BCF) relates the concentration of a chemical in plants and animals to the concentration of the chemical in the medium in which they live. It was estimated that the BCFs of inorganic tin were 100, 1000, and 3000 for marine and freshwater plants, invertebrates, and fish. Marine algae can bioconcentrate stannic tin by a factor of 1900. The BCF of tributyltin was estimated to be 473, but measured BCFs were always higher. Bioconcentration factors for bis(tributyltin)oxide with marine oysters were measured as 2300–11 400. Seven-day BCFs were derived for seven organotin compounds for muscle, liver, kidney, and vertebra tissue of carp. The BCFs ranged from 12 to 5012; the highest factors were found for tributyltins. However, these factors were not based on steady-state conditions, and may be low estimates. No information was obtained on the food chain and biomagnification of inorganic or organic tin.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value (TLV) – time-weighted average for tin metal, tin oxide, and inorganic compounds (except SnH_4) is $2\,mg\,m^{-3}$. The TLV for tin oxide is $1\,mg\,m^{-3}$. The TLV for organic tin compounds is $0.1\,mg\,m^{-3}$ with a skin exposure warning.

See also: Metals; Neurotoxicity; Organotins; Pollution, Water.

Further Reading

Bingham E, Cohrssen B, and Powell CH (2001) *Patty's Toxicology*, 5th edn., vol. 2, pp. 576–597. New York: Wiley.

Dart RC (2004) *Medical Toxicology*, 3rd edn., pp. 1465–1467. Baltimore, MD: Williams and Wilkins.
Goyer RA, Klaassen CD, and Waalkes MP (1995) *Metal Toxicology*. San Diego, CA: Academic Press.
Rudel H (2003) Case study: Bioavailability of tin and tin compounds. *Ecotoxicology and Environmental Safety* 56(1): 180–189.

Relevant Websites

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Tin.
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Tin.

Tissue Repair

Udayan M Apte and Harihara M Mehendale

Introduction

Tissue repair (TR) refers to compensatory regeneration of a tissue followed by surgical, mechanical, or chemical-induced injury resulting in restoration of structure and function of the tissue. TR is characterized by cell division to increase the number of cells, differentiation, and specification of the newly divided cells, angiogenesis, that is, regeneration of blood vessels to restore blood supply, and regeneration of extracellular matrix (ECM), which holds the tissue together. TR is a complex and comprehensive process that encompasses various aspects of tissue rebuilding and is governed by an intricate molecular signaling (**Figure 1**). Not all the tissues in the body are capable of stimulating TR. The so-called 'postmitotic' tissues such as muscles and nervous tissue cannot undergo TR while other tissues such as skin, liver, kidney, and lungs are capable of undergoing a TR upon surgical resection, mechanical, or chemical injury.

TR following chemical-induced injury has its special characteristics. Unlike surgical resection, chemicals cause injury at a slower pace and the progression of injury takes place long after the offending chemical is removed from the body. In most cases chemicals are metabolized in the body by various drug-metabolizing enzymes (DMEs) to generate reactive metabolites. These highly reactive metabolites bind and inactivate macromolecules in the cell necessary for cellular functions leading to cell death. Initiation of injury in the tissue leads to stimulation of compensatory TR, directed toward replacing the dead cells. Thus, the extent of TR following chemical injury often depends on the dose of the offending toxicant, the extent of injury, the toxicokinetics of the toxicant (half-life), and exposure time (acute versus chronic exposure). Additionally, TR is governed by a number of other factors

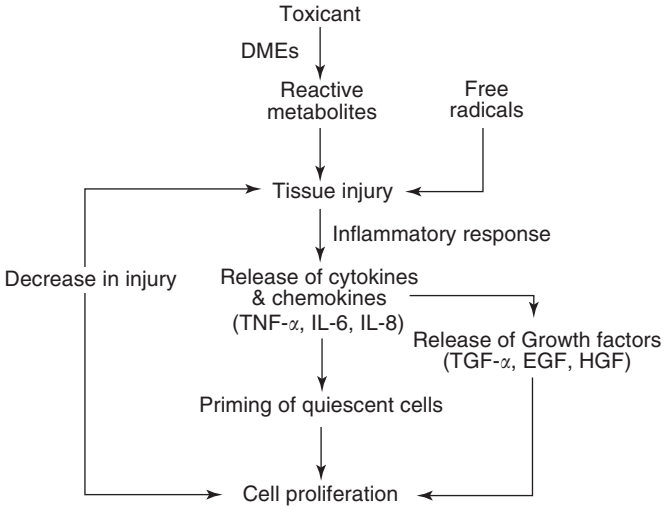


Figure 1 Schematic representation of stimulation of tissue repair. Chemicals and drugs are metabolized in the tissue to their reactive metabolites, which initiate injury. An inflammatory response follows the injury stimulating release of cytokines and chemokines, which prime the quiescent cells to enter cell cycle. Additional stimulation to complete cell division comes from growth factors. As cell proliferation increases, the dead tissue is replaced by viable cells and injury regresses.

such as the age, species and strain difference, nutrition, and presence of disease.

The Cells Involved in Tissue Repair

There are three main types of cells in the body depending upon their regenerative capacity – labile, stable, and permanent. The labile cells are under continuous active division and replace the cells that are lost from the body. Examples of labile cells include the epithelia of ducts, hematopoietic stem cell, and epidermis. Injury to labile cells is rapidly repaired due to an aggressive TR response.

The stable cells have a long life span and divide at a very slow rate. The parenchymal cells of the most solid glandular organs such as liver and kidney are stable cells. They are capable of undergoing rapid division upon injury to the organ to replace the dead cells. Regeneration following injury in the tissues that contain stable cells requires having at least some healthy cells remaining to undergo proliferation and regeneration. Liver is a classic example of such type of tissue where, under normal conditions, less than 1% cells are in division. Upon partial hepatectomy or acute chemical-induced injury, the healthy hepatocytes are stimulated for division resulting in complete restoration of tissue mass and liver function. In case of a chronic injury, the repeated injury caused by the chemicals results in failure of repair mechanisms leading to scar formation (fibrosis), cirrhosis and may develop into cancer.

The central nervous system and the cardiac and skeletal muscles are permanent tissues that do not divide in postnatal life. Due to the inability in proliferation, injury to such tissue results in a scar formation and a permanent loss of function. Extensive investigations are underway to compensate the inability of proliferation in these tissues by using stem cells.

Tissue Repair in Specific Organs

The main organs that exhibit capacity to regenerate following chemical-induced injury (and surgical/mechanical injury) include liver, kidney, lungs, skin, and gastric mucosa. The regeneration of skin has been mainly studied using various models of wound repair and are not a major form of toxicant-induced injury. TR following toxic injury in true sense is observed mainly in liver, kidney, lungs, and gastrointestinal tract, out of which, liver remains to be the most widely studied organ.

Tissue Repair in Liver

The remarkable capacity of liver to regenerate upon surgical resection or toxicant-induced injury has been studied extensively and is still at the center of research in hepatobiology. Liver regeneration has been known since ancient times as indicated by the myth of Prometheus. According to the myth, Prometheus, a Greek god, stole fire from Zeus and gave it to the humans. Zeus punished Prometheus for this by subjecting Promethues's liver to be eaten by an eagle. Each night the eagle would eat Prometheus's liver, which would grow back the next morning, only to be eaten again by the eagle, thus subjecting Prometheus to an eternal torture. Thus, the myth of Prometheus can be viewed as an evidence that ancient humans knew about the regenerative capacity of the liver.

Two-third partial hepatectomy (PH) is the main model used to study liver TR. Surgical removal of 70% tissue mass of liver results in stimulation of a massive regeneration response. Hepatocytes are generally in a differentiated, nondividing stable state. Upon PH, hepatocytes dedifferentiate and enter cell cycle and proliferate. Substantial cell division is observed within 2 days following PH along with regeneration of ECM, angiogenesis, and regeneration of hepatobilliary ducts. In experimental animals such as rats subjected to PH, the complete tissue mass is replaced within seven days following PH.

In the last two decades extensive information has been gathered about TR following chemical-induced liver injury. Liver is a prime target for a variety of chemicals including pharmaceutical drugs, mainly by virtue of its role as a major site of drug metabolism in the body. Hepatocytes are the main reservoirs of DMEs, which metabolize drugs and toxicants to more water-soluble metabolites. During this process, sometimes, toxic metabolic intermediates arise, which attack the macromolecules of hepatocytes directly, or by generating free radicals, resulting in injury and death of the hepatocytes. In response to such drug-induced injury, liver TR is stimulated, which opposes progression of injury by replacing the dead cells and restoring the structure and function of liver. Liver TR following chemical-induced toxicity holds a great clinical significance since acute liver failure (ALF) induced by drugs and toxicants is a prevalent clinical condition. It is known that more than 800 pharmaceutical drugs and chemicals are associated with ALF. In such conditions, ability of the patient to stimulate an effective TR may have a lasting effect on the final outcome (survival versus death) of the ALF. Extensive research is being conducted to develop regenerative therapies against drug-induced hepatotoxicity and ALF.

A number of models have been used to study the TR following chemical-induced injury in

experimental animals. Liver TR has been studied in rodents following liver injury induced by carbon tetrachloride (CCl_4), acetaminophen, thioacetamide, galactoasmine, trichloroethylene (TCE), allyl alcohol, and lipopolysaccharide (bacterial endotoxin). The major finding of studies with a diverse group of chemicals and a number of different animals models is that TR plays an important role in determining survival following toxicant exposure. It is observed that animals treated with nonlethal doses of toxicants develop relatively lower liver injury, and recover as soon as TR replaces the dead cells. In contrast, animals exposed to very high, lethal doses exhibited inhibition of TR resulting in progression of injury and death.

Tissue Repair in Kidney

Due to the filtration and excretory function, kidney receives extensive blood supply, which makes it a prime target for toxicant induced injury. Kidney damage due to toxicants leads to necrosis of tubular cells, resulting in renal failure and death. The renal tubular epithelium is known to regenerate following toxicant- or ischemia-induced renal injury. The renal tubule or nephron is the functional unit of kidney and exhibits regiospecific differences in regenerative capacity in its different structures. The glomeruli do not exhibit regenerative capacity and thus, glomerular damage is irreversible. In contrast, the tubular epithelium exhibits extensive ability for TR, and as a result, the damage to tubular epithelium is completely reversible. Similarly, the cortical tubules exhibit much higher regenerative capacity as compared to the medullary tubules.

A number of toxicants and drugs are known to induce renal injury resulting in acute renal failure, a prevalent clinical condition. These include antibiotics such as gentamycin, anticancer agents such as cisplatin, radiocontrast agents such as diatrizoates, heavy metals such as mercury, and metabolites of environmental toxicants such as *S*-(1-2-dichlorovinyl)-L-cysteine (DCVC, a metabolite of potent carcinogen TCE, which is a liver toxicant itself). Renal TR has been studied in great detail using various toxicants including DCVC, cisplatin, and folic acid and these studies indicate that TR plays a significant role in survival following exposure to renal toxicants.

Tissue Repair in Lung

Lung is a complex tissue formed of a variety of histologically different cell types. Lung is damaged by a number of airborne toxicants such as ozone, arsenic, asbestos and isocyanates. Many of the lung toxicants are important from the occupational health point of view. Lung exhibits extensive regenerative capability, especially in the epithelium of trachea and bronchi, which regenerate rapidly after abrasive action of airborne toxicants. Lung injury initiates an inflammatory response (which is also true in the case of all other organs), following which the alveolar type II pneumocytes undergo rapid proliferation and migration, finally differentiating into alveolar type I pneumocytes. The type I pneumocytes repopulate the damaged areas and restore the epithelial lining.

Tissue Repair and Dose Response

One of the most impressive characteristics of TR following chemical-induced toxicity is its ability to follow a dose response (**Figure 2**). It has been observed that increasing doses of toxicant induce TR in increasing order, until a threshold is reached.

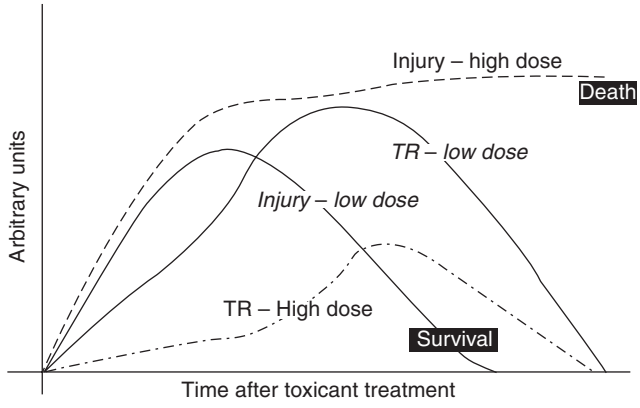


Figure 2 Schematic representation depicting that tissue repair follows dose response. Tissue repair (TR) increases as the dose of the offending chemical increases, until a threshold dose (high dose in this graph), where TR is inhibited resulting in progression of injury and animal death.

Doses higher than the threshold dose inhibit TR leading to progression of injury and death. The extent of TR is, in most cases, governed by the extent of injury. At very high (and mostly lethal) doses, the molecular signaling that stimulates repair is inhibited resulting in inhibition of tissue repair. It is known that TR follows such dose response following administration of individual toxicants and chemical mixtures and is universally observed in all the organ systems.

Factors Affecting Tissue Repair

Studies with a diverse group of chemicals, various organ systems and various animal models such as mice, rats, guinea pigs, hamsters, and dogs have revealed that TR following toxicant exposure depends upon a number of factors such as age, species and strain difference, nutrition and disease condition.

Under experimental conditions, age difference seems to affect the ability of animal to mount an effective TR. It has been observed that neonatal animals are capable of stimulating a prompt and robust TR as compared to adult animals. This is due to the fact that most of their organ systems are still developing and rapid cell division is underway in those organs. Interestingly, old animals such as 12- and 24-month-old rats, exhibit increased ability to stimulate TR following toxicant-induced injury as compared to young adults (3 months old). Certain species and strains exhibit higher TR following toxicant treatment as compared to others. Gerbils have much sluggish TR as compared to rats while F344 rats have higher TR as compared to Sprague–Dawley rats.

Nutritional status changes the ability of animal to stimulate repair. In general, it is observed that high glucose levels inhibit TR while animals fed diet supplemented with fatty acids increases TR response. Interestingly, caloric restriction is known to stimulate TR in various organs including the liver and gastrointestinal tract. The modulation of molecular signaling that underlies the TR by these various nutritional components is central to their unique effects on TR.

Diabetes, known to inhibit the wound repair process, has also been shown to inhibit TR following toxic injury. Diabetes represents a special condition where the extent of injury does not determine the extent of TR since the ability of the animal to mount TR following injury is hampered by the disease condition. In diabetic rats, no matter how low the injury might be, it progresses due to inability of the animal to stimulate repair leading to organ failure.

Molecular Signaling Involved in Tissue Repair

TR is a complex process involving proliferation of parenchymal cells, regenerations of ECM, angiogenesis and reorganization of tissue and is governed by an equally complex network of molecular signaling. Studies with PH in liver, unilateral and five-sixth nephrectomy, chemical-induced liver and kidney injury, and wound repair have collectively generated extensive information about the signal transduction pathways involved in TR. Generally, the first cells to sense the injury are the resident macrophages of the organ such as Kupffer cells in the liver. These macrophages release a variety of proinflammatory cytokines and chemokines, which stimulate and attract the neutrophils and monocytes to the site of injury. Recent evidence suggests that the chemokines and cytokines also stimulate proliferation of other healthy parenchymal cells. These cytokines include tumor necrosis factor-α, interleukin-6 (IL-6), IL-8, and others. It is now known that these cytokines prime the resting nondividing parenchymal cells to enter into cell cycle and undergo division. The primed cells are further stimulated by various growth factors such as transforming growth factor-α (TGF-α), hepatocyte growth factor, and epidermal growth factor. These growth factors stimulate the cells via their cell surface receptor and an intricate intracellular network of kinase enzymes and nuclear transcription factors such as nuclear factor-κB, and AP-1 to complete the cell division. The regeneration of ECM is known to be governed by a complex balance between the ECM-degrading matrix metalloproteases and TGF-β. The role of nuclear receptors such as peroxisome proliferators-activated receptor, retinoid-x-receptor, liver-x-receptor, etc. has also been demonstrated. One of the main mysteries of TR is the timely termination of cell division, a process important in maintaining the critical balance between compensatory repair and unregulated cancerous growth. The molecular signaling involved in the termination of TR is not completely clear, though TGF-β has been implicated in this process.

Significance of Tissue Repair

Extensive evidence gathered from experiments with a number of diverse toxicants, animal models and interventional experiments indicates that TR plays a critical role in the final outcome (survival versus death) of the chemical-induced injury. An effective, timely, and robust TR results in regression of injury, restoration of structure and function of the tissue,

while inhibition of TR leads to progression of injury, and organ failure resulting in animal death. This is important in clinical settings where therapies directed toward stimulation of TR of a patient can lead to survival. Similarly, early detection of factors stimulating TR (such as increase in growth factors) may provide doctors a good prognostic marker helpful in deciding future treatment. In risk assessment area, assessment of TR may indicate additional information about the outcome of toxic exposure and have significant impact on setting guidelines of toxicant exposure.

See also: Diabetes, Effect of Toxicity; Dose–Response Relationship; Kidney; Liver.

Further Reading

Chanda S and Mehendale HM (1996) Hepatic cell division and tissue repair: A key to survival after liver injury. *Molecular Medicine Today* 2: 82–89.

Diehl AM (2002) Liver regerenation. *Frontiers in Bioscience* 7: e301–e314.

Liu Y (2004) Hepatocyte growth factor in kidney fibrosis: Therapeutic potential and mechanisms of action. *American Journal of Physiology. Renal Physiology* 287: F7–F16.

Price PM, Megyesi J, and Safirstein RL (2003) Cell cycle regulation: Repair and regeneration in acute renal failure. *Seminars in Nephrology* 23: 449–459.

Soni MG and Mehendale HM (1998) Role of tissue repair in toxicologic interactions among hepatotoxic organics. *Environmental Health Perspectives* 106: 1307–1317.

Titanium

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 7440-32-6
- Synonyms: Titanate; T40
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formula: Ti^{n+} ($n = 2, 3, 4$)

Uses

Titanium metal is lightweight and has high strength; thus, it is used in aircraft and other structures where cost is not a major factor. It also resists corrosion, making it especially useful in surgical implants and prostheses. Titanium fibers are used as an asbestos substitute. Titanium's most widely used compound, titanium dioxide, is used as a white pigment in paints and plastics and as a food additive to whiten flour, dairy products, and candies. It is also used in cosmetics and sunscreen formulations.

Exposure Routes and Pathways

Ingestion is the primary exposure pathway. Corn oil, butter, and white wheat products are perhaps the main sources of titanium. In industrial settings, inhalation is an important pathway. Titanium is not absorbed dermally.

Toxicokinetics

Approximately 3% of ingested soluble titanium is absorbed. The lungs are the main depot for inhaled titanium; it tends to remain in the lungs for long periods. Titanium is also found in the kidneys, the liver, and some fat tissue. Titanium crosses the blood–brain and placental barriers. Absorbed titanium is excreted in the urine.

Acute and Short-Term Toxicity (or Exposure)

Human

Titanium dioxide appears to be relatively nontoxic. Ti has been used extensively in food products without apparent adverse effects. Upper airway irritation is the principle sign of acute overexposure. Increased pulmonary dust disposition may lead to alveolar cell hyperplasis and fibrosis.

The liquid titanium tetrachloride is corrosive to skin and membranes of the eye. This may be due to liberation of hydrochloric acid on hydrolysis.

Some people may be hypersensitive to titanium.

Chronic Toxicity (or Exposure)

Animal

Titanium is neither mutagenic nor carcinogenic. Titanocene (an organic compound), however, induced fibrosarcoma when injected intramuscularly in rats. This same compound was carcinogenic against the Ehrlich ascites tumor in mice. There have been reports of tumors induced with the pure metal. Titanium dioxide did not induce tumors when administered orally; however, a few lung tumors were detected after titanium dioxide dust was inhaled by rats.

Certain titanium compounds may be nephrotoxic and hepatoxic to animals.

Human

Generally, titanium dioxide is considered physiologically inert by all routes; however, if relatively high concentrations of titanium dioxide dusts are inhaled, toxicological actions are noted. A weak fibrosis of the lung tissue occurs but is not fatal.

Clinical Management

Because of the low toxicity of titanium dioxide, there have not been any reports of therapy. Generally, titanium dioxide is biologically nonreactive when administered orally or intravenously.

Exposure Standards and Guidelines

Titanium is classified as a nuisance particulate with an ACGIH (American Conference of Governmental Industrial Hygienists) threshold limit value time-weighted average of 10 mg kg^{-1}.

Miscellaneous

Titanium was discovered by the Reverend William Gregor in 1791, and is named after the 'Titans' of Greek mythology.

See also: Metals; Toxicity Testing, Inhalation.

Further Reading

Murthy LI, Dankovic DA, and Murthy RC (2001) Titanium, zirconium, and hafnium. In: Bingham E, Cohrssen B, and Powell CH (eds.) *Patty's Toxicology*, 5th edn., vol. 2, pp. 677–689. New York: Wiley.

Wong SL, Nakamoto L, and Wainwright JF (1995) Detection and toxicity of titanium from pulp and paper effluents. *Bulletin of Environmental Contamination and Toxicology* 47: 115–123.

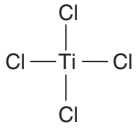
Relevant Website

http://www.inchem.org – Titanium (Environmental Health Criteria 24) from the International Programme on Chemical Safety. Titanium Dioxide (Summaries and Evaluations from the International Agency for Research on Cancer).

Titanium Tetrachloride

Robert Kapp

- Related Compounds: Titanium dichloride (CAS 10049-06-6); Titanium trichloride(CAS 7705-07-9); Titanium dioxide (CAS 13463-67-7); Titanium sulfate (CAS 13693-11-3)
- Chemical Abstracts Service Registry Number: CAS 7550-45-0
- Synonyms: Tetrachlorotitanium; Titanic chloride; Titanium chloride; Titanium chloride ($TiCl_4$); Titanium tetrachloride; Titantetrachlorid (German); Titaantetrachloride (Dutch)
- Chemical/Pharmaceutical/Other Class: Inorganic halide
- Chemical Formula: $TiCl_4$
- Chemical Structure:



Uses

Titanium tetrachloride is used as an intermediate in the production of titanium metal, titanium dioxide, and titanium chloride pigments, as a polymerization catalyst, in the manufacture of iridescent glass and faux pearls, and with ammonia to produce smoke screens. It is also used as a catalyst in many organic syntheses in the chemical industry. Titanium tetrachloride was formerly used with potassium bitartrate as a mordant in the textile industry, and with dyewoods in dyeing leather.

Background Information

Titanium tetrachloride is a colorless to light yellow liquid with a pungent odor.

Exposure Routes and Pathways

Exposure to titanium tetrachloride is primarily occupational with titanium industry workers having the highest potential for exposure. Titanium tetrachloride enters the environment in the air primarily

from factories that use the material in various chemical processes, or as a result of accidental spills. The material reacts quickly with water in the air to form hydrochloric acid, titanium hydroxide, and titanium oxychlorides. Titanium tetrachloride in its pure form is not present in water, soil, food, or air, except in manufacturing sites that use or manufacture the material. Because titanium tetrachloride breaks down rapidly, it is unlikely that anyone outside the workplace would be exposed to it. Minimal exposure could occur from breathing vapors or touching the material during accidental spills in the workplace.

Toxicokinetics

No human or animal studies were found on adsorption, distribution, metabolism, or excretion of titanium tetrachloride. Because of the nature of titanium tetrachloride, it is suggested that the major route of exposure is via inhalation, with the lungs as the major target organ. Dermal exposure can also result where accidental spills have occurred. It has been shown that titanium dioxide was present in the lungs of workers occupationally exposed to titanium tetrachloride.

Mechanism of Toxicity

The instability of titanium chloride leads to its hydrolysis, giving off heat and producing hydrochloric acid among other materials. The hydrochloric acid is partially responsible for the corrosive effects noted following titanium tetrachloride exposure. The hydrolysis of titanium tetrachloride occurs in several steps, which produces titanium oxide hydrate that can absorb the hydrochloric acid vapors and carry them into deeper parts of the lungs. There are no studies done on the mechanism of toxicity following ingestions of titanium tetrachloride. Following dermal exposure to titanium tetrachloride, the hydrochloric acid produced in the reaction with water is responsible for the serious thermal and acid burns that occur.

Acute and Short-Term Toxicity (or Exposure)

Animal

The dermal LD_{50} in the rabbit is $3160\,mg\,kg^{-1}$, while the inhalation LD_{50} in the rat is $400\,mg\,l^{-1}$. Eye injury, including corneal opacity, necrotic keratitis, and conjunctivitis, occurred in rats acutely exposed to titanium tetrachloride vapors. Acute animal tests in rats and mice have demonstrated titanium tetrachloride to have high to extreme acute toxicity via inhalation.

Human

Titanium tetrachloride is a severe skin, eye, and respiratory irritant and corrosive. It can also severely irritate the mucous membranes and the lungs. Inhalation of high levels of titanium tetrachloride can be fatal due to the ensuing lung injury from the hydrochloric acid produced. Acute (short-term) exposure may result in constriction of various sections of the upper respiratory tract in humans.

Chronic Toxicity (or Exposure)

Animal

The major effect of exposure of experimental animals to titanium tetrachloride was an increase in the incidence of rhinitis in the respiratory tract with increasing levels of concentration of titanium tetrachloride (0.1, 1.0, and $10.0\,mg\,l^{-1}$). Tracheitis also increased with duration of exposure and concentration of the material. Gross pathology and histology revealed compound-related changes in the thoracic lymph nodes and the lungs of the treated animals, and the severity of alveolar hyperplasia was noted as increased at increasing concentrations. In addition, high dose animals had a significant increase in neutrophils and a concomitant decrease in lymphocytes. Lesions described as lung squamous cell carcinoma and keratinizing squamous cell carcinoma were observed in rats in a 2 year chronic inhalation study performed in 1986. A 1994 reevaluation of the slides from this study by a group of pathologists concurred that these lesions should have been more properly characterized as either squamous metaplasia or proliferative keratin cysts. Based upon this new evaluation, titanium tetrachloride was not found to be carcinogenic in rats.

Human

Pleural thickening and decreased pulmonary function have been associated with chronic exposure to titanium tetrachloride in titanium metal workers. Chronic inhalation exposure may result in upper respiratory tract irritation, chronic bronchitis, cough, bronchoconstriction, wheezing, chemical pneumonitis, or pulmonary edema in humans. Because titanium tetrachloride rapidly hydrolyzes upon contact with water, the negative findings from the limited studies performed are insufficient to reach any conclusion about titanium tetrachloride's ability to induce genotoxic effects.

Epidemiological studies conducted on titanium tetrachloride are inadequate to determine whether this material can cause carcinomas in occupationally

exposed workers or not. No adequately conducted definitive studies on either humans or animals could be located with respect to the potential of titanium tetrachloride to produce reproductive or developmental effects. The (US) Environmental Protection Agency (EPA) has not established a reference dose or a reference concentration for titanium tetrachloride.

Clinical Management

Upon ocular exposure, the eye should be generously washed with tap water. Refer for medical attention. In case of dermal exposure, contaminated clothing should be removed and the skin should be rinsed with tap water. Titanium tetrachloride ingestion should be referred for medical attention and vomiting should not be induced. Upon inhalation, the victim should be removed to fresh air and given artificial respiration if indicated. The body should be placed in a half-upright position. Refer for medical attention.

Environmental Fate

Environmental exposure to titanium tetrachloride is unlikely because it hydrolyzes rapidly upon contact with moist air to form a vapor of hydrochloric acid, titanium dioxide, and titanium oxychloride.

Exposure Standards and Guidelines

The American Industrial Hygiene Association recommended Workplace Environmental Exposure Level: 8 h time-weighted average is $500\,mg\,m^{-3}$. Titanium tetrachloride is not listed as a carcinogen by the (US) Environmental Protection Agency, the International Agency for Research on Cancer, the (US) National Institute of Environmental Health Sciences National Toxicology Program, the (US) Occupational Safety and Health Administration, and the American Conference of Governmental Industrial Hygienists. The (US) Agency for Toxic Substances, and Disease Registry has calculated a chronic inhalation minimal risk level (MRL) of $0.0001\,mg\,m^{-3}$ based on respiratory effects in rats. The MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure. Exposure to a level above the MRL does not mean that adverse health effects will occur. The MRL is intended to serve as a screening tool.

See also: Alkyl Halides.

Further Reading

Murthy LI, Dankovic DA, and Murthy RC (2001) Titanium, zirconium, and hafnium. In: Bingham E, Cohrssen B, and Powell CH (eds.) *Patty's Toxicology*, 5th edn., vol. 2, pp. 687–688. New York: Wiley.

Roy PK, Bhatt A, and Rajagopal C (2003) Quantitative risk assessment for accidental release of titanium tetrachloride in a titanium sponge production plant. *Journal of Hazardous Materials* 102: 167–186.

Relevant Websites

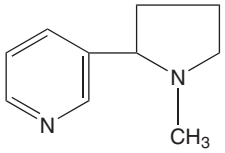
http://www.epa.gov – US Environmental Protection Agency (EPA), Air Toxics. Titanium Tetrachloride.

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

Tobacco

C Lynn Humbertson

- Chemical Abstracts Service Registry Number: CAS 54-11-5 (nicotine)
- Synonyms: *Nicotiana tabacum* (cultivated tobacco); *Nicotiana rustica*; Methylpyridylpyrrolidine
- Description: Tobacco products contain dried tobacco leaves, which are used to take advantage of the psychoactive effects of the alkaloid nicotine. Snuff has a pH of 7.8–8.2. Cigarettes are acidic. Chewing tobacco has alkali added and is basic
- Chemical Formula: $C_{10}H_{14}N_2$ (nicotine)
- Chemical Structure:



Nicotine

Uses

Tobacco products do not have a therapeutic use and can produce physiologic addiction. Commonly used products include cigarettes, chewing tobacco, snuff,

and cigars. Tobacco enemas have been used to treat intestinal parasites. Nicotine is used as a pesticide.

Background Information

The tobacco plant is a tall annual that belongs to the Nightshade or Solanaceae family. The Nightshade family includes at least 2400 species, including crop plants, poisonous plants, herbs, shrubs, trees, and perennial flowering plants. Commonly known members of this family include: tomato, potato, eggplant, tobacco, bittersweet, and petunia. Nightshades include plants that contain medicinally active and highly toxic constituents. These include alkaloids (such as nicotine, atropine, and scopolamine), belladonna, and mandrake.

Tobacco includes numerous species that are grown all over the world. The species *Nicotiana tabacum* is known as common tobacco. Common tobacco is what is primarily used in cigarettes. It is native to the West Indies, Mexico, and South America. The two varieties of common tobacco that account for most of what is grown in the United States are the large-leaf 'burley' and 'bright-leaf' tobacco.

Naturally occurring active constituents in common tobacco include alkaloids, organic acids, and nicotine. The nature and concentration of the active constituents vary with the species variety and strain of plant as well as growing conditions.

Exposure Routes and Pathways

Tobacco is smoked, nasally insufflated, or chewed to make the nicotine bioavailable for absorption.

Toxicokinetics

The absorption of the nicotine in tobacco is incomplete after ingestion. Rectal administration via an enema may bypass the first-pass metabolism and result in higher serum levels and toxicity. Snuff is well absorbed nasally. Cigarette tobacco contains 15–20 mg nicotine per gram of tobacco and cigars contain 15–40 mg nicotine. Cigarette butts contain 25% of the total cigarette nicotine content. By the 1980s, cigarettes contained 15 mg tar and 1.3 mg nicotine. Snuff is made from powdered tobacco leaf and contains from 4.6 to 32 mg g^{-1} nicotine in the moist material; dry snuff contains 12.4–15.6 mg g^{-1}.

Peak plasma levels occur 15–30 min after ingestion and 2–10 min after smoking cigarettes. Nicotine undergoes a large first-pass effect during which the liver metabolizes 80–90%. Smaller amounts are metabolized in the lungs and kidneys. The metabolites include isomethylnicotinium ion, nornicotine, cotinine, and nicotine-1-*N*-oxide. Protein binding ranges from 4.9% to 20%. The presence of significant amounts of nicotine in the gastrointestinal tract after intravenous dosing suggests that passive diffusion or enterohepatic circulation occurs. The apparent volume of distribution in animals is $\sim 1\,l\,kg^{-1}$. In one clinical study, it was $2\,l\,kg^{-1}$ in smokers and $3\,l\,kg^{-1}$ in nonsmokers. Nicotine passes into breast milk in small quantities. Nicotine and its metabolites are excreted in the urine. At a pH of 5.5 or less, 23% is excreted unchanged. At a pH of 8, only 2% is excreted in the urine. Nicotine can be found in the urine of nonsmokers.

Mechanism of Toxicity

Tobacco smoke includes ~4000 chemical species with varying potential which cause adverse effects. Nicotine is stimulating to the autonomic nervous system ganglia and neuromuscular junction. The most prominent effects relate to stimulation of the adrenal medulla, central nervous system (CNS), cardiovascular system (release of catecholamines), gastrointestinal tract (parasympathetic stimulation), salivary and bronchial glands, and the medullary vomiting center. There is subsequent blockade of 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

A dose of 10 mg kg^{-1} (buccally) is fatal in dogs. Symptoms include initial hyperexcitability, hyperpnea, salivation, vomiting, diarrhea, then depression, incoordination, and paralysis.

Human

Nicotine is highly toxic. Ingestion of more than one cigarette or three cigarette butts, one cigar, or a pinch of snuff is toxic. Symptoms begin within 30–90 min of ingestion and persist for 1 or 2 h after mild exposure and 18–24 h after severe intoxication. Vomiting usually occurs within minutes of absorption, which helps to decrease the severity of intoxication. Abdominal pain and delayed diarrhea are possible. CNS symptoms include headache, dizziness, agitation, incoordination, convulsions, and/or coma. Cardiovascular effects seen include initial hypertension followed by hypotension, tachycardia, then bradycardia, and cardiac arrhythmias. Respiratory symptoms include initial tachypnea followed by

dyspnea, increased bronchial secretions, respiratory depression, cyanosis, and/or apnea. Infants are especially sensitive to the effects of tobacco.

Chronic Toxicity (or Exposure)

Human

Exposure to tobacco in several forms is associated with an increased risk of cancer; in addition, several active ingredients, such as nicotine, have been demonstrated to be addictive. Tobacco smoke is a significant indoor air pollutant. It includes ~4000 components, some regulated as human carcinogens. Second-hand smoke is a particular concern for children's health and is not only associated with an increase in lifetime risk of cancer, but an increased risk of developing respiratory conditions such as bronchitis, pneumonia, and asthma.

Chronic use of snuff has caused oropharyngeal cancer. Tobacco and alcohol ambylopia is seen in chronic smokers who are malnourished and alcoholic. Green tobacco sickness occurs in young workers who do not smoke but work with wet, uncured tobacco. Withdrawal symptoms can occur when use of a tobacco product is stopped.

The occurrence of various cancers and decreased cardiovascular function are increased with tobacco use. These effects may also occur via passive inhalation of cigarette or cigar smoke.

In Vitro Toxicity Data

Tobacco has tested positive in the Ames assay. Tobacco grown in some areas has been reported to show differing results in the Ames assay. A recent study reported tobacco smoke aerosols generated at temperatures greater than 400°C to be positive in the Ames assay (activated with rat liver S9) (strains TA98 and TA100). Aerosols generated at lower temperatures did not test positive in the same study.

Clinical Management

If ingested, syrup of ipecac-induced emesis should be avoided since seizures or lethargy can occur rapidly. Activated charcoal should be administered. Seizures should be treated with diazepam or phenytion. Atropine can be used to control signs of excess parasympathetic stimulation. If hypotension does not respond to intravenous fluids, dopamine or norepinephrine may be indicated. Antacids should be avoided since nicotine has greater absorption in an alkaline media. Vital signs and level of consciousness should be monitored closely. Further care is symptomatic and supportive. Nicotine laboratory determination is only of diagnostic value and does not direct therapy.

Ecotoxicology

Growth and production of tobacco and tobacco products use significant natural resources, from land to materials such as wood used to dye and cure tobacco. This has led to deforestation in some areas of the world with subsequent erosion and flooding of agricultural lands.

The common tobacco plant depletes soil nutrients (e.g., nitrogen, phosphorus, and particularly, potassium) at a higher rate than most food and cash crops (e.g., cotton, coffee). One of the reasons for tobacco's high uptake of soil nutrients is the practice of 'topping' the plants to increase the growth of leaves and increased nicotine content contributes to the increased uptake of soil nutrients.

Other Hazards

Use of cigarettes and matches is the leading cause of deaths from fires in the United States.

Dried tobacco should not be used in animal feed. Pets and livestock should not drink water that has been in contact with tobacco or tobacco products (e.g., ashtrays or puddles where tobacco is being harvested and processed) as the water may contain high levels of nicotine. There is some controversy over risks associated with pregnant sows ingesting tobacco leaves. Grazing on tobacco is not recommended until risks are better understood.

See also: Carcinogenesis; Developmental Toxicology; International Agency for Research on Cancer; Neurotoxicity; Nicotine; Tobacco Smoke.

Further Reading

Conti-Fine BM, Navaneetham D, Lei S, and Maus AD (2000) Neuronal nicotinic receptors in non-neuronal cells: New mediators of tobacco toxicity? *European Journal of Pharmacology* 393(1–3): 279–294.

Relevant Websites

http://www.who.int – World Health Organization (2000) Final Report: Advancing Knowledge on Regulating Tobacco Products Oslo, Norway, 9–11 February 2000.

http://www.ash.org.uk – Clive Bates, Martin Jarvis, Tobacco additives and Gregory Connolly (1999) Cigarette engineering and nicotine addiction. Action on Smoking and Health. London (14 July 1999).

http://www.oehha.ca.gov – Office of Environmental Health Hazard Assessment (OEHHA) California. Health Effects of Exposure to Environmental Tobacco Smoke.

http://www.hhs.gov – The Health Consequences of Smoking: A Report of the Surgeon General (2004).

Tobacco Smoke

Robert Kapp

Chemical

Cigarettes, cigars, and pipes produce tobacco smoke during the combustion process. Tobacco smoke is an aerosol, formed when tobacco is incompletely burned during the smoking of cigarettes, pipes, or cigars. Temperatures in burning cigarettes range from ambient to ~950°C, depending on the amount of oxygen present.

During the burning of tobacco (itself a complex mixture), thousands of chemical substances are generated. These compounds are typically classified into a particulate phase (trapped on a glass-fiber pad, and termed 'TPM' (total particulate matter)) and a gas/vapor phase (which passes through such a glass-fiber pad). 'Tar' is a mathematically derived material determined by subtracting the weight of the nicotine and water from the TPM. Typical smoke components include nicotine (CAS 54-11-5) (mostly in the particulate phase), and carbon monoxide (CAS 630-08-0) (gas phase). However, several components of tobacco smoke (e.g., hydrogen cyanide (CAS 74-90-8), and formaldehyde (CAS 50-00-0)) do not fit neatly into this rather arbitrary classification. Analysis of tobacco smoke has yielded a number of toxicologically significant chemicals and groups of chemicals, including polycyclic aromatic hydrocarbons, tobacco specific nitrosamines, aldehydes, hydrogen cyanide, nitrogen oxides, benzene (CAS 71-43-2), toluene (CAS 103-38-3), phenols, and aromatic amines. The radioactive element polonium-210 (CAS 013981-52-7) and benzopyrene (a carcinogen (CAS 50-32-8)) are also known to occur in tobacco smoke.

Cigarettes are designed and produced with various tar yields. The Federal Trade Commission designated a test to measure the amount of 'tar', nicotine, and carbon monoxide using smoking machines. Tobacco companies use terms such as 'full flavor', 'medium', 'mild', 'light', and 'ultra light' to describe the strength of the taste of cigarettes. These terms are commonly referred to as 'descriptors' and facilitate smokers' ability to distinguish among different product offerings. Descriptors are generally used as a point of comparison for a cigarette brand in order to distinguish it from other brands on the market.

Some researchers report that smokers of 'light' cigarettes inhale as much 'tar' and nicotine as from full-flavor brands. Volume 13 of the National Cancer Institute's Smoking and Tobacco Control Monograph Series concluded "that people who switch to low-'tar' or 'light' cigarettes from 'full flavor' cigarettes are likely to inhale the same amount of cancer-causing toxins and they remain at high risk for developing smoking-related cancers and other diseases." Generally, as 'tar' yield increases, the amounts of individual constituents increase. Several studies indicate that across brands, the relative proportions of constituents remain similar.

Physical

The particles in tobacco smoke are liquid aerosol droplets (~20% water), with a mass median aerodynamic diameter that is submicrometer (and thus, fairly 'lung-respirable' by humans). The droplets are present in high concentrations (some estimates are as high as 10^{10} droplets per cm^3). Most cigarettes today contain a filter, consisting principally of cellulose acetate although other materials have been used (e.g., charcoal granules, paper, etc.) The filter can reduce 'tar' and nicotine smoke yields up to 50% by several different mechanisms, with an even greater removal rate for other classes of compounds (e.g., phenols). Selective filtration of the vapor-phase components of tobacco smoke is conceptually much simpler than selective removal of the permanent gas or particulate components. Cigarette filters containing charcoal granules (either as a cavity filter or embedded into the cellulose acetate) appear to be effective in reducing concentrations of such toxicologically important tobacco smoke components such as 1,3-butadiene (CAS 106-99-0) and acrolein (CAS 107-02-8), but are completely ineffective in reducing other such toxicologically important components such as carbon monoxide.

Toxicology

Cigarette smoking causes lung cancer, heart disease, chronic obstructive pulmonary disease, emphysema, and other serious diseases in smokers. Smokers are

far more likely to develop serious diseases, like lung cancer, than nonsmokers. Given the high prevalence of smoking (at least in the Western world), smoking tobacco is considered the single most preventable cause of human disease. Public health officials have concluded that secondhand smoke from cigarettes causes disease, including lung cancer and heart disease, in nonsmoking adults, as well as causes conditions in children such as asthma, respiratory infections, cough, wheeze, otitis media (middle ear infection), and sudden infant death syndrome. It has also been shown that serum immunoglobulin levels and T killer cell activity decrease in smokers. Immunological studies in tobacco-smoke exposed animals have demonstrated suppression of antibody responses and enhanced susceptibility to murine sarcoma virus and influenza virus. There are currently no validated animal models to predict human disease from smoking. Animal inhalation carcinogenicity studies with tobacco smoke are negative. Tobacco smoke is mutagenic in most *in vitro* assays with and without metabolic activation. Tobacco smoke condensate promotes the formation of papillomas in the 'mouse skin painting assay'. Tobacco smoke is not teratogenic in animal studies; however, it is recognized as producing low birth weight in humans. In humans, the number of alveolar monocyte/macrophage cells (MOs) has been shown to increase several-fold in smokers versus nonsmokers. This increase may be a result of an increased production of IL-1 by the alveolar MOs, which results in an influx of polymorphonuclear cells and peripheral blood mononuclear cells into the lung. While these MOs appear to be in an activated state, they manifest decreased phagocytic and bactericidal activity.

Mechanisms

Despite the strength of the epidemiological associations, the actual mechanisms by which smoking can cause so many diseases remain largely unknown. A major problem in establishing the mechanism is the inability to reproduce the human diseases in animal models. In particular, many attempts have been made to produce lung cancer in animals exposed to tobacco smoke by the inhalation route, without success. A recent review stated that "significant increases in the numbers of malignant tumors of the respiratory tract were not seen in rats, mice, hamsters, dogs or nonhuman primates exposed for long periods of time to very high concentrations of mainstream cigarette smoke." It is only by collecting the 'tar' and repeatedly painting this on to mice that tumors are produced, and these tumors (along with the test material and the target organ) are very different from those tumors exhibited by smokers. While there is no direct information to indicate that nicotine is responsible for any of the major diseases associated with smoking, nicotine does accelerate heart rate, elevate blood pressure, and constrict blood vessels within the skin. These are considered to be the result of stimulation of the ganglionic sympathetic nervous system.

Addiction

The overwhelming medical and scientific consensus is that cigarette smoking is addictive. It is not clear exactly which components of the smoke are responsible for the addiction. Scientific data indicate that nicotine contributes to the addiction; however, other factors may be involved in the addiction process. It is believed that nicotine may exert an effect by binding to a subset of cholinergic receptors that are located at the neuromuscular junction and in the central nervous system where psychoactive and addictive properties reside. In addition, nicotine is associated with alterations of electroencephalographic recordings in humans. Nicotine replacement therapy is not completely successful in aiding in quitting smoking on the one hand and very large numbers of smokers are able to spontaneously stop smoking on the other. Hence, the specific etiology of tobacco addiction remains unknown.

Future Considerations

Given the major health problems that result from smoking, and the high prevalence of people who are unwilling to quit, there is a need for the development of potentially reduced exposure products, or PREPs, as described in a recent publication from the National Academy of Sciences. Cigarette manufacturers have responded in a number of ways including changes in the blend composition, novel filter designs to assist in selective filtration, and the complete removal of burning tobacco as the heat source for the release of flavorful components of tobacco smoke. Animal models for smoke-induced disease are needed to further validate these novel products as reduced-risk products. Additionally, validated biomarkers of effect are needed to more precisely predict the effects in humans.

See also: Carcinogenesis; Cardiovascular System; Developmental Toxicology; Immune System; International Agency for Research on Cancer; Neurotoxicity; Respiratory Tract.

Further Reading

Davis DL and Nielsen MT (1999) *Tobacco Production, Chemistry and Technology.* Ames, IA: Iowa State University Press. ISBN 0632047917.

Institute of Medicine (2001) *Clearing the Smoke: Assessing the Science Base for Tobacco Harm Reduction*. ISBN 0-309-07282-4.

Relevant Websites

http://www.hhs.gov – The Health Consequences of Smoking: A Report of the Surgeon General, 2004.

http://www.cdc.gov – National Center for Chronic Disease Prevention and Health Promotion – Tobacco Information and Prevention Source.

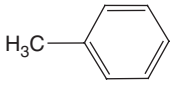
http://cfpub2.epa.gov – Respiratory Health Effects of Passive Smoking (also known as Exposure to Secondhand Smoke or Environmental Tobacco Smoke –ETS).

http://www.oas.samhsa.gov – Results from the 2002 National Survey on Drug Use and Health: National Findings Department of Health and Human Services.

Toluene

Stephen R Clough

- Chemical Abstracts Service Registry Number: CAS 108-88-3
- Synonyms: Methylbenzene; Phenylmethane; Toluol (DOT); Antisal 1a; Methacide; Methylbenzol; NCI C07272; Toluen (Dutch); Toluen (Czech); Tolueno (Spanish); Toluolo (Italian); Tolu-sol
- Chemical/Pharmaceutical/Other Class: Aromatic hydrocarbon
- Chemical Formula: C_7H_8
- Chemical Structure:



Uses

Most toluene is added to automobile or aviation gasoline mixtures (benzene $\geqslant$ xylene $\geqslant$ toluene) to increase octane ratings. Toluene is an excellent organic solvent and is used extensively in the manufacture of benzene derivatives, caprolactam, saccharin, medicines, dyes, perfumes, TNT, toluenediisocyanates (polyurethane resins), toluene sulfonates (detergents); as a solvent for scintillation counting; in paints and coatings, gums, resins, rubber; and as a diluent and thinner in nitrocellulose lacquers, plastic toys, and model airplanes. Toluene is also used extensively in the production of glues and is responsible for the narcosis and permanent brain damage seen in 'glue sniffers'.

Exposure Routes and Pathways

Because toluene is fairly volatile, exposure for humans would occur principally by inhalation. It has a human odor threshold of $\sim 0.1\,\mathrm{mg\,m^{-3}}$ (~ 26 ppb). Dermal exposure may also be significant, especially in an industrial setting, where skin may be exposed for long periods of time. Oral exposure is the least probable route and would occur primarily as a result of accidental poisoning or suicide.

Toxicokinetics

Toluene is readily absorbed from the lung and gastrointestinal tract, although studies in animals suggest absorption occurs more slowly in the gastrointestinal tract. Slow absorption also occurs through skin. Studies of humans and animals indicate that inhaled toluene distributes to tissues that are high in fat content (e.g., body fat, bone marrow, and brain) or well supplied with blood (e.g., liver). It seems reasonable to assume that similar distribution would occur for other routes of exposure.

In both humans and animals, toluene is rapidly excreted as both the unchanged compound in expired air and as a metabolite in the urine. Toluene is converted in the liver to water-soluble hippuric acid and conjugated cresols, which are then excreted in the urine. This conversion has been demonstrated in man and animals exposed via inhalation, although it is expected to occur for other exposure routes as well. Another excretion route for toluene is exhalation of the unchanged chemical. This excretion route might be expected to operate for all exposure routes but be more effective for exposures via inhalation.

Mechanism of Toxicity

Although the exact biochemical mechanism of toxicity has not been identified for toluene, it is known that the primary toxic effect of toluene is dysfunction of the brain and central nervous system (CNS-narcosis). The main function of neurons is to conduct electrochemical signals to one, several, or thousands of other cells. The normal physiology of these neurons is, in turn, largely dependent on the integrity of the cell membrane, which polarizes and depolarizes during the transmission of these signals. Thus, the most probable mechanism of toxicity is the unique

sensitivity of the cell membranes of neurons to the solvent-like property of toluene, which disrupts the normal transmission of nerve impulses.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute toxicity of toluene in laboratory animals is very low. The oral rat LD_{50} is $\sim 5\,g\,kg^{-1}$ and the inhalation LC_{50} in mice is ~ 400 ppm over 24 h.

Human

Much of the information on toxicity of toluene to humans comes from studies of solvent abuse (such as glue sniffing) and during exposure in the workplace (e.g., painters and printers). Interpretation of the data can be difficult due to the fact that these individuals are simultaneously exposed to mixtures of other chemicals. Both acute experimental and occupational exposures to toluene in the range of 100–1500 ppm ($\sim$325–5600 mg m^{-3}) have elicited dose-related CNS alterations, such as fatigue, confusion, and incoordination, as well as impairments in reaction time and perceptual speed. At 200–500 ppm, headache, nausea, eye irritation, loss of appetite, a bad taste, lassitude, and incoordination are reported but not accompanied by significant laboratory or physical findings. For high acute exposures ($\sim$30 000 ppm), initial lightheadedness and exhilaration is followed by progressive development of narcosis and CNS depression.

Chronic Toxicity (or Exposure)

Animal

Toxicity to the embryo or fetus and teratogenic effects have been rarely observed in animal studies. These effects were seen in only one experiment in which the dose was high enough to be toxic to the mother as well. More frequently, when maternal toxicity was not present, fetal toxicity or teratogenicity was not found. Growth inhibition of rat pups born during inhalation exposure to toluene through two generations has been observed.

CIIT conducted a 2 year inhalation toxicology study in Fischer 344 rats exposed to atmospheric toluene. The concentrations used were 30, 100, or 300 ppm (113, 377, or 1130 mg m^{-3}) for 6 h per day, 5 days per week. The only finding was a dose-related reduction in hematocrit values (number of red blood cells) in female rats exposed to 100 and 300 ppm toluene. This is not considered a significant toxic effect. Therefore, a no-observed-adverse-effect level was set at the highest exposure level – 300 ppm (equivalent to 29 mg kg^{-1} day^{-1}).

National Toxicology Program (NTP) also conducted a 2 year inhalation study in mice and rats (doses of 600 or 1200 ppm in rats and 120, 600, and 1200 ppm in mice, 6.5 h day^{-1}, 5 days per week). Lesions of the nasal cavity (in rats) and abnormal growth (hyperplasia) of the bronchial lining (in mice) were seen, but no deaths and significant body weight changes were observed during the course of the study. There was no evidence of cancer induction in this study.

In a recent study by NTP, rats and mice were given oral doses of toluene, ranging from 312 to 5000 mg kg^{-1}, 5 days per week for 13 weeks. General toxic effects, which included decreased movement or prostration, tearing and salivation, and body tremors were seen in both species at 2500 mg kg^{-1}. A few animals died at this dose. There were changes in organ weights and microscopic pathologic changes of several organs at 1250 mg kg^{-1} in rats. Organ weight changes, but not pathologic changes, were seen at 2500 mg kg^{-1} in mice but not at lower doses. The only adverse effect seen at the lowest dose was increased liver and kidney weights at 625 mg kg^{-1} in rats.

Human

After long-term exposure, blood abnormalities, psychomotor disorders, changes in the lens of the eye, immune system changes, kidney effects, menstrual disorders, and birth defects have been observed in some, but not all, studies of workers or abusers, and the possible confounding effect of mixed chemical exposure is mentioned in most. Liver effects, which figure prominently in animal studies, have not been observed in occupationally exposed individuals. Based on epidemiological studies, there is no evidence that toluene can cause cancer in humans. Although there was no evidence of cancer in the CIIT or NTP studies, and most mutagenicity tests have been negative, US Environmental Protection Agency (EPA) considers the data inadequate to classify toluene relative to its carcinogenicity; it is rated D (not classified, inadequate evidence in animals) in the current weight-of-evidence system.

Clinical Management

Persons who have been overcome by toluene fumes or gases should be removed from the area of exposure to fresh air. Should breathing become labored or shallow, medical intervention (e.g., artificial respiration) may be necessary. Following accidental or intentional ingestion, vomiting should not be induced and prompt medical attention should be

obtained. Liquid toluene spills on exposed skin should be immediately dried with an absorbent towel and then washed with soap and water.

Environmental Fate

Automobile emissions contribute the majority of toluene that is found in the atmosphere. Toluene is the most prevalent aromatic hydrocarbon in the air, with levels ranging from 0.14 to 59 ppb. Toluene has also been detected in surface water and treated wastewater effluents at levels generally below $10\,\mu g\,l^{-1}$. Toluene is readily biodegradable and will not bioconcentrate or bioaccumulate within a food web. In a study of edible aquatic organisms, 95% of the tissues sampled had levels <1 ppm.

Ecotoxicology

According to the US EPA ECOTOX aquatic toxicity database, the saltwater organism that is the most sensitive to toluene is the pink salmon, with respective acute (48 h) and chronic (96 h) LC_{50}s of 6190 and $6410\,\mu g\,l^{-1}$. For freshwater organisms, the most sensitive organism for both an acute (48 h) and a chronic (96 h) exposure is the rainbow trout, with respective LC_{50} values of 6780 and $5800\,\mu g\,l^{-1}$. These organisms are exposed in a laboratory setting and the concentrations of toluene used are many, many times higher than what would be anticipated to occur in natural waters.

Exposure Standards and Guidelines

Under US EPA current guidelines for risk assessment, the acceptable exposure dose for humans (or reference dose) is $0.2\,mg\,kg^{-1}\,day^{-1}$. For an average human weighing 70 kg, this dose is equivalent to $\sim$1/2000th of an ounce.

Under the Safe Drinking Water Act, the maximum contaminant level (MCL) is the standard criterion for drinking water and the maximum contaminant level goal (MCLG) is the goal. The MCL and MCLG for toluene in drinking water are $1000\,\mu g\,l^{-1}$, based on health protective limits developed from the CIIT study. The Occupational Safety and Health Administration recommends workplace air concentrations do not exceed 100 ppm; the American Conference of Governmental Industrial Hygienists recommends 50 ppm (based on potential skin exposure).

Miscellaneous

Toluene is a clear, flammable liquid with a sweet odor that is widely used in both the chemical and the pharmaceutical industries. In terms of production, it is the 24th highest volume chemical in the United States. It is derived mainly from petroleum refining and only a small percentage of that produced is used directly.

See also: Pollution, Air; Pollution, Air Indoor; Pollution, Water; Sensory Organs; Skin.

Further Reading

Filley CM, Halliday W, and Kleinschmidt-DeMasters BK (2004) The effects of toluene on the central nervous system. *Journal of Neuropathology & Experimental Neurology* 63(1): 1–12.

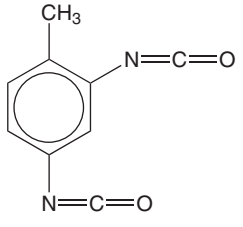
Relevant Websites

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

Toluene Diisocyanate

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 584-84-9
- Synonyms: Toluene 2,4-diisocyanate; 2,4-Diisocyanatotoluene; TDI; Nacconate 100
- Chemical Formula: $CH_3C_6H_3(NCO)_2$
- Chemical Structure:



Uses

Toluene diisocyanate is used in the production of polyurethane foams, elastomers, and dyes. It is a cross-linking agent for nylon-6.

Exposure Routes and Pathways

The respiratory and dermal routes of exposure are of most concern.

Toxicokinetics

Toluene diisocyanate has rapid linear absorption via inhalation with persistence in tissues at low levels for up to 2 weeks. Absorption of toluene diisocyanates by inhalation is reflected by high acute toxicity following such exposure. Little information is available on the distribution of toluene diisocyanates in mammals. Reaction of toluene diisocyanate with serum albumin yields protein conjugates. Toluene diisocyanate is hydrolyzed into 2,4-diaminotoluene in man.

Mechanism of Toxicity

Toluene diisocyanate is a cross-linking agent and both a pulmonary and dermal sensitizer. Most toxicity occurs with repeated exposure.

Acute and Short-Term Toxicity (or Exposure)

Animal

Rat oral $LD_{30} = 6.17\,g\,kg^{-1}$. It is irritating to the gastrointestinal tract upon ingestion. The RD_{50} (50% respiratory depressive concentration) in mice is 0.4 ppm. The LC_{50} (inhalation) is 14 ppm per 4 h in rats and 10 ppm per 4 h in mice.

Human

Toluene diisocyanate is a strong irritant to the eyes, skin, gastrointestinal tract, and respiratory system. It is a lacrimating agent and a strong dermal and pulmonary sensitizer. It can cause euphoria, ataxia, and mental aberrations. Signs and symptoms of acute exposure are nonspecific and include irritation of the nose and throat, shortness of breath, choking, coughing, nausea, vomiting, and abdominal pain. A common response to inhaled toluene diisocyanate is both acute and chronic decrease in ventilatory capacity, that is, decreased FEV1 (FEV = functional exhalation volume), even in the absence of overt signs or symptoms. The onset of signs and symptoms may be delayed and may persist for several days following removal from exposure.

Chronic Toxicity (or Exposure)

Animal

Experimental studies have shown that dermal application of toluene diisocyanate can elicit pulmonary sensitization. International Agency for Research on Cancer lists toluene diisocyanate as an animal carcinogen (from studies using gavage administration).

Human

Pulmonary sensitization is a serious complication following repeated toluene diisocyanate exposures. Signs may become more pronounced with continued exposure over days to months. Initial symptoms are nocturnal dyspnea and/or cough, progressing to bronchitis. In occupational settings, the time from initial employment to the development of asthmatic symptoms has ranged from 6 months to 20 years. Given sufficient exposure, virtually any person may become sensitized: the proportion of individuals with chemical asthma in working populations varied from 4.3% to 25%. Skin sensitization may also occur with repeated exposures. Urticaria, dermatitis, and allergic contact dermatitis have been reported in workers exposed to toluene diisocyanate-based resins. The dermatological symptoms included eczema and erythema. Toluene diisocyanate is a suspected carcinogen, but it is unclear whether it can lead to cancer with inhalation exposures.

Clinical Management

Affected eyes should be irrigated with running water. Contaminated areas of skin should be washed with soap and water. Patients asymptomatic of respiratory effects should receive oxygen and ventilatory support.

Environmental Fate

Ten days after a spill of 13 tons of toluene diisocyanate onto wet forest soil, the area was covered with sand. The soil concentration of toluene diisocyanate and toluenediamine declined from parts per thousand to parts per million from 10 days to 12 weeks after the spill. Six years later, only polyureas were found. Under controlled conditions, 5 kg of toluene diisocyanate was covered with 50 kg of sand and 5 kg of water and samples taken from the top and bottom of the sand. After 24 h, $<6\%$ toluene diisocyanate remained. Toluene diisocyanate is rapidly hydrolyzed

in aquatic environments. Elimination of toluene diisocyanate from the atmosphere is by reaction with hydroxyl radicals and by dry deposition.

Ecotoxicology

Little information is available on the ecotoxicology of toluene diisocyanate.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value for toluene diisocyanate is 0.005 ppm. The reference exposure level for toluene diisocyanate is 0.01 ppb.

See also: Respiratory Tract; Sensitivity Analysis.

Further Reading

Bingham E, Cohrssen B, and Powell CH (2001) *Patty's Toxicology*, 5th edn., vol. 4, pp. 1439–1443. New York: Wiley.

Bolognesi C, Baur X, Marczynski B, *et al.* (2002) Carcinogenic risk of toluene diisocyanate and 4,4′-methylenediphenyl diisocyanate: Epidemiological and experimental evidence. *Critical Reviews in Toxicology* 31: 737–772.

Collins MA (2002) Toxicology of toluene diisocyanate. *Applied Occupational and Environmental Hygiene* 17: 846–855.

Dart RC (2004) *Medical Toxicology*, 3rd edn. Philadelphia, PA: Lippincott.

Relevant Website

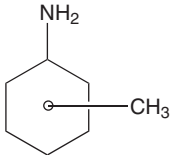
http://www.intox.org – Toluene Diisocyanate (UKPID Monograph from the International Programme on Chemical Safety).

Toluidine

Shayne C Gad

This article is a revision of the previous print edition article by Shayne C Gad and Jayne E Ash, volume 3, pp. 251–252, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Numbers: *m*-Toluidine (CAS 108-44-1); *o*-Toluidine (CAS 95-53-4); *p*-Toluidine (CAS 106-49-0)
- Synonyms: Aminotoluene; *m*-Toluidine; *o*-Toluidine; *p*-Toluidine; 2-Methylaniline
- Chemical/Pharmaceutical/Other Class: Substituted aromatic
- Chemical Formula: $C_7H_8NH_2$
- Chemical Structure:



Uses

Toluidine is used to produce dyes for textiles and other substances and as an accelerator in vulcanization. It is also used in organic synthesis.

Exposure Routes and Pathways

Inhalation and dermal contact are possible routes of exposure.

Toxicokinetics

In urine, 83.9% of *o*-toluidine is excreted after 48 h. Urinary excretion for *m*-toluidine and *p*-toluidine over a 24 h period is 10%. Hydroxy- and *N*-acetyl derivatives have been identified as urinary metabolites.

Mechanism of Toxicity

Toluidine interferes with enzymes associated with the detoxification process and monooxygenase system. It defats membranes.

Acute and Short-Term Toxicity (or Exposure)

Animal

Toluidine is an irritant primarily due to defatting. It is a mild skin irritant and moderate eye irritant in rabbits. Oral LD_{50}s in rats are 450 mg kg^{-1} for *m*-toluidine, 670 mg kg^{-1} for *o*-toluidine, and 3360 mg kg^{-1} for *p*-toluidine. Oral LD_{50}s in mice are 740 mg kg^{-1} for *m*-toluidine, 520 mg kg^{-1} for *o*-toluidine, and 330 mg kg^{-1} for *p*-toluidine.

Human

o-Toluidine is highly toxic to humans when absorbed through the skin, inhaled as vapor, or absorbed through the gastrointestinal tract. Acute exposure causes methemoglobinemia and central nervous system depression.

Chronic Toxicity (or Exposure)

Animal

In male and female mice exposed to 50 000 ppm *o*-toluidine for 7 weeks, pigment deposition was noted in the spleen, kidneys, and liver. Chronic exposure to *o*-toluidine can cause effects on the spleen, liver, urinary bladder, and blood (methemoglobinemia and reticulocytosis) in laboratory animals. The hydrochloride salt of *o*-toluidine was carcinogenic in rats and mice.

Human

Chronic effects in workers exposed to *o*-toluidine include anemia, anorexia, weight loss, skin lesions, central nervous system depression, cyanosis, and methemoglobinemia. *o*-Toluidine and *p*-toluidine are suspected carcinogens (bladder cancer).

In Vitro Toxicity Data

o-Toluidine has been found genotoxic in the Ames test, sister chromatid exchange, mouse lymphoma assay, and unscheduled DNA synthesis.

Clinical Management

All contaminated areas should be washed, including inside ear canals and under nails. The exposed person should be monitored for methemoglobinemia. If contamination is 30% or less, bed rest is recommended. If contamination is over 30%, the patient should be observed and given oxygen therapy. If contamination is over 50%, the exposed person should be given intravenous glucose solution. If contamination is $\geqslant$60%, methylene blue should be administered.

Environmental Fate

In soil, *o*-toluidine will be eliminated by biodegradation, oxidation, and binding to soil components. In water, toluidine will be eliminated by biodegradation, oxidation, and photooxidation as well as some adsorption to sediment. In the atmosphere, toluidine will photodegrade (half-life about 2 h).

Ecotoxicology

The 24 h LC_{50} in *Medaka* was 60 mg l^{-1}. In fathead minnows, the 96 h LC_{50} was >160 mg l^{-1}. Bioconcentration in aquatic species should not be a concern.

Exposure Standards and Guidelines

The threshold limit value for *o*-toluidine is 2 ppm. The permissible exposure limit is 5 ppm. No reference concentration or reference dose has been established for *o*-toluidine.

See also: Carcinogenesis; International Agency for Research on Cancer; Genetic Toxicology.

Further Reading

ortho-Toluidine (2000) *IARC Monographs on Evaluating Carcinogenesis Risks in Humans* 77: 267–322.

Woo Y and Lai DL (2001) Aromatic amino and nitro-amino compounds. In: Bingham E, Cohrssen B, and Powell CH (eds.) *Patty's Toxicology*, 5th edn., vol. 4, pp. 1009–1014. New York: Wiley.

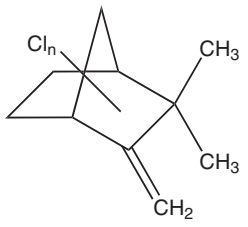
Relevant Website

http://www.inchem.org – *o*-Toluidine (Concise International Chemical Assessment Document Number 7 from the International Programme on Chemical Safety).

Toxaphene

David R Wallace

- Chemical Abstracts Service Registry Number: CAS 8001-35-2
- Synonyms: Chlorinated camphene; Camphochlor; Compound 3956; Melipax; Toxadust, Toxakill, Attac, Anatox; Strobane-T and others
- Chemical/Pharmaceutical/Other Class: Organochlorine insecticide
- Chemical Formula: Toxaphene contains over 670 chemicals
- Chemical Structure:



Uses

Toxaphene is an insecticide that contains over 670 chemicals and can exist as a yellow to amber solid or gas. Heavily used in the United States until 1982, its use was completely banned in 1990. Toxaphene was used primarily to control insects on cotton crops in the southern United States, it has also been used to control pests on livestock and to control unwanted fish in aquatic environments.

Exposure Routes and Pathways

Exposure can occur via oral, inhalation, or dermal routes. Principle exposure appears to have occurred during the manufacturing process, from dust or mists. Therefore, people with the greatest risk of toxaphene exposure were those involved in the manufacturing of toxaphene, cotton farmers, and registered insecticide applicators. It does not have significant solubility in water, but will accumulate in sediment on the lake or river bottom. Individuals can be exposure by breathing air near a toxaphene-contaminated waste site or in agricultural areas where toxaphene was used. Infants and children who consume soil contaminated with toxaphene are also at risk. Since toxaphene will accumulate in the sediment on lake or river bottoms, consumption of fish or shellfish that can concentrate toxaphene residues may pose a higher risk.

Toxicokinetics

Since toxaphene is a mixture of 670 chlorinated camphenes, some have greater toxicity than technical grade toxaphene alone. Toxaphene components A and B have been shown to have 6 and 14 times greater toxicity than the technical mixture, respectively. Component A is a mixture of 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane and 2,2,5-*endo*,6-*exo*,8,9,9,10-octachlorobornane. Component B has been identified as 2,2,5-*endo*, 6-*exo*,8,9,10-heptachlorobornane. Rats dechlorinate toxaphene either by reductive dechlorination or dehydrochlorination. Toxaphene can also be metabolized by NADPH-dependent mixed function oxidases in microsomal preparations.

Mechanism of Toxicity

The neuroexcitatory properties of toxaphene are due to its ability to reduce chloride uptake into neurons, leading to depolarization of the cells and hyperactivity. It is believed that toxaphene acts on the picrotoxin-binding site on the $GABA_A$ receptor. Toxaphene may also impair calcium transport, which will interfere with numerous neuronal pathways and function.

Acute and Short-Term Toxicity (or Exposure)

Short-term exposure to toxaphene above the levels established by the Environmental Protection Agency (maximum contaminant level (MCL)) has been shown to cause effects in the central nervous system (CNS), which include restlessness, hyperexcitability, tremors, spasms, or convulsions.

Animal

Oral administration of toxaphene caused generalized CNS hyperactivity in dogs and rats. Changes in both hepatic and renal function were described in both species.

Human

Short-term exposure of $0.5\,g\,m^{-3}$ for 30 min a day for 10 days caused no discernible effects in individuals inhaling toxaphene vapor. If toxaphene is spilled on skin or clothing, or if exposed skin comes in contact with toxaphene solid, pain and reddening of the tissue may occur. Oral ingestion of toxaphene has produced the most robust toxic responses. Toxaphene is readily absorbed through the intestines and an oral dose of 0.6 g can result in convulsions, nausea, vomiting, a bluish coloration of the skin (resembling cyanosis), and eventually coma or death. The estimated lethal dose for toxaphene in humans following oral ingestion is 2–7 g.

Chronic Toxicity (or Exposure)

Exposure to toxaphene above the MCL has been shown to cause liver and kidney degeneration, excitotoxicity of the CNS, suppression of the immune system and possibly cancer.

Animal

Mice that were fed a diet of technical grade toxaphene showed a significant increase in hepatic carcinomas (98% in the high dose versus only 8% in the control group). There also appeared to be disruption of the endocrine system. Toxaphene increases the hepatic metabolism of estradiol and estrone in rats, thus reducing their effects on the reproductive cycle.

Human

Workers that have been chronically exposed to toxaphene have exhibited genetic changes including

acentric fragments and chromatid exchanges. Whether toxaphene is carcinogenic in humans is unknown, but it can cause cancer in laboratory animals.

In Vitro Toxicity Data

There is no data of carcinogenicity in humans, but toxaphene is classified as 'B2; probable human carcinogen' based on bioassays in laboratory animals and positive mutagenesis results in *Salmonella* assays.

Clinical Management

Blood tests are available to determine the levels of toxaphene. Emesis should not be induced in individuals expected to have suffered from acute exposure as this may trigger potential CNS toxicity. Activated charcoal (25–100 g for adults and 25–50 g for children) should be administered to inactivate unabsorbed toxaphene. Gastric lavage should be used in cases where a lethal dose of toxaphene has been ingested. For seizures and other CNS hyperactivity, a CNS depressant such as an intravenous benzodiazepine (diazepam or lorazepam) or barbiturate (phenobarbital) should be administered. For injury to lungs, ventilation and oxygenation should be maintained. Arterial blood gases should be checked frequently and nonselective adrenergic agonists should not be administered (β-2 agonists result in localized bronchodilation with minimal cardiovascular effects, which may increase the risk for cardiac arrhythmias). Individuals who have experienced ocular contact with toxaphene should have eyes irrigated with copious amounts of water at room temperature. For dermal exposure, extensively wash hair and skin using soap, then alcohol and then soap again. Any contaminated clothing should be discarded.

Environmental Fate

At peak production in 1977, 40 million pounds of toxaphene were being used each year. Toxaphene has a very long half-life in soil and can persist for up to 14 years. Current evidence suggests that it does not leach out of the soil and into ground water; nor is it metabolized by bacteria in the soil. Toxaphene can evaporate and be degraded by photolysis. Run-off from contaminated soil can carry toxaphene into nearby bodies of water where it can be concentrated in fish.

Ecotoxicology

As with many pesticides, toxaphene poses a great concern to wildlife species. In many of the species examined, the LD_{50} value for toxaphene is in the same range as that determined for the rat (80–90 mg kg^{-1}) or below. Therefore, toxaphene could present a significant ecotoxicological problem if released into the environment in significant quantities. Dredging of contaminate sediments could release toxaphene and increase availability for aquatic organisms.

Exposure Standards and Guidelines

The Environmental Protection Agency has established a limit of 0.003 mg l^{-1} of drinking water and also requires that spills in excess of 1 lb be reported. The Occupational Safety and Health Administration (OSHA) has established a permissible exposure limit of 0.5 mg toxaphene per m^3 for an 8 h day/40 h work week. The National Institute for Occupational Safety and Health recommends that toxaphene levels should at the lowest dose/concentration as possible in the workplace due to the potential for toxaphene to be carcinogenic in humans. The American Conference of Governmental Industrial Hygienists recommends a limit equal to that established by OSHA and that 1 mg m^{-3} should not be exceeded over a 15 min period.

See also: Organochlorine Insecticides.

Relevant Websites

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

http://www.epa.gov – Environmental Protection Agency. EPA Ground water and drinking water: Consumer Factsheet on Toxaphene. United States Environmental Protection Agency, Washington, DC. March 6, 2003.

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

Toxic Substances Control Act, US

Robert Kapp

- AGENCY: US Environmental Protection Agency (EPA)
- YEAR OF ENACTMENT/AMENDMENTS: Toxic Substances Control Act, 1976; Asbestos Hazard Emergency Response Act Amendment, 1986; Radon Program Development Act Amendment, 1988; Asbestos School Hazard Abatement Reauthorization Act Amendment, 1990; Residential Lead-Based Paint Hazard Reduction Act Amendment, 1992

Background Information

Beginning in the late 1960s and the early 1970s, there was recognition within the United States that existing laws did not adequately regulate the use of toxic chemical substances. One response was that President Nixon created the President's Council of Environmental Quality (CEQ) in 1969, followed by the formation of the EPA in December 1970. In April 1971, the CEQ drafted the first version of the Toxic Substances Control Act, which was debated for several years among Congress, the CEQ, the EPA, numerous chemical industry trade groups and other stakeholders. In the midst of these debates, the Kepone incident in Hopewell, Virginia was publicized on a popular television program, CBS's '60 Minutes', in 1976. Kepone was an insecticide that was being manufactured in a poorly controlled environment near the James River, which lead to an outbreak of severe neurological disorders among workers. The James River was closed to commercial and sport fishing as a result. The national exposure on television coupled with the increased public pressure over controlling exposure to polychlorinated biphenyls led to an agreement in the US Senate-House Conference Committee. The Toxic Substances Control Act (TSCA) (Public Law 94-469) was finalized and signed by President Ford in October 1976.

Overview of the Toxic Substances Control Act

The goal of this legislation was to help control the hazards of chemicals in commercial production in the United States. Specifically, TSCA expanded existing federal authority to regulate manufacturing, disposal, importing, distributing, and processing of all toxic chemicals. Under TSCA all such chemicals must be inspected and approved by the EPA before they enter the market including new and existing chemicals. This Act was originally passed in 1976 and was amended in 1986, 1988, 1990, and 1992.

The 1976 TSCA regulations covered all organic and inorganic chemical substances and mixtures, both synthetic and naturally occurring with the following exceptions: food, food additives, drugs, cosmetics, nuclear materials, tobacco, and pesticides. With respect to the qualified chemicals, the legislation gave EPA the authority to:

1. Require manufacturers and importers to submit information on all new chemical substances prior to manufacture for commercial purposes.
2. Require that manufacturers and processors collect, maintain, and possibly submit specified information on the chemical substances.
3. Regulate both old and new chemical substances that are expected to present or are presenting unreasonable risks to health and the environment.

The TSCA legislation provided the EPA Administrator with the authority to review and evaluate data from new and existing chemicals intended to be sold in commerce with respect to manufacture, processing, distribution, use, or disposal. If the data are insufficient or if the data show that the material or its use can cause adverse health or environmental effects, the EPA Administrator can limit, delay, or completely prohibit the manufacture and distribution of the material. The Act further specifies that the risks of using a particular substance must be compared with the benefits derived from its use. The objective is to create a balance between preventing health or environmental risk and not curbing innovative technology.

TSCA currently has four titles as noted below:

- *Title I – Control of Toxic Substances*: This section includes provisions for testing chemical substances and mixtures; processing notices; regulating hazardous chemicals substances and mixtures; managing imminent hazards; and reporting and retaining information.
- *Title II – Asbestos School Hazard Emergency Response Amendment*: This section was added by the Asbestos Hazard Emergency Response Act (AHERA, Pub. L. 99-519), which was enacted by Congress on October 22, 1986. It authorized EPA to amend its TSCA regulations to impose more requirements on asbestos abatement in schools. AHERA provides for the promulgation of federal regulations requiring inspection for asbestos and

appropriate response actions in schools and mandates periodic reinspection. In addition, it required the EPA Administrator to determine "the extent of the danger to human health posed by asbestos in public and commercial buildings and the means to respond to any such danger."

AHERA was amended in 1990 by the Asbestos School Hazard Abatement Reauthorization Act Amendment (ASHARA, Pub. L. 101-637) to require accreditation of persons who inspect for asbestos-containing material in school, public, and commercial buildings. This amendment also mandated the accreditation of persons who design or conduct response actions with respect to asbestos-containing material in such buildings.

- *Title III – Indoor Radon Abatement*: This change was made on October 28, 1988 (Pub. L. 100-551). The purpose of this legislation was to assist states in responding to the threat to human health posed by exposure to radon. EPA is required to publish and keep current a citizen's guide to radon health risk, and to perform studies of the radon levels in schools and radon contamination in federal buildings.
- *Title IV – Residual Lead-Based Paint Hazard Reduction Act Amendment*: This section was added on October 28, 1992 (Pub. L. 102-550). The purpose of this legislation is to reduce environmental lead contamination and prevent adverse health effects as a result of lead exposure, particularly in children. Provisions include identifying lead-based paint hazards, defining levels of lead allowed in various products, including paint and toys, and establishing state programs for the monitoring and abatement of lead exposure levels, including training and certification for lead abatement workers.

The law is divided into a number of sections, which deal with various issues. The following table outlines the major TSCA Sections:

Section	*Subject*	*40 CFR reference*
4	Authority to require chemical testing	Parts 790–799
5	New chemicals	Part 720
	Premanufacturing notification exemptions (PMN)	Part 723
5(a)	Significant new use rules (SNUR)	Part 721
6, 7	Existing chemicals control	Part 750
8(a)	Chemical use reporting	Parts 740 and 712
8(b)	Inventory reporting guidelines	Part 710
8(c)	Adverse reactions	Part 717
8(d)	Health and safety data reporting	Part 716
12	Export rules	Part 707
13	Import rules	Part 707

At the date of this publication, the US EPA's Office of Pollution Prevention and Toxic Substances enforces TSCA.

Existing Chemicals

TSCA mandates that EPA identify, compile, keep current, and publish the TSCA Chemical Substance Inventory. The Inventory defines what chemicals exist in US commerce for TSCA purposes and not only contains chemical substances that have been manufactured or imported since January 1, 1975, but also includes intermediates used in the manufacture of other chemicals. The TSCA Inventory list currently contains ~80 000 chemicals. EPA can require companies to maintain records and submit reports revealing production and processing, significant adverse reactions, health and safety studies and substantial risk reports. Once an imported substance is found to be on the TSCA Inventory, it is subject to any rule deemed appropriate by EPA. Likewise, if the substance is not on the TSCA Inventory, the manufacturer or importer must comply with the PMN requirements before importation (see 'New Chemicals' below). If deemed necessary, EPA can require testing if there is evidence that a substance presents an unreasonable risk to health or the environment or if a substance is produced in substantial quantity and there is insufficient data to allow proper evaluation. If, in fact, EPA determines a substance presents an unreasonable risk, production can be prohibited, limited, or more substantial labeling can be required. If EPA determines there is an imminent hazard, significant legal action may be pursued including product seizure or recall. Importers of chemical substances must comply with the same regulations including an additional certification requirement. Importers of chemical substance must certify as follows:

- *Negative certification*: The importer must certify that all of the chemicals in the imported product are not subject to TSCA and are regulated under another statute. A negative certification is generally required for imports of pesticides (but not pesticide intermediates), nuclear materials, firearms and ammunition, food, food additives, drugs, registered pesticides, cosmetics, or medical devices.
- *Positive certification*: A positive certification is required for all imports of chemical substances or mixtures (other than articles) subject to TSCA regulations. The importer must certify that all chemicals in the imported product comply with all applicable rules or orders under TSCA and that

they are not offering the product for entity in violation of TSCA or any applicable rule under TSCA.
- *No certification*: No certification is required for chemical substances or mixtures as part of an article (unless required by a rule or order under TSCA), or for tobacco or tobacco products.

Exporters are also required to notify EPA before shipping any product abroad for which test data are required, regulatory action has been proposed or occurred, or action of some sort is pending or relief granted. Substances subject to export notification are listed on the Chemicals on Reporting Rules (CORR).

New Chemicals

Substances not on the Inventory or are not otherwise excluded or exempt are considered 'new' and are subject to a premanufacture notice (PMN). Examples of exclusions would include mixtures, substances subject to another statute, impurities, by-products and nonisolated intermediates. Additional exemptions also include test marketing products, low volume products, polymer exemptions, LoREX (low release and exposure exemption), and R&D substances. By statute, chemical manufacturers must notify the Agency at least 90 days before manufacturing a chemical substance that is not listed on the TSCA Chemical Substance Inventory. However, TSCA does not empower the US EPA to require routine testing of new chemicals to permit a valid evaluation of the potential risks. This has been a limitation in the overall effectiveness of the PMN process. Frequently, very little data accompanies the PMN (50% of submissions present no safety data and 90% have only an LD_{50} and an Ames test); however, the EPA must decide within 90 days if the submitted chemical will pose a health or environmental hazard.

The PMN generally requires the following items:

- chemical identity,
- manufactured amounts,
- number of employees exposed,
- method of disposal,
- categories of use,
- by-products,
- releases to the environment,
- any relevant health or environmental effects data, and
- other 'reasonably ascertainable' data.

If there is a paucity of data and/or the chemical may present an unreasonable risk, EPA has the authority to require various testing to fill data gaps or limit or ban the manufacture or importation of the chemical altogether. However, EPA must review the PMN within 90 days of receipt of the Notification. The EPA assesses the potential risks associated with the manufacture, processing, distribution, use, and disposal of the substance in question. The review period may be extended for 'good cause' under extenuating circumstances. Upon completion of the review, EPA may take regulatory action if the substance in question:

1. may present an unreasonable risk;
2. may enter the environment in substantial amounts; and
3. may result in substantial human exposure.

On the other hand, the EPA may take no action if the data show that there is no substantial exposure or risk. In that case, the substances deemed 'new' are then added to the TSCA Inventory when EPA receives a Notice of Commencement (NOC) from the manufacturer or importer following the completion of the 90 day review period. The NOC must be filed within 30 days of manufacture or import. At this time, the substance in question becomes an 'existing' chemical for regulatory purposes under TSCA and anyone can then begin to manufacture or import the chemical.

Sustainable Futures as a TSCA-Related Voluntary Pilot Project

On December 11, 2002, EPA announced in the US Federal Register, a TSCA-related voluntary pilot project, entitled Sustainable Futures. The goal of this pilot project has been to encourage the application of pollution prevention principles and the development of inherently low hazard new chemicals submitted as PMNs under Section 5 of TSCA. The Agency seeks to gain additional data and experience regarding the pollution prevention, risk reduction, and source reduction benefits of the use of hazard, exposure, and risk screening methodologies such as EPA's Pollution Prevention (P2) Framework in new product development efforts.

To encourage industry participation in this voluntary pilot project, the Agency has provided regulatory flexibility in the form of certain expedited reviews of PMNs. For purposes of this voluntary pilot project, EPA implemented a program leading to the opportunity for simultaneous submissions of Test Market Exemption applications and PMNs on chemical substances for which the submitter demonstrates the application and use of the P2 Framework or other scientifically acceptable hazard and exposure

screening methodologies. This regulatory flexibility has the effect of reducing the time to market for select new chemicals from 90 to 45 days.

In order to qualify for this pilot project, and associated expedited review, companies subject to TSCA Section 5 reporting requirements must demonstrate experience and competence with the P2 Framework or other scientifically acceptable approaches to chemical risk screening. In order to do this, companies need to:

1. take necessary training;
2. use hazard and exposure screening tools and demonstrate to EPA that model results were used to inform corporate decision-making. EPA wants submitters to use these tools to select safer new chemical alternatives to submit as new chemical notifications (and, where appropriate, to identify opportunities to eliminate or control exposures through process controls); and
3. submit 5–10 successful (i.e., not regulated by EPA) PMNs or PMN exemption notices which have been developed using chemical hazard and exposure screening tools. These submissions should also include documentation of chemicals evaluated, models used, endpoints on which decisions were based, and the submitter's perspectives on the extent to which the screening tools provided useful information to compare alternatives and select safer chemicals.

The Federal Register notice provides additional detail relating to the expedited review available under this pilot project and discusses criteria or factors EPA will consider to determine eligibility for the pilot project and associated expedited review.

As indicated above, there is considerable complexity (and potential future change) in the TSCA regulations and the PMN process, and readers would do well to consult the rules and regulations linked to below.

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 Torts.

Relevant Websites

http://www.epa.gov – US Environmental Protection Agency, Office of Pollution Prevention and Toxics (OPPT). Sustainable Futures Project. Substances subject to export notification are listed on the Chemicals on Reporting Rules (CORR) available at this website.

http://www4.law.cornell.edu – Toxic Substances Control (from the US Code).

Toxic Torts

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Introduction to Toxic Torts

According to Black's Law Dictionary (1990, 6th edn., p. 1489) in American civil law, a tort is a "legal wrong committed upon the person or property independent of contract. It may be either (1) a direct invasion of some legal right of the individual; (2) the infraction of some public duty by which special damage accrues to the individual; or (3) the violation of some private obligation by which like damage accrues to an individual."

During the last half century, a complex form of tort action known as the 'toxic tort' has developed to address some of the challenges of modern industrial society. Typically, the toxic tort is a civil action that seeks damages for injury to person or to property arising from alleged exposure to a toxic substance, emission, or product. Toxic tort claims most commonly are filed in classic civil lawsuits, but toxic tort issues also arise, for example, in workers' compensation claims and in administrative actions for cleanup of hazardous waste sites. In the majority of toxic tort actions, the plaintiff must show: (1) exposure to a toxin, (2) that the toxin caused a compensable injury, and (3) that a compensable injury, in fact, occurred.

Distinguishing Features of Toxic Torts

Toxic tort litigation has several distinguishing characteristics, including issues of exposure, latency, prospective damages, causation, risk, and complex challenges involving expert testimony and multiplicity of parties.

Regarding 'exposure', the plaintiff or claimant alleges knowing or unknowing 'exposure' (e.g., absorption, contact, ingestion, inhalation, implantation, or injection) to one or more environmental (e.g., chemical, biological, radiological, nuclear, or explosive) agents alleged to be 'toxic'. The Toxic Substances Control Act, 15 USCA § 2606(f), defines

*The author would like to acknowledge Michele A Reynolds' first edition from which several points were adapted.

toxicity in terms of 'imminent hazard', which is described as involving "the manufacture, processing, distribution in commerce, use, or disposal of [a substance that] is likely to result in ... injury to health or the environment." Similarly, in the Hazard Communication Standard promulgated by the Occupational Safety and Health Administration (OSHA), a 'health hazard' is defined as "a chemical for which there is statistically significant evidence based on at least one study conducted in accordance with established scientific principles that acute or chronic health effects may occur in exposed employees." 29 C.F.R. § 1910.1200(c).

The environmental agents that appear in toxic tort actions tend to be many of the same agents selected by governmental agencies (e.g., EPA, FDA, CPSC, OSHA, and DOT) for regulation. Agents of concern under TOSCA, 15 USCA § 2603(b) (2) (A), include those causing effects such as 'carcinogenesis, mutagenesis, teratogenesis, behavioral disorders, and cumulative or synergistic effects'. By contrast, under the Hazard Communication Standard, OSHA requires actions to be taken with regard to agents that may be classified as "carcinogens, toxic or highly toxic agents, reproductive toxins, irritants, corrosives, sensitizers, hepatotoxins, nephrotoxins, agents which act on the hematopoietic system, and agents which damage the lungs, skin, eyes, or mucous membranes." 29 C.F.R. § 1910.1200.

Regarding 'latency', in some toxic tort actions, the alleged adverse effects of exposure may not be immediately apparent because the injuries have not yet manifested themselves or because the harm goes undetected for a period of time. Latency often becomes an issue in lawsuits involving cancer, birth defects, and genetic mutations, and statutes of limitation or rules of accrual may be modified to accommodate those situations where the moment of the defendant's action and the discovery of the injury are separated by substantial intervals of time (typically years).

Regarding 'prospective damages', the diseases and illnesses that form the basis of damage claims in many environmental tort actions may develop over long periods, may derive from extended periods of exposure to toxic substances, and often are characterized as disorders whose underlying mechanisms are not well understood. Consequently, plaintiffs may assert claims for 'future harms' that have not yet, and possibly never will, manifest themselves, or whose progression from an early stage is highly speculative. Courts have addressed at least the following proposed kinds of prospective damages:

1. The plaintiff is suffering an existing physical injury that may worsen or develop into or be related to more serious consequences (e.g., asbestosis and possible lung cancer).
2. The plaintiff is not suffering from any existing injury or disease, but due to the exposure to the toxic substance is, or may be, at an increased risk of developing a particular disease in the future.
3. The plaintiff, because of his or her enhanced susceptibility to contracting such a disease, suffers present emotional distress, usually in the form of fear or anxiety about the prospective harm, sometimes accompanied by physical manifestations.
4. The plaintiff, again because of enhanced risk of future serious disease or physical injury, incurs or should incur present and future medical expenses in the nature of surveillance and monitoring costs to ascertain the presence or development of the disorder.

Regarding 'causation', the often lengthy interval between 'exposure' and 'manifestation' increases the challenge for any plaintiff seeking to establish the necessary causal link between a 'toxic' agent and legally cognizable injury. The passage of time increases not only the likelihood of onset of multiple intervening causes, but also the likelihood of developing a condition otherwise known to have significant background incidence and prevalence in the general, nonexposed population. Consequently, it is frequently impossible to determine with any measure of certainty whether a plaintiff's health problem arose from the defendant's product or conduct, or whether that plaintiff would have developed her health problem in the absence of objective evidence of exposure.

Attributes of Causation

In toxic or environmental tort litigation, the plaintiff must prove that the defendant's product was a cause or a substantial factor contributing to her harm. In many cases, however, the evidence of direct causation is difficult to acquire.

Frequently, plaintiff's counsel will rely upon the testimony of experts to prove causation. The basis for their opinions will most likely include epidemiologic studies, case studies, animal studies, and/or *in vitro* studies. These experts will attempt to explain complicated scientific issues to members of a jury who are not trained to assess the reliability of scientific or medical testimony. Because even the most discerning jurors may be 'dazzled' by an expert's credentials and apparent knowledge, some authorities worry that jurors may not derive a 'true' understanding of biomedical thought on a particular subject. Not surprisingly, many judges have struggled with the standards for admissibility of evidence as a way to

limit the 'dazzling' effect and to keep inaccurate or 'junk' science out of the courtroom.

The standards for admissibility of expert testimony to prove causation clearly will continue to impact the future of toxic tort litigation. Therefore, an understanding of the Daubert decision and the continuing debate over the admissibility of expert scientific or medical testimony will benefit anyone dealing with toxic or environmental tort issues.

The Daubert Decision

The Federal Rules of Evidence (FRE) were adopted in 1975. Subsequently most states (at least 37) have adopted their own codified rules of evidence modeled closely on the FRE. For scientific evidence, the most relevant of the Rules are found in Article VII of the FRE in a section known as Opinions and Expert Testimony. Prior to 1993, some federal appellate courts had applied Rule 702 of the Federal Rules of Evidence to medical and scientific experts. (Rule 702 authorizes scientific testimony whenever it will assist the trier of fact to understand the evidence or to determine a fact in issue.) In 1993, in Daubert v. Merrell Dow Pharmaceuticals, Inc., 509 US 579, the Supreme Court of the United States issued an important interpretation of Rule 702. Seven of the nine justices ruled that judges must serve as evidentiary gatekeepers who determine whether proffered evidence is scientifically valid and relevant. The Court suggested several factors for judges to consider in determining whether to admit a particular theory or technique: Is the theory or hypothesis testable? Has it been tested? Has the theory or technique been subjected to peer review and publication? For a particular scientific technique or methodology, what is the known or potential rate of error? What (if any) are the standards that control the technique's operation? To what extent is the theory or technique generally accepted in the scientific community?

Although Daubert involved an interpretation of the Federal Rules of Evidence that binds only federal courts, the decision has influenced many state courts grappling with novel scientific evidence. Experts seeking to testify about scientific or medical matters that are novel or not generally accepted should therefore be prepared to address each of the concerns articulated by the Supreme Court. In addition, experts should remember that:

- In qualifying an expert to offer testimony, courts are typically more concerned with degree of familiarity with the pertinent subject matter than with title or specialty designation.
- Regarding causation analysis, courts often accept testimony from nonphysicians, especially in toxic tort, workers' compensation, and product liability cases.
- In general, experts *cannot* offer legal conclusions or express opinions about the credibility of other witnesses.
- In general, expert testimony that a conclusion is 'possible' does not suffice to meet the standard for admissibility with respect to the party who bears the burdens of production and persuasion. An expert's testimony that a certain thing is possible is no evidence at all. His opinion as to what is possible is no more valid than the jury's own speculation as to what is or is not possible.

A number of problems remain for post-*Daubert* courts. Does *Daubert* apply to jury as well as nonjury trials? Does the decision apply to all, or just novel expert testimony? Does *Daubert* apply only to 'scientific' experts? In appellate courts, how much *de novo* review is warranted? As gatekeepers, judges may need to look at several aspects of an expert's testimony, including the theory or reasoning behind it, the methodology or technique employed, the protocols followed, the data generated, the conclusion reached, or the interpretation of the opinion in a legal context. Some commentators have suggested that *Daubert* seems to indicate that the Federal Rules only require the reasoning or methodology underlying the testimony to be scientifically valid. If either of these aspects of an expert's opinion have the indicia of validity, it would appear that testimony governed by the Federal Rules should be admitted.

But is the *Daubert* approach restricted to assessment of the validity of theory or method, or should courts look behind apparently legitimate reasoning or technique and evaluate the legitimacy of the results as embodied in proper following of protocols, generation of data, and reaching of conclusions? In the same vein, is the apparent *Daubert* distinction between theory and methodology realistic or useful? Perhaps, but courts must recognize that two experts operating under the same generally accepted theory may employ radically different methods, each of which may be generally accepted in one scientific community but not in the other. In those situations, courts must recognize that experts from different disciplines often make certain assumptions that can never by 'scientifically' proved, and that these assumptions may lead legitimate experts to equally logical, but clearly opposite conclusions.

Regarding the indicia of validity and reliability, how should courts weigh the factors enunciated by the *Daubert* Court? How do courts factor the

importance of peer review, publication, testing, rates of error, the existence or lack of standards, and the notions of widespread or general acceptance? The Supreme Court did not state that any one indicator of validity or reliability is essential under the Federal Rules of Evidence. And finally, what is meant by reliability and validity? The scientific and medical literature definition of these terms is quite different from the definitions used by the Supreme Court and legal commentators. In science and medicine, reliability refers to precision or reproducibility, while validity basically refers to accuracy. By contrast, many legal commentators and courts have equated reliability with accuracy or the probability of accuracy, and validity with sound reasoning. Jurists should not be surprised that scientists or physicians may not understand or accept the meanings or connotations that courts and some members of the bar have applied to the terms 'reliability' and 'validity'. Thus, it would appear that uniformity of approach to the admissibility of scientific evidence will not easily be accomplished on the heels of Daubert.

The Categories of Admissibility Standards

Jurisdictions vary drastically in their standards for admission of scientific studies and expert opinions. At least three methods of screening have been characterized, including: (1) the 'pure' Frye approach, (2) the relevance approach, and (3) the discretionary approach.

The Frye Test

Although the Supreme Court stated that the Frye decision did not survive the enactment of the Federal Rules of Evidence, the Frye test remains influential in American courts. The Frye test refers to the standard for admission of scientific evidence applied by the US Court of Appeals for the District of Columbia in Frye v. United States, 293 F. 1013 (D.C. Cir. 1923). In refusing to admit the results of a lie detector test, the court stated in pertinent part:

Just when a scientific principle or discovery crosses the experimental and demonstrable stages is difficult to define. Somewhere in this twilight zone the evidential force for the principle must be recognized, and while the courts will go a long way in admitting expert testimony deduced from a well-recognized scientific principle or discovery, the thing from which the deduction is made must be sufficiently established to have gained general acceptance in the particular field in which it belongs.

Therefore, in Frye jurisdictions, scientific research, studies, techniques, or methods can be admitted into evidence only pursuant to a 'general acceptance' by the applicable scientific community; that is, nonmainstream studies are excluded.

The continued viability of the Frye approach has been a subject of much debate, both before and after Daubert. The purpose of the Frye test was to "prevent...the introduction into evidence of specious and unfounded scientific principles or conclusions based upon such principles." Advocates of this conservative approach argue that it protects the legal system from the 'junk science' that plagues the litigation process. As E.A. Firestone has explained:

> The accumulation of scientific knowledge is an additive process in which short steps of progress are created out of techniques and concepts already in existence. In this manner, a scientist will propose theories to the relevant community with the knowledge, and stoic acceptance, that these facts will be re-examined and redefined by a number of subsequent investigators ... In science, facts arise only as a result of collective acceptance ... good science is defined by consensus, not by credentials. The scientific community rightly treats new theories with skepticism, an attitude which does not reflect on the proponent of the theory but on the training of the scientific community.

In other words, proponents of Frye believe that scientific consensus is a strong indicator of the reliability of a theory.

Some Frye proponents also contend that admission of evidence or opinions unsupported by the scientific or medical community opens the courtroom door to any expert willing to testify on a party's behalf. Consequently, juries may award large verdicts based on evidence which is fundamentally flawed. These awards, in turn, may lead to an 'avalanche of litigation' and 'endless, baseless claims'. Furthermore, recent problems of availability and affordability of insurance have been attributed to controversial scientific and medical opinions admitted into evidence.

By contrast, the critics of Frye proclaim that the test should be abolished because it bans useful groundbreaking studies or theories from the courtroom. Plaintiffs have argued that the people who have been harmed by exposure to toxic substances should not have to wait for proof of others being similarly hurt before they can receive relief. This argument is especially significant in the context of toxic tort litigation, where anecdotal (case) reports have been cited by some experts as evidence that a toxic substance causes a specific type of injury.

Many toxic tort plaintiffs argue against the application of the Frye test. Specifically, as innocent victims, they contend that they should not have to wait several years for adequate epidemiologic studies

to be completed before they seek compensation. Defendants will argue the reverse: that until there is adequate proof of a causal connection between a toxic substance and the problems alleged to have been caused by that agent, they should not be held legally responsible under 'medical certainty', 'scientific certainty', 'general acceptance', or 'consensus' standards. Consequently, conservative standards, on the Frye end of the spectrum, will be difficult for many toxic tort plaintiffs to meet.

Other Standards for Admissibility of Scientific Evidence and Expert Testimony

Critics of Frye saw an opportunity to loosen the admission standards for expert testimony upon the promulgation of the FRE in 1975. The FRE did not mention Frye or the 'general acceptance' test. In fact, according to the court in United States v. Downing, 753 F.2d 1224, 1234 (3d Cir. 1985), "neither the text…nor the accompanying notes of the advisory committee…explicitly set forth the appropriate standard by which the admissibility of novel scientific evidence is to be established."

The arguably more liberal standards permit scientific evidence which is not generally accepted by the medical community to bypass the judge and be presented to the jury. As the court explained in Ferebee v. Chevron Chemical Co., 736 F.2d 1529 (D.C. Cir. 1984), cert. denied, 469 US 1062 (1984):

Judges, both trial and appellate, have no special competence to resolve the complex and refractory causal issues raised by the attempt to link low-level exposure to toxic chemicals with disease. On questions such as these, which stand at the frontier of current medical and epidemiologic inquiry, if experts are willing to testify that such a link exists, it is for the jury to decide whether to credit such testimony.

In federal court, admissibility of scientific evidence and expert testimony depends upon the application of the Federal Rules of Evidence to the facts of any case. After Daubert, these rules generally permit the judge (as 'gatekeeper') to admit evidence which is helpful to the trier-of-fact, reliable, and nonprejudicial.

In practice, application of the liberal standards varies by jurisdiction. The most lenient, or 'let-it-all-in', approach has been implemented by a few federal and state courts. This approach is based upon the theory that "any lack of foundation for an expert's opinion goes to the weight and not the admissibility of the opinion." However, even in these ultra-liberal courts, the party seeking to admit the evidence will still be required to demonstrate helpfulness, reliability, and lack of prejudice.

By contrast, other courts have struggled to reach a compromise between the 'general acceptance' and the 'let-it-all-in' standards. Some have significantly modified the Frye rule to ease the standard for admission of expert testimony, while others have remained quite conservative in their approaches to admissibility. Of note, a 'balancing test' espoused by Judge Weinstein in *In re Agent Orange*, 611 F.Supp. 1223 (D.C.N.Y. 1985), *aff'd*, 818 F.2d 187 (2d Cir.), *cert. denied*, 487 US 1234 (1987) requires the court to determine probativity of evidence by weighing reliability, helpfulness, and relevance against prejudice, confusion, and waste of time. Thus, despite the guidance provided by Daubert, courts do not agree on how to handle expert evidence generally, and on how to apply Frye specifically.

In the first post-Daubert decade, however, the Supreme Court's apparently more liberal approach to admissibility has not had the effect of further opening the courtroom doors to dubious scientific evidence. Indeed, the federal courts now are scrutinizing the evidence under several criteria, not just general acceptance. Such close attention has led to greater exclusion of evidence because each of the Daubert factors offers an opportunity for the court to find deficiencies in the proffered evidence.

Causation in the Trenches

Though there may be some dispute as to what comprises the relevant technical community, 'general acceptance' within that community is usually the beginning and end of the inquiry. Typically, the judge does not ask whether the community is correct in accepting or rejecting the relevant principle, technique, or methodology. By contrast, there are multiple indicia of 'scientific validity' to be examined under Daubert, but it does not appear that any one of these indicia is essential to the court's inquiry. Unfortunately, neither the *Frye* approach nor the Daubert indicia encourage the gatekeeper to undertake the intense, 'behind the scenes' analysis that is required to unearth the basis of expert opinion in many toxic tort cases. Any court that does take a 'hard look' will find that conceptual boundaries of technical 'communities' are increasingly difficult to define, peer review is not a uniform process, publication is easily accomplished as each value-laden community defines itself and creates its own journals, and tests are performed in laboratories that are not certified or accountable.

Importantly, however, there are some science-based criteria that both Frye and Daubert courts can employ in toxic tort cases. These criteria are found in the ontologic framework of scientific

materialism, which displaced vitalism many centuries ago and continues to dominate Western biomedical concepts of disease, causation, and pathogenesis. Realistic views of this ontology hold that the integration of insights derived from epidemiology, basic science, and clinical science remains the best way to generate and test causal hypotheses. To support an opinion with reasonable medical or scientific certainty that exposure to an environmental agent(s) is a cause of a disease, illness, or disorder, the overwhelming majority of scientists, physicians, and epidemiologists demand that the following criteria be satisfied:

1. The prevalence and incidence rate of the disorder should be significantly higher in those exposed to the hypothesized cause than in controls not so exposed (the cause may be present in the external environment or as a defect in host responses).
2. Exposure to the hypothesized cause should be more frequent among those with the disorder than in controls without the disorder when all other risk factors are held constant.
3. In the course of time, the disorder should *follow* exposure to the hypothesized causal agent.
4. A spectrum of host responses should follow exposure to the hypothesized agent along a logical biologic gradient from mild to severe.
5. A *measurable* host response following exposure to the hypothesized cause: (a) should have a high probability of appearing in those lacking this response before exposure, or (b) should increase in magnitude if present before exposure. This response pattern should occur infrequently in persons not so exposed.
6. Experimental reproduction of the disorder should occur more frequently in animals or man appropriately exposed to the hypothetical cause than in those not so exposed; this exposure may be deliberate in volunteers, experimentally induced in the lab, or demonstrated in a controlled regulation of natural exposure.
7. Elimination or modification of the hypothesized cause or of the vector carrying it should decrease the incidence of the disorder.
8. Prevention or modification of the host's response on exposure to the hypothesized cause should decrease or eliminate the disorder.
9. All of the relationships and findings should make biologic and epidemiologic sense.

Importantly, the number of these validation criteria that must be satisfied in the mind of any expert before he or she will render an opinion supporting a causal connection between exposure to agent X and onset of effect (or injury) Y varies from just one to all nine. There is no reliable evidence indicating consistency in the use of these criteria among individual experts or among members of a particular scientific discipline or medical specialty. Furthermore, experts typically do not identify those principles of causation analysis which underlie the basis of their opinions. The failure to make explicit (and to consistently apply) a consensus methodology of causation analysis has created much confusion in workers' compensation, product liability, toxic tort, hazardous waste, and adverse drug reaction litigation.

Even if all of the above validation criteria (for proof of general causation) were met, the alleged causal link between a toxic substance and an objectively verifiable injury merely becomes a possibility. The case-specific causation analysis must now be undertaken. Assuming the causative agent(s) can be identified, the expert must: (a) establish actual and biologically significant exposure; and (b) link the exposure to a reproducible, reasonably well-defined disorder. 'Exposure' does not typically mean 'in the vicinity of' for purposes of scientific causation analysis. There must be some evidence of inhalation, absorption, or ingestion by an individual of measurable quantities of specific substance(s). Assuming some credible evidence of exposure, the expert typically identifies a reproducible, reasonably well-defined, measurable health effect. Not all effects, however, are adverse or detrimental to an individual, and, of course, not all adverse effects constitute disease or disability (in medicine or in law).

Several mistakes of inference should be avoided by those attempting to assign cause-and-effect relationships. For example, although anecdotes and case reports can suggest testable hypotheses, they should not, by themselves, provide a basis for causal inference, especially in the absence of unbiased selection of subjects, examination of patients for other explanations of the adverse event, and measurement of the frequency of the same adverse event in appropriate control patients.

Bias in the selection of experimental subjects should also be avoided. Findings in clinic populations that are not randomly selected may not be representative of (or apply to) the general population. The phenomenon known as Berkson's Paradox (selection bias) is frequently overlooked by those attempting to causally relate environmental exposures to various health problems.

Many people, and some medical and legal professionals, fall into the trap of attributing an adverse effect to a procedure, mishap, or medication simply because the event occurred *sometime after* the performance of the procedure or the administration of

the drug. This fallacy of logic is known as *post hoc, ergo propter hoc*, which loosely translated means 'if condition B temporarily follows situation A, then A must have caused B'. Just because one sees a lot of worms and toads on the sidewalk after a thunderstorm does not mean it has been raining worms and toads.

Interpretations of population means or averages must be made with caution. Some data sets display a Gaussian (bell-shaped) distribution while others manifest biphasic or other distributions. Calculations of averages for the latter population distributions can be misleading, for example, when estimating future costs based on 'average' survival times.

Finally, the role of statistical associations and correlation coefficients in the proof of causality remains controversial. In particular, *meta-analysis*, or the use of formal statistical techniques to provide a 'quantitative synthesis' of a body of separate but similar experiments or studies, has provoked substantial disagreement in many disciplines. Critics of meta-analysis warn that: (a) meta-analysis itself is not an experiment; (b) it is difficult to avoid mixing the results of well-designed studies with poorly designed ones; (c) the investigator never knows if she has included *all* the relevant studies; (d) overlooking unpublished 'negative' studies may produce bias toward 'positive' results; (e) investigators often erroneously assume that exposure conditions are equal among the studies; (f) meta-analysis often abandons quantitative scientific evaluation of the magnitude of some effects; (g) inconsistent use of statistical methods to generate the results of meta-analyses decreases both the ability to extrapolate (decreases external validity) and to undertake comparative risk assessment. The role of meta-analysis as a tool for proof of causation in environmental tort, product liability, and workers' compensation cases remains to be determined.

Causation in the Law

To probe the controversy surrounding occupational disease, workers' compensation, toxic tort, and hazardous substance litigation, one must understand the traditional legal approach to causation. First, alleged wrongful conduct must be a *cause-in-fact* of harm. Proof of 'factual causation' usually involves proof of logical relationships between events linked in a deductive 'causal chain'. Cause-in-fact corresponds to the use of causation in everyday language. It is also called 'but for' or '*sine qua non*' causation, suggesting that the consequences would have been different if the cause-in-fact had not occurred. Courts, however, frequently confront multiple-cause events and experienced jurists recognize that harm is not necessarily the result of antecedent *individual* events. In addition, courts which confront excessively long causal chains must decide where a causal chain should end. Thus, the concept of *proximate cause* evolved to allow a jurist to discriminate between many so-called causes-in-fact and to incorporate policymaking into identification of *the* cause or causes which the legal system holds ultimately responsible for harm.

A third notion of causation – *probabilistic causation* – has also evolved over the last century. Probabilistic causation relies on probabilistic reasoning rather than on simple, deductively derived causal chains. Problems have arisen, however, because, as explained below, probabilistic reasoning serves two analytically distinct purposes in legal proceedings.

Traditionally, a plaintiff has two tasks known as burdens of proof. First, he or she must meet the *burden of production* by providing factual evidence for each element of a particular cause-of-action (e.g., negligence, battery, etc.) Second, he or she has a *burden of persuasion*. That is, she must convince the jury that her version of the facts is worthy of their collective belief with a minimum level of certainty, as defined by a standard of persuasion. The four commonly used standards are: (a) 'beyond a reasonable doubt' in criminal cases; (b) 'by clear and convincing evidence' in some civil cases; and (c) 'more likely than not'; or (d) 'by a preponderance of the evidence' in most civil cases, including workers' compensation, toxic tort, products liability, and occupational disease claims.

Qualitative concepts of probability (as embodied in the above standards) have long and explicitly influenced jury deliberations as to whether or not a plaintiff has met his burden of *persuasion*. By contrast, in conventional personal injury litigation, probability and inductive reasoning have not explicitly played a role in fact-finding *per se*. That is, the facts themselves, defined as elements on which one party has the burden of *production*, are generally deemed true or false – with a probability of either 0 or 1. For example, the light was either red or green, the brakes either did or did not work, or the pedestrian either did or did not fall.

Among the elements of a case which the plaintiff has the burden of proving is causation-in-fact. This element is common to toxic tort, hazardous waste, occupational disease, and conventional traumatic injury claims. As noted above, causation-in-fact *probability* is not an issue in most conventional injury cases. The jury simply decides which version of the facts it believes in an all-or-none, yes-or-no fashion, with no room for intermediate probabilities. Causation evidence is not expressed probabilistically.

This is not so in late twentieth-century environmental claims where, given the frequent impossibility of proving individual causation, statistical causation evidence (expressed probabilistically) is required as a factual estimate of the defendant's contribution to the plaintiff's risk. For example, the issue in a typical trauma case may be whether or not a car could have stopped at a red light. Evidence might be heard on speed, braking ability, and driver reaction time *for that particular vehicle* (car X). The jury then finds that car X either could or could not have stopped. However, in the absence of facts concerning the individual car, undisputed evidence may show that of 100 cars chosen at random, 55 would have been able to stop. As to whether or not plaintiff has met the burden of production, the jury could find either way, depending on how it responds to probabilistic (statistical) evidence. Jury response is, in turn, likely to be influenced by judicial instructions on inferences to be drawn from group-based information.

The jury may believe that 55% of cars could have stopped, but have no idea whether car X is among that group. Thus, the jury would say the plaintiff had not met the burden of production. Alternatively, the jury may believe that 55% of cars could have stopped *and* infer that car X (assuming it is not atypical) more likely than not would have stopped since most cars would have. This finding, however, incorporates a leap of faith from established fact about a population to a conclusion about a particular car.

The propriety of this kind of mental leaping is one of the most controversial aspects of toxic tort and occupational disease cases, where causation often cannot be properly formulated as a yes-or-no fact. Instead, parties rely on evidence of increased risk or enhanced probability of disease which may or may not be attributable to defendant's conduct. The inquiry becomes one of the existence and magnitude of a fact *probability*. Therefore, understanding the dual nature of probability, as both a factual statistical quantity (fact probability) and a measure of strength of belief (belief probability), becomes important. Unfortunately, fact probability and belief probability have not been kept analytically distinct. Courts have 'collapsed' the requirements for burden of production and burden of persuasion into *one* test that blurs plaintiff's twofold task of defining not only the facts or elements to be proved but also the amount of credence to be accorded a fact in support of a finding. When a judge tells a jury that "plaintiff must show that causation is more likely than not," she/he risks confusion. Does she/he mean that the *fact* of causation which plaintiff must prove (burden of production) is not traditional true-or-false (100% vs. 0%) causation but only the existence of a statistical probability of causation greater than 50%? Or does she/he refer to the burden of persuasion guided by a standard of *belief* that causation is 'more likely than not' true; that is, does the jury believe a knowable fact with more than 50% confidence?

Concern over haphazard and unrecognized transfer of 'preponderance of evidence' or 'more likely than not' standards from the burden of persuasion to the burden of factual proof (burden of production) involves more than idle semantics. The adverse effects of failure to undertake a deliberate, two-step probabilistic analysis include: (a) undue preference for particular probabilities of causation found in *one* epidemiologic study, especially when meta-analysis of multiple studies is not possible or available; (b) unrecognized lowering of the burden of production with concomitant stiffening of the burden (standard) of persuasion; (c) inappropriate fixation on simplistic quantitative rules such as the '$>50\%$ likelihood' rule; and (d) poorly reasoned opinions because courts fail to explain exactly how they apply the $>50\%$, 'more-likely than-not' rule.

Courts that apply the rule only to fact probabilities essentially seek a yes-or-no belief in a $>50\%$ fact probability. By contrast, traditional courts that apply the rule only to belief probabilities seek a $>50\%$ belief in a yes-or-no fact. In toxic tort/occupational disease claims where both fact probability and belief probability are issues, there are at least two other approaches. Courts could apply the 'more-likely than-not' standard *jointly*, reducing alleged fact probability by a factor reflecting the jury's doubt about its truth. By contrast, the rule could be applied *sequentially* to require only a $>50\%$ belief in a fact probability which itself may barely exceed the $>50\%$ threshold. It is important to see that joint application stiffens the causation burden-of-production/burden-of-persuasion, while sequential application substantially lessens the causation production/persuasion requirements. The point here is that, regardless of approach, a court that deals with causal indeterminacy characteristic of toxic tort/occupational disease claims should be explicit about what it is doing, especially if defendant's culpability of conduct or duty to prevent risk is factored into determination of the causation issue.

Theories of Liability

The developing law of toxic and environmental torts exhibits a blending of principles from judge-made 'common law' (i.e., court cases) and standards and approaches from regulatory aspects of 'public law' (i.e., statutes and regulations). Some public

law statutes may seek to lessen the burden on plaintiffs for establishing causation (when compared with traditional tort requirements) by creating a presumption that once the plaintiff makes a basic (threshold) showing of evidence, the burden shifts to the defendant to prove that he did not cause the plaintiff's injury…in effect requiring that the defendant prove a negative. Other public law statutes might require the court to accept animal data as evidence of causation when human epidemiologic data are not available. Still other statutes (e.g., Veterans' Dioxin & Radiation Exposure Compensation Standards Act of 1984, 38 USC § 1154) may establish an administrative schedule that provides a fixed amount of compensation to individuals who meet certain criteria. Some statutes, like the Comprehensive Environmental Response Compensation Act (CERCLA), 42 USCA §§ 9601-9675, do not offer remedies for personal injury. Instead, CERCLA creates remedies to be pursued by administrative and judicial action of the government, and in limited circumstances by private parties, in matters related to cleanup of hazardous substances released into the environment.

Toxic tort theories of liability can be organized into three categories: (a) claims against sellers of products, (b) claims related to activities on the land, and (c) miscellaneous torts. Theories associated with claims against sellers of products include breach of express or implied warranty, misrepresentation, fraud, negligence, regulatory duty to disclose (e.g., Hazard Communication Standard, 29 C.F.R. § 1910.1200), and no-fault (strict) product liability based on defective design, defective manufacture, or failure to warn of foreseeable risks or hazards. Theories associated with claims related to activities on the land include trespass, public nuisance, private nuisance, breach of fiduciary duty, and strict liability for unreasonably dangerous, abnormally dangerous, or ultrahazardous activities. Miscellaneous theories include assault, battery, negligence *per se*, intentional or negligent infliction of emotional distress, and violation of the federal Racketeer and Corrupt Organizations Act (RICO), 18 USCA §§ 1961-1968.

Common Law Claims against Sellers of Products

Article 2 of the Uniform Commercial Code, adopted in virtually every state, supplies the basic rules governing claims under express and implied warranties. UCC § 2-313 provides that a seller makes an express warranty by 'affirmation of fact or promise made', by 'description of the goods', or by a 'sample or model', any of which must have been part of the basis of the bargain between the seller and buyer. Breach of an express warranty essentially creates a strict liability because the product need not be shown to be defective under this theory. By contrast, UCC § 2-314 creates an implied warranty of merchantability in a contract of sale in which a seller is 'a merchant with respect to goods of that kind'. To be merchantable, the product must, at a minimum, be 'fit for the ordinary purposes for which such goods are used', be 'adequately contained, packaged, and labeled as the agreement of sale may require', and 'conform to the promises or affirmations of fact made on the container or label if any'. Importantly, a claim for breach of implied warranty of merchantability will not survive if the use of the product was not its 'ordinary use'. Finally, UCC § 2-315 states that "where the seller at the time of contracting has reason to know any particular purpose for which the goods are required and that the buyer is relying on the seller's skill or judgment to select or furnish goods, there is ... an implied warranty that the goods shall be fit for such purpose." For a legally enforceable warranty of fitness for a particular purpose, the seller does not have to be a 'merchant' of the type of goods sold, the seller should know that the buyer will be relying on the seller's skill or judgment, and the particular purpose must be different from the ordinary purpose for which the product is used. The seller's knowledge that the consumer intends to use the product for a certain purpose would not trigger this warranty if that purpose falls within the ordinary range of uses for that product.

Under Section 9 of the Restatement (Third) of Torts, a product seller can be held liable for misrepresentations that are fraudulent, negligent, or innocent. Comment b provides that "one engaged in the business of selling chattels who, by advertising, labels, or otherwise, makes to the public a misrepresentation of a material fact concerning the character or quality of a chattel sold by him is subject to liability for physical harm to a consumer of the chattel caused by justifiable reliance upon the misrepresentation, even though (a) it is not made fraudulently or negligently, and (b) the consumer has not bought the chattel from or entered into any contractual relation with the seller." To prove actual fraud in a toxic tort case, the plaintiff must show (1) a misrepresentation of fact, (2) that the defendant had knowledge of the falsity, (3) that the defendant intended to induce the plaintiff to act in reliance on the factual misrepresentation, (4) plaintiff's justifiable reliance on the misrepresentation, and (5) damage or loss as a result of the plaintiff's reliance.

Negligence began to emerge as a separate cause of action for unintentional torts in the early 1800s

coinciding with the Industrial Revolution in England. The textbook elements of the tort of negligence are: (1) duty, an obligation recognized by the law, requiring the actor to conform to a certain standard of conduct, for the protection of others against unreasonable risks of harm; (2) breach of duty, or the failure to comply with a recognized standard of care; (3) proximate or legal cause, a reasonably close causal connection between the conduct and the resulting injury, which includes both cause-in-fact and certain legal limitations on the extent to which the law will recognize 'cause'; and (4) actual loss or damage to the interests of another. The standard of care defining duty, and the breach of that duty, may be difficult to prove when the interval (latency) between the time of exposure and the manifestation of illness is measured in months to years. Proof of negligence typically requires proof of defendant's knowledge of the hazards at the time of exposure as well as proof of foreseeability of harm to the plaintiff.

Regarding strict liability for design defects, at least three approaches to defining 'defective condition' have emerged. Some courts apply the 'consumer expectation test', derived from comment i of § 402A of the Restatement (Second) of Torts, which defines 'unreasonably dangerous' as "dangerous to an extent beyond that which would be contemplated by the ordinary consumer who purchases it, with the ordinary knowledge common to the community as to its characteristics." However, because this test is difficult to apply in cases involving alleged toxic products, where the expectations of the reasonable consumer may not be clear, other courts have embraced a risk-utility or risk-benefit balancing test, wherein a product will be deemed defective if the danger outweighs the products utility. A somewhat more rigorous approach appears to be adopted by the Restatement (Third) of Torts: Products Liability, comment d to § 2, where the test for a design defect is "whether a reasonable alternative design would, at reasonable cost, have reduced the foreseeable risks of harm posed by the product and, if so, whether the omission of the alternative design by the seller or a predecessor in the distributive chain rendered the product not reasonably safe." Importantly, in *Potter v. Chicago Pneumatic Tool Co.*, 694 A.2d 1319 (1997), the Connecticut Supreme Court rejected an absolute requirement of safer alternative design, concluding that "the feasible alternative design requirement imposes an undue burden on plaintiffs that might preclude otherwise valid claims from jury consideration."

Regarding strict liability for manufacturing defects (e.g., decomposed mouse in a bottle of soda, or excessive amounts of arsenic in a cattle dip), § 2A of the third Restatement invokes liability "when the product departs from its intended design even though all possible care was exercised in the preparation and marketing of the product." This rule of absolute liability is based on a policy of encouraging manufacturers, distributors, and retailers to invest in product safety measures and to raise the level of quality control during production processes.

Regarding strict liability for failure to warn, the seller of a product generally has a duty to disclose only foreseeable risks. However, in Davis v. Wyeth Laboratories, Inc., 399 F.2d 121 (9th Cir. 1968), the court viewed even small foreseeable risks as requiring a warning when it held that a manufacturer of polio vaccine had a duty to warn consumers of the risk that one person in a million would contract polio by receiving the vaccine. In addition, "the manufacturer's status as an expert means that at a minimum he must keep abreast of scientific knowledge, discoveries, and advances and is presumed to know what is imparted thereby. But even more importantly, a manufacturer has a duty to test and inspect his product. The extent of research and experiment must be commensurate with the dangers involved." (See Borel v. Fibreboard Paper Products Corp., 493 F.2d 1076 (5th Cir. 1973)).

Common Law Claims Related to Activities on the Land

Toxic tort cases can involve microscopic substances in air, soil, or water that invade and interfere with a person's possessory interest in property. According to Restatement (Second) of Torts § 158, "one is subject to liability to another for trespass, irrespective of whether he thereby causes harm to any legally protected interest of the other, if he intentionally: (a) enters land in the possession of the other, or causes a thing or third person to do so, or (b) remains on the land, or (c) fails to remove from the land a thing which he is under a duty to remove." The invasion, even by invisible substances, may be on the surface of the land, beneath the land, or in the air above the land. In toxic tort trespass claims, however, courts may be reluctant to proceed in the absence of proof of actual damages.

To bypass some of the historically rigid requirements for proof of trespass, and to avoid numerous privileges that may be asserted as defenses to trespass claims, toxic tort plaintiffs may rely on the theory of nuisance, which is generally defined in the Restatement (Second) of Torts § 821 as "an unreasonable interference with a right common to the general public," or as an interference with the use and enjoyment of one's property. Nuisance may arise

from intentional or negligent conduct, or it may be associated with abnormally dangerous activities. Proof of nuisance does not require physical invasion of property, and the nature of the interest protected (use and enjoyment) is broader than the possessory interest protected by the law of trespass. Two separate doctrines of nuisance have evolved – public nuisance and private nuisance.

A cause of action sounding in public nuisance must allege harm, injury, inconvenience, or annoyance arising out of the invasion of a public interest. The Restatement (Second) of Torts states that analysis of the reasonableness of the challenged interference should ask: (a) whether the conduct involves a significant interference with the public health, the public safety, the public peace, the public comfort, or the public convenience; or (b) whether the conduct is proscribed by a statute, ordinance, or administrative regulation; or (c) whether the conduct is of a continuing nature or has produced a permanent or long-lasting effect, and, as the actor knows or has reason to know, has a significant effect upon the public right. The goal of this legal theory is the protection of community rights, and a private person does not have a claim for damages under public nuisance unless she can establish that she suffered special damage different from that sustained by other members of the general public. Where an injunction is the remedy sought, this requirement may be less stringently imposed by some courts.

By contrast, although no unitary precise definition has emerged, private nuisance is typically defined as an unreasonable nontrespassory interference with a private individual's use and enjoyment of his property. Some courts state that plaintiffs can recover for inconvenience, discomfort, and annoyance in addition to damages for injury to their persons and proprietary interests, while others have limited the scope of private nuisance claims by denying recovery based solely on fear of future injury or on decline in property value.

The recognition and enforcement of fiduciary duties can also play a significant role in toxic tort actions. For example, when property contaminated with hazardous substances, or property in proximity to a hazardous condition is acquired without notice of the condition, plaintiffs may sue real estate brokers who may or may not have known of the existence of the hazard. Under the traditional doctrine of *caveat emptor*, in the absence of outright fraud, a buyer has little recourse against a seller or broker for claims arising out of the defects on the property. However, with increased recognition during the past 30 years of fiduciary relationships between brokers and purchasers, some courts may hold brokers liable when they make representations without determining the actual condition of the property, especially if the buyer inquired about the specific condition, or if the broker had information or a suspicion that should have prompted investigation. Other courts may go further, either imposing broker liability for innocent transmission of misrepresentations by the seller, or imposing on the broker a full duty to investigate. In Strawn v. Canuso, 657 A.2d 420 (N.J. 1995), the Supreme Court of New Jersey went so far as to hold that developers and brokers of new homes have an affirmative obligation to disclose to prospective purchasers the existence of off-site hazards that materially affect the value of the property. These new trends in the law of fiduciaries are based on the inequitable bargaining positions of purchasers of residential property when compared with developers and brokers, and on the differences among the parties in relative access to information about hazards.

Toxic tort plaintiffs may also allege that a defendant's conduct was unreasonably dangerous or ultrahazardous (e.g., storing hazardous chemicals on the property). A plaintiff who relies on this theory does not have to show a lack of due care on the part of the landowner, and does not have to prove fault. Rather, the determination of unreasonable or abnormal danger, and the proof of strict (no-fault) liability, typically require a showing of: (a) the existence of a high degree of risk of harm, (b) a likelihood that the harm that results from it will be great, (c) an inability to eliminate the risk by the exercise of reasonable care, (d) the extent to which the activity is not a matter of common usage, (e) the inappropriateness of the activity to the place where it is carried on, and (f) the extent to which the value of the activity to the community is outweighed by its dangerous attributes. (Restatement (Second) of Torts, § 520.) Importantly, in most courts, no single factor is considered dispositive, and not all of the factors need apply for a finding of abnormal danger. The policy basis for this form of strict liability in toxic tort law is the perceived need to require defendants who place products or services into commerce to pay their way by compensating for any harm they may cause. As of 2004, courts in the United States are split in their decisions as to whether the handling, storage, and/or disposal of hazardous substances necessarily constitutes an abnormally dangerous activity as defined by the Restatement (Second) of Torts.

Miscellaneous Theories of Liability

Assault and battery are two intentional tort causes of action which have been employed in toxic tort cases.

Assault is an intentional, unlawful threat or offer to touch another person under circumstances that create in the mind of the other person a well-founded fear of an imminent battery, coupled with an apparent present ability to complete the attempt. Alternatively, an assault is an act intended to put another person in reasonable apprehension of an immediate battery, accompanied by success in causing such apprehension. The defendant must have been in a position to carry out the threat immediately and he must have taken some affirmative action to do so. By contrast, battery is an intentional harmful or offensive touching or contact with another person. A defendant may be liable for battery where she acts intending to cause such contact or an imminent apprehension of such contact, and the harmful contact indirectly or directly results. In Werlein v. United States, 746 F.Supp. 887 (D.Minn. 1990), *Vacated in part pursuant to settlement agreement*, 793 F.Supp. 898 (D.Minn. 1992), the court defined the standard for battery as requiring the plaintiff to prove that the defendant disposed of the toxic substances intending to cause an offensive or harmful contact, or with the knowledge that such contact was substantially certain to occur. Battery claims are infrequent in toxic tort litigation, but plaintiffs seeking punitive damages may include such a claim in order to prove intentional conduct which is more 'egregious' than mere negligence.

As previously noted, toxic tort cases involve a mixture of common law and statutory law claims. Plaintiffs may attempt to show that a violation of a standard of conduct established by statute or regulation should be viewed as negligence *per se*. In jurisdictions where negligence *per se* is recognized as a basis for a legal claim, the statute giving rise to the claim typically must have been enacted to protect the class of persons of which the plaintiff is a member against the kind of harm that the plaintiff has suffered. The presumption of negligence which flows from judicial recognition of this legal theory, however, has been disfavored in American toxic tort litigation. Numerical standards in statutes or regulations are frequently based on scant (albeit sophisticated) scientific or biomedical data of varying degrees of uncertainty, or data open to multiple reasonable interpretations, or data that are more complete or different at the time the action is brought when compared with data that formed the basis of the standard. Not surprisingly, courts have uniformly held that violations of the Occupational Safety and Health Act may provide evidence of negligence, but do not create legally operative presumptions of negligence. Elliott v. S.D. Warren Co., 134 F.3d 1 (1st Cir. 1998).

In the early part of the twentieth century, the Restatement of Torts concluded that one's interest in freedom from emotional or mental distress was not of sufficient importance to require others to refrain from conduct intentionally designed to cause such distress upon pain of adverse legal consequences. The interest in emotional and mental tranquility was simply one for which the law formerly provided no protection. More recently, according to Prosser and Keeton on Torts, § 12 (1984, 5th edn.), a plaintiff who successfully proves physical personal injury is entitled to compensation for all damages for injury past, present, and future associated with the circumstances giving rise to the action. Consequently, a plaintiff who proves physical injury causally related to exposure to an environmental agent may recover for both the physical injuries and for any associated 'emotional distress'.

Two basic types of claims for emotional distress have been proffered in toxic tort lawsuits. If the defendant's conduct is viewed as extreme and outrageous, courts may allow a claim for intentional or reckless infliction of emotional distress. By contrast, if the defendant's conduct is alleged to be negligent, a claim for negligent infliction of emotional distress may be allowed.

One of the most important issues in any jurisdiction that recognizes claims for emotional distress in toxic tort cases is whether an allegation or some level of proof of physical injury must accompany any such distress claim. For intentional, reckless, or outrageous conduct, the Restatement (Second) of Torts § 46 recognizes claims for intentional infliction of emotional distress, even in the absence of allegation or proof of physical injury or risk of physical injury. By contrast, in the Restatement (Second) of Torts § 436A concludes that "if the actor's conduct is negligent as creating an unreasonable risk of causing either bodily harm or emotional disturbance to another, and it results in such emotional disturbance alone, without bodily harm or other compensable damage, the actor is not liable for such emotional disturbance." The physical harm may be, but is not required to be, caused by the defendant's conduct; physical harm that results from the emotional distress is sufficient to satisfy the requirement. The nature and scope of the physical injury requirement (either leading to or pursuant to the emotional distress) has been addressed by a handful of American courts. For example, in Temple-Inland Products Corp. v. Carter, 993 S.W.2d 88 (Tex. 1999), the Texas Supreme Court held that, in the absence of manifest disease, mere inhalation of asbestos fibers was not a physical injury that would trigger a claim for negligent infliction of emotional distress. Similarly, in

Simmons v. Pacor, Inc., 543 Pa.664 (Pa. 1996), the Pennsylvania Supreme Court held that 'non-impairing, asymptomatic pleural thickening' did not constitute sufficient physical injury to be compensable as a matter of law. Consequently, pleural thickening did not satisfy the physical injury requirement needed to trigger a claim for emotional distress.

In 1993, however, the California Supreme Court broke new ground in toxic tort law. In Potter v. Firestone Tire and Rubber Company, 863 P.2d 795 (Cal. 1993), the court held that landowners (who alleged increased, but unquantified, risk of cancer due to alleged exposure to toxic substances due to the proximity of an adjacent landfill containing 'toxic waste') could nevertheless file a claim for negligent infliction of emotional distress in the absence of physical injury. The court concluded that "the physical injury requirement is a hopelessly imprecise screening device – it would allow recovery for fear of cancer whenever such distress accompanies or results in any physical injury, no matter how trivial, yet would disallow recovery in all cases where fear is both serious and genuine but no physical injury has yet manifested itself." Importantly, the Potter court limited these types of recoveries to plaintiffs proving distress that is reasonable, serious, and based upon a knowledge that the likelihood of developing cancer is more likely than not. Furthermore, claims based on fear of latent disease must be distinguished from claims that seek compensation for the increased risk itself. Most American courts have not as yet recognized or embraced the latter types of claims.

Defenses in Toxic Tort Litigation

Defendants in toxic tort litigation can and do assert a host of defenses in order to avoid liability. Some of these defenses substantively negate a specific cause of action, some limit claims against particular defendants, some arise in the law of procedure, some derive from the plaintiff's culpable conduct, and some arise because public law obligations can preempt the operation of common law. This chapter will conclude with brief descriptions of some of the important defenses that have been asserted in toxic tort cases.

State-of-the-Art

The state-of-the-art defense is asserted in strict liability (failure to warn) claims where the defendant alleges that he did not know, and could not reasonably have known, of the hazards of the product at the time of the plaintiff's exposure. A minority of courts have occasionally rejected this defense, reasoning that the imposition on manufacturers of the costs of failure to discover hazards creates an incentive for them to invest more actively in safety research.

Learned Intermediary

The learned intermediary doctrine is raised when the defendant claims that other parties with superior knowledge had the responsibility to warn the user of the product of its hazards. The third Restatement, § 6(d) (1) retains the learned intermediary doctrine, providing that a commercial seller or distributor of prescription drugs or medical devices is shielded from liability to the ultimate consumer where it has given reasonable warnings of foreseeable harm to "prescribing and other health care providers who are in a position to reduce the risks of harm in accordance with instructions or warnings." Where the manufacturer knows that the physician or other health care provider has a limited decision making role in the therapeutic relationship with patients, then the manufacturer is required to directly warn the patients. Restatement (Third) of Torts: Products Liability, § 6(d) (2) and comment b.

Sophisticated User

Courts allow the sophisticated user defense when the party to whom the product is delivered knows better than the seller the ultimate uses of the product, and the seller provides adequate warning of the hazards of the product to the purchaser. This defense typically arises in cases involving bulk suppliers of chemicals to knowledgeable intermediaries. Where the employer can be shown to be in a position to adequately warn its employees, the existence or the requirement of adequate warnings from the supplier may be irrelevant. In addition, chemical manufacturers may reasonably rely on the industrial user of a chemical to comply with the OSHA Hazard Communication Standard and pass on the manufacturer's warnings to its employees.

Unavoidably Unsafe Products

Some products (e.g., experimental drugs and some prescription drugs) cannot be made safe for the use for which they were intended, but their usefulness may outweigh the risk of harm. Comment k of § 402A of the second Restatement of Torts provides that "such a product, properly prepared, and accompanied by proper directions and warning, is not defective, nor is it unreasonably dangerous." This defense is designed to encourage development of useful and necessary products by allowing the seller to avoid liability associated with the risks of these products.

Sovereign Immunity

In the absence of a statute to the contrary, sovereign immunity generally protects governmental entities from liability in toxic tort actions. Even when a statute waives some aspects of governmental immunity, the statute often contains exceptions. For example, the US Government has waived its sovereign immunity for tort claims in the Federal Tort Claims Act, 28 USCA § 1346(b), which provides that federal district courts shall have exclusive jurisdiction over claims for money damages against the Unites States alleging personal injury, property damage, or wrongful death as a result of a negligent act or omission by a government employee within the scope of employment.

Key exceptions to this waiver of immunity include claims "based upon the exercise or performance or the failure to exercise or perform a discretionary function or duty on the part of a federal agency or an employee of the government, whether or not the discretion be abused." 28 USCA § 2680(a). In general, discretionary function involves judgment or choice by the actor, and the conduct also must be of the sort that the discretionary function exception was designed to protect, so as to prevent "judicial 'second-guessing' of legislative and administrative decisions." United States v. Varig Airlines, 467 US 797 (1984). By contrast, this exception will not apply automatically to all agency activities, and will not apply "when a federal statute, regulation, or policy specifically prescribes a course of action for an employee to follow."

A second exception to the waiver of sovereign immunity arose in the case of Feres v. United States, 340 US 135 (1950), where the Supreme Court held that members of the armed forces cannot bring tort actions against the government for harms that 'arise out of or are in the course of activity incident to service'. This exception was extended in Stencel Aero Engineering Corp. v. United States, 431 US 666 (1977), where the Court said that the government cannot be required to indemnify defendants in an action in which the plaintiff could not have sued the government directly. The Feres doctrine was further extended in Minns v. United States, 155 F.3d 445 (4th Cir. 1998), where the court held that children who claimed severe birth defects were caused by their servicemen-father's exposure to toxic chemicals during the Persian Gulf War were barred from suing the United States.

Government or Military Contractors

Some defendants will argue that the manufacture and provision of toxic chemicals according to government specifications should protect them from toxic tort liability. The accepted elements of the government contractor defense, as outlined in the *Agent Orange Litigation*, 534 F.Supp. 1046 (E.D.N.Y. 1982), include the following: (1) the government must have established the specifications for the product; (2) the product manufactured by the defendant must have met the government's specifications in all material respects; and (3) the government must have known as much or more than the defendant about the hazards to people that accompanied use of the product.

Assumption-of-the-Risk

This defense arises when the defendant can show that the plaintiff had knowledge of the hazard, appreciated the magnitude of the hazard, and voluntarily encountered it.

Comparative Negligence or Fault

Restatement (Third) of Torts § 17(a) provides that "a plaintiff's recovery of damages for harm caused by a product defect may be reduced if the conduct of the plaintiff combines with the product defect to cause the harm and the plaintiff's conduct fails to conform to generally applicable rules establishing appropriate standards of care." These rules clearly vary among jurisdictions, but product misuse, alteration, and/or modification are considered relevant to the determination of comparative responsibility, and evidence of these activities are frequently incorporated into the apportionment assessment.

Statutes of Limitation

These statutes (SOL) are designed to protect defendants from stale claims and to impose a degree of finality on litigation. The period of time within which the plaintiff may assert a claim begins at the time the cause of action *accrues*. In toxic tort litigation, it is not always clear when the action accrues for purposes of triggering the running of the statutory period. At least four approaches have been developed for determining the point of accrual of a toxic tort cause of action.

First, the 'exposure rule' states that the action accrues at the time of last exposure, even if the claimant's illness did not manifest until many years after the final exposure. Since typical statutory periods for commencement of tort claims run between 2 and 4 years, application of the exposure rule effectively bars many claimants who allege development of latent injuries.

Second, the 'judicial discovery rule' states that the judicially imposed date of accrual of a toxic tort cause of action is the earliest date on which the plaintiff knew or should have known the presence of

the injury. Thus, once the potential plaintiff 'discovers' that he may have suffered 'an actionable wrong', the period of the SOL begins to run.

Third, the 'statutory discovery rule' codifies the SOL period, which begins to run at the time of the discovery of the injury or when the injury should have been discovered through the exercise of reasonable diligence. Occasional statutes further provide that if the plaintiff has not discovered the cause of the illness or injury within a stated number of years, the claim will be barred.

Fourth, the 'time of discovery rule' focuses on the point in the disease process at which the potential plaintiff is deemed to have sufficient information to proceed with legal action. Typically, the date of accrual is recognized as the date when the claimant became aware of a potentially compensable injury *and* its potential cause. If the claimant becomes ill, but remains unaware of the precise cause of the illness, then the running of the SOL may be triggered on the date the potential plaintiff acquired knowledge of a 'reasonable possibility' that the particular exposure could be a cause of her injury. In Evenson v. Osmose Wood Preserving Company of America, Inc., 899 F.2d 701 (7th Cir. 1990), the court stated that "a reasonable possibility, while less than a probability, requires more than the mere suspicion possessed by [the plaintiff], a layperson without technical or medical knowledge." Consequently, most potential plaintiffs are not likely to become aware of a 'reasonable possibility' until after hearing a biomedical professional express an opinion regarding that 'reasonable possibility'.

Statutes of Repose

In contrast to statutes of limitation, which bar actions at a specified time period after the cause accrued, statutes of repose bar the institution of an action a specified number of years after a particular event, such as the date of first sale of a product or the date of improvements to real property. After that time, no action can be brought, even though the elements of a claim may not have all yet occurred and only occur years after the period has run. Thus a cause of action may be extinguished before it ever accrues, with the repose conferring immunity upon the defendant. In First United Methodist Church v. US Gypsum Co., 882 F.2d 862 (4th Cir. 1989), the Fourth Circuit Court of Appeals noted that "statutes of repose are based on considerations of the economic best interests of the public as a whole and are substantive grants of immunity based on legislative balances of the respective rights of potential plaintiffs and defendants struck by determining a time limit beyond which liability no longer exists ... as a general rule, a statute of limitations is tolled by a defendant's fraudulent concealment of a plaintiff's injury because it would be inequitable to allow a defendant to use a statute intended as a device of fairness to perpetrate a fraud. Conversely, a statute of repose is typically an absolute time limit beyond which liability no longer exists and is not tolled for any reason because to do so would upset the economic balance struck by the legislative body."

Res Judicata or Claim Preclusion

When a valid and final judgment has been taken on a particular claim, *res judicata*, or claim preclusion, prevents a subsequent action on the same claim. The modern approach to determining what constitutes a claim focuses on 'transaction'. According to the Restatement (Second) of Judgments § 24, "the claim extinguished includes all rights of the plaintiff to remedies against the defendant with respect to all or any part of the transaction, or series of connected transactions, out of which the action arose…[a transaction is] to be determined pragmatically, giving weight to such considerations as whether the facts are related in time, space, origin, or motivation; whether they form a convenient trial unit; and whether their treatment as a unit conforms to the parties' expectations or business understanding or usage."

In toxic tort litigation, the scope of the 'transaction' is not always clear. For example, should the asbestos claimant be able to split his transaction into separate claims for plaques, fibrosis, cancer, and fear or risk of any one or more of those three conditions? If a particular jurisdiction has a discovery statute of limitations and forbids claim splitting, a plaintiff's entire claim would accrue at the time of the first injury. Effectively, a plaintiff could be barred from bringing any future claim long before he even knew about it. Traditional *res judicata* (claim preclusion) rules bar any accrued action arising out of the same circumstances as a claim previously adjudicated. A handful of jurisdictions, however, enable a plaintiff to split claims under limited circumstances, especially when that plaintiff might reasonably be expected to encounter difficulty in trying to prove with reasonable certainty that a disorder will develop in the future. Obviously, the recognition of claims for increased risk of future illness in any particular jurisdiction will depend on the future evolution of both accrual rules and claim-splitting rules.

Preemption

Many of the activities and events underlying private toxic tort actions are also regulated by public laws

embodied in statutes and regulations. Sometimes, the duties defined by public law and common law exist independently, but other times they overlap. Sometimes the overlap creates contradictory, conflicting, or ambiguous obligations, enabling the defendant to assert a defense that the public law obligation preempts the operation of the common law.

In general, the law starts with a presumption against preemption. However, express language in a statute may provide explicit evidence of legislative intent to preempt common law. Hopefully, that language of preemption is clear and unambiguous as to the scope of the preemption. Occasionally, these same statutes will contain other language that attempts to 'save' or retain or reserve all rights under the common law, so as to restrict the scope of the public law preemption and make it easier to interpret the 'express preemption' enunciated in the statute.

By contrast, when the statute is silent on preemption, or the preemptive language is ambiguous or unclear as to the scope of the preemption, courts often undertake an 'implied preemption' analysis in order to determine whether the legislature intended to 'occupy the field' with legislation so sweeping that no room was left for common law, or to determine whether the common law actually conflicts with the scheme of the statute. A court may find an actual conflict where it is not possible to comply with both common law and public law, or the court may decide that common law acts as an obstacle to fulfillment of the statutory objectives.

Toxic Torts and the Future

As noted in previous editions of this chapter, the law of toxic torts continues to develop. Traditional legal rules continue to be strained and stretched. The tension created by the juxtaposition of scientific uncertainty and unsettled law continues to impact toxic tort litigants. The emerging interface of genetic and environmental forces creates new challenges for the proof of causation and injury, and further complicates emerging concepts of latency between exposure and either onset or manifestation of injury. Epidemiological studies continue to vary widely in their attempts and in their ability to detect in populations objectively verifiable effects that can be attributed to chronic, low-dose exposures to various environmental agents. Finally, among the most interesting challenges offered by toxic tort litigation is the persistent need for medical, scientific, legal, and regulatory professionals to improve their capacities to communicate and understand the complex concepts, language, and approaches used by the various participants in this fascinating sphere of human activity and concern.

See also: Clean Air Act (CAA), US; Clean Water Act (CWA), US; Federal Insecticide, Fungicide, and Rodenticide Act, US; Food Quality Protection Act, US; Food, Drug, and Cosmetic Act, US; National Environmental Policy Act; Occupational Safety and Health Act, US; Pollution Prevention Act, US; Resource Conservation and Recovery Act, US; Safe Drinking Water Act, US; Toxic Substances Control Act, US.

Further Reading

Black B and Lilienfeld DE (1984) Epidemiologic proof in toxic tort litigation. *Fordham Law Review* 52: 732.

Boston GW and Madden MS (2001) *Law of Environmental and Toxic Torts*. St. Paul, MN: West Group.

Brennan TA (1988) Causal chains and statistical links: The role of scientific uncertainty in hazardous substance litigation. *Cornell Law Review* 73: 469.

Eggen JM (2000) *Toxic Torts in a Nutshell*. St Paul, MN: West Group.

Gold S (1986) Note, causation in toxic torts: Burdens of proof, standards of persuasion, and statistical evidence. *Yale Law Journal* 96: 376.

Snyder JW (1997) Silicon breast implants: Can emerging medical, legal, and scientific concepts be reconciled? *Journal of Legal Medicine* 18: 133.

Snyder JW, Casey-Maier J, and Love BD (1997) Injury and causation on trial: The phenomenon of 'multiple chemical sensitivities'. *Widener Law Symposium Journal* 2: 97.

Toxics Law Reporter. *A Weekly Review of Toxic Torts, Hazardous Waste, and Insurance Litigation (BNA)*. Washington, DC: Bureau of National Affairs.

Toxicity Testing, Alternatives

Shayne C Gad

Since the early 1980s, public perception of the value of (and benefits from) animal testing has been strongly influenced by what is now called the animal rights movement. This concern has done a great deal of good because it has caused careful consideration of why and how testing is performed, with significant alterations being made in practices across the range of safety assessment. Its impact has been uneven, however, in the degree of sensitivity of the issue of

animal testing in different industries and organizations. Some organizations no longer perform any animal testing, having it conducted externally if it is required. Others are simply quiet about it.

The guiding principles subscribed to by many in the field are the four Rs. The historical beginnings of this concept date to 1959 when Russell and Burch first proposed what have come to be called the three Rs of humane animal use in research: replacement, reduction, and refinement. These three principles have served as the conceptual basis for reconsideration of animal use in research. To these has been added a fourth principle: responsibility.

Replacement means utilizing methods which do not use intact animals in place of those that do. For example, veterinary students may use a canine cardiopulmonary resuscitation simulator, Resusci-Dog, instead of living dogs. *In vitro* techniques include utilizing cell or tissue cultures, isolated cells, tissue slices, subcellular fractions, transgenic cell cultures, and cells from transgenic organisms. Cell cultures may replace mice and rats in discovering substances poisonous to humans. In addition, using the preceding definition of animal, an invertebrate (e.g., a horseshoe crab) could replace a vertebrate (e.g., a rabbit) in a testing protocol. Further, computer software programs and their associated databases of toxicology-related structure–activity data can be used to do *in silico* modeling to predict the toxicity profile of a new chemical.

Reduction refers to the use of fewer animals. For instance, changing practices allow toxicologists to estimate the lethal dose of a chemical with as few as 1/10 the number of animals used in traditional tests. In biomedical research, long-living animals such as primates may be used in multiple sequential protocols assuming that the protocols are not deemed inhumane or scientifically conflicting. Designing experimental protocols with appropriate attention to statistical inference can lead to either decreases or increases in the numbers of animals used. Through coordination of efforts among investigators, several tissues may be simultaneously taken from a single animal. Reduction can also refer to the minimization of any unintentionally duplicative experiments, perhaps through improvements in information resources.

Refinement entails the modification of existing procedures so that animals are subjected to less pain and distress. Refinements may include administration of anesthetics to animals undergoing otherwise painful procedures; administration of tranquilizers for distress; humane destruction prior to recovery from surgical anesthesia; and careful scrutiny of behavioral indices of pain or distress followed by cessation of the procedure or the use of appropriate analgesics. Refinements also include the enhanced use of noninvasive imaging technologies that allow earlier detection of tumors, organ deterioration, or metabolic changes and the subsequent early euthanasia of test animals.

Responsibility is the fourth R, which was not in Russell and Burch's initial proposal. To toxicologists this is the cardinal R. They may be personally committed to minimizing animal use and suffering and to doing the best possible science of which they are capable, but at the end of it all, toxicologists must stand by their responsibility to be conservative in ensuring the safety of the people using or exposed to the drugs and chemicals produced and used in our society. This is particularly true for medical devices and includes in it the element of ensuring adherence to regulatory requirements and standards.

Toxicology, and particularly the portion of it associated with the assessment of commercial products for safety, is philosophically a conservative scientific practice. If a choice is to be made as to whether to accurately identify human hazard or to over-predict any hazard, with both predictions having a degree of uncertainty, the latter course will be chosen. Likewise, if an evaluation process, which is dated but very familiar is challenged by a new technology which is scientifically, economically, and ethically superior but with which there is no precedent or prior history of use, the former will be selected. Both of these two choices are made with reference to what (1) is regulatorily accepted (i.e., codified in law) and (2) what has to date been the standard in litigation defense. The key assumptions currently underlying safety assessment are (1) that other organisms can serve as accurate predictive models of toxicity in humans, (2) that selection of an appropriate model to use is essential to the accurate prediction of adverse health effects in humans, and (3) that an understanding of the strengths and weaknesses of any particular model is required before the relevance of specific findings to humans can be established. When we refer to models, we usually mean test organism, though in fact the manner in which parameters are measured (and which parameters are measured) to characterize an endpoint of interest is also a critical part of the model (or, indeed, may actually constitute the model). To an increasing degree, both *in vivo* (intact, higher organism) and *in vitro* and *in silico* models are used, though the degree of utilization of *in vitro* and *in silico* models has lagged behind its potential use.

Mechanisms of chemical toxicity are largely identical in humans and animals. Our increased understanding of mechanisms on the molecular and cellular level has caused some of the same people who question the general principle of predictive

value of animal tests to suggest that the state of knowledge is such that *in silico* models or simple biochemical or cell culture systems could always be used in place of intact animals to accurately predict or warn of toxicities in humans. This last suggestion also misses the point that the final expressions of toxicity in humans or animals are frequently the summation of extensive and complex interactions occurring at cellular and biochemical levels. For example, although it was once widely believed (and still is believed by many animal rights activists) that *in vitro* mutagenicity tests would replace animal bioassays for carcinogenicity, this is clearly not the case on either scientific or regulatory grounds. Although there are differences in the responses of various species (including humans) to carcinogens, the overall predictive value of such results (when tempered by judgment) is clear.

Increasingly, alternative models that use other than intact higher organisms are being used in toxicology for a number of reasons. These reasons include desires for specificity of response, use of small quantities of test materials, and expedited development, all of which are particularly important in the biotechnology industries. Well-reasoned use of *in vitro* or other alternative test model systems is essential to the development of a product safety assessment program that is both effective and efficient.

The 'ideal' test to answer a safety assessment question should have an endpoint measurement that provides data such that dose–response relationships can be obtained. Furthermore, any criterion of effect must be sufficiently accurate in the sense that it can be used to reliably resolve the relative toxicity of two test chemicals that produce distinct yet similar responses (in terms of hazard to humans). In general, it may not be sufficient to classify test chemicals into generic toxicity categories. For instance, if a test chemical falls into an intermediate toxicity category but is borderline to the next, more severe toxicity category, it should be treated with greater concern than another test chemical that falls at the less toxic extreme of the same immediate category. Therefore, it is essential for a test system to be able to place test chemicals in an established toxicity category as well as to rank materials relative to others in that category.

The endpoint measurement of the ideal test system must be objective. This is important so that a given test chemical will yield similar results when tested using the standard test protocol in different laboratories. If it is not possible to obtain reproducible results in a given laboratory over time or between various laboratories, then the historical database against which new test chemicals are evaluated will be time or laboratory dependent. If this is the case, then there will be significant limitations on the application of the test system because it could potentially produce conflicting results. From a regulatory point of view, this possibility would be highly undesirable. Along these lines, it is important for the test protocol to incorporate internal standards to serve as quality controls. Thus, test data could be represented utilizing a reference scale based on the test system response to the internal controls. Such normalization, if properly documented, could reduce interest variability.

From a practical point of view, there are several additional criteria that the ideal test should meet. Alternatives to current *in vivo* test systems basically should be designed to evaluate the observed toxic response in a manner as closely predictive of the outcome of interest in humans as possible. In addition, the test should be fast enough so that the turnaround time for a given test chemical is reasonable for the intended purpose (very rapid for a screen and timely for a definitive test). The speed of the test and the ability to conduct tests on several chemicals simultaneously will determine the overall productivity. The test should be inexpensive so that it is economically competitive with current testing practices. Finally, the technology should be easily transferred from one laboratory to another without excessive capital investment (relative to the value of the test performed) or the need for special skills for test implementation.

Table 1 Rationale for using *in vivo* test systems

Evaluate actions/effects on intact animals and assess organ/tissue interactions
Allow either neat chemicals or complete formulated products (complex mixtures) to be evaluated
Yield data on the recovery and healing processes
Are currently predominantly the required statutory tests for agencies worldwide
Afford quantitative and qualitative evaluations using a scoring system that is generally capable of ranking materials according to their relative hazards
Are amenable to modifications to meet the requirements of special situations (such as multiple dosing or exposure schedules)
Allow the use of an extensive available database and have cross-reference capabilities for evaluation of relevance to human situation
Are associated with ease of performance and relatively low capital costs in many cases
Are generally both conservative and broad in scope, providing for maximum protection by erring on the side of over prediction of hazard to humans

The point is that these characteristics of the ideal test system provide a general framework for evaluating alternative test systems in general. No test system is likely to be ideal. Therefore, it is necessary to weigh the strengths and weaknesses of each proposed test system in order to reach a conclusion as to the effectiveness of a particular test.

In recent years, tremendous progress has been made in our understanding of mechanisms of biological action down to the molecular level. This has translated to multiple modifications and improvements to *in vivo* testing procedures, which now give us tests which (1) are more reliable, reproducible, and predictive of potential hazards in humans; (2) use fewer animals; and (3) are considerably more humane than earlier test forms. Since 1971 *in vitro* alternative test systems have been proposed, developed, and validated to at least some extent. Yet the perception persists that little has changed in how safety assessment is performed by or for industry.

In both theory and practice, *in vivo* and *in vitro* tests each have potential advantages, as summarized in **Tables 1** and **2**. It should be noted that the relative

Table 2 Limitations of *in vivo* testing systems that serve as a basis for seeking *in vitro* alternatives for safety assessment tests

May involve complications and/or confounding or masking of findings
May assess only the short-term site of application or immediate structural alterations produced by agents; however, specific *in vivo* tests may only be intended to evaluate acute local effects, so this may be a purposeful test system limitation
Require stringent technician training and monitoring (particularly because of the subjective nature of evaluation)
May not perfectly predict results in humans if the objective is to exclude or identify severe acting agents
Structural and biochemical differences between test animals and humans that make extrapolation from one to the other difficult
Lack of standardization
Variable correlation with human results
Large biological variability between experimental units (i.e., individual animals)
Large, diverse, and fragmented databases that are not readily comparable
Require a comparatively longer time to express/evaluate endpoints
Require comparatively larger quantities of test material
May be conducted using either a single endpoint (e.g., lethality and corrosion) or a so-called 'shotgun' or multiple-endpoint approach (e.g., a 13 week oral toxicity study)
Are the accepted norm for evidence in courts of law for litigation cases

Table 3 Levels of models for toxicity and research

Level/model	*Advantages*	*Disadvantages*
In vivo (intact higher organism)	Full-range of organismic responses similar to target species	Cost Ethical/animal welfare concerns Species-to-species variability
Lower organisms (earthworms, fish)	Range of integrated organismic responses	Frequently lack responses typical of higher organisms Animal welfare concerns
Isolated organs	Intact but isolated tissue and vascular system Controlled environmental and exposure conditions	Donor organism still required Time-consuming and expensive No intact organismic responses Limited duration of viability
Cultured cells	No intact animals directly involved Ability to carefully manipulate system Low cost Ability to study a wide range of variables	Instability of system Limited enzymatic capabilities and viability of system No (or limited) integrated multicell and/or organismic responses
Chemical/biochemical systems	No donor organism problems Low cost Long-term stability of preparation Ability to study a wide range of variables Specificity of response	No *de facto* correlation to *in vivo* system Limited to investigation of a single defined mechanism
In silico (computer) simulations	No animal welfare concerns Speed and low per-evaluation cost	May not have predictive value beyond a narrow range of structures Expensive to establish

Table 4 Possible interpretations when *in vitro* data do not predict results of *in vivo* studies

Chemical is not absorbed at all or is poorly absorbed in *in vivo* studies
Chemical is well absorbed but is subject to 'first-pass effect' in the liver
Chemical is distributed so that less (or more) reaches the target tissue than would be predicted on the basis of its absorption
Chemical is rapidly metabolized to an active or inactive metabolite that has a different profile of activity and/or different duration of action than the parent drug
Chemical is rapidly eliminated (e.g., through secretory mechanisms)
Species of the two test systems used are different
Experimental conditions of the *in vitro* and *in vivo* experiments differed and may have led to different effects than expected. These conditions include factors such as temperature or age, sex, and strain of animal
Effects elicited *in vitro* and *in vivo* by the particular test substance in question differ in their characteristics
Tests used to measure responses may differ greatly for *in vitro* and *in vivo* studies, and the types of data obtained may not be comparable
The *in vitro* study did not use adequate controls (e.g., pH, vehicle used, volume of test agent given, and samples taken from sham-operated animals), resulting in 'artifacts' of methods rather than results
In vitro data cannot predict the volume of distribution in central or in peripheral compartments
In vitro data cannot predict the rate constants for chemical movement between compartments
In vitro data cannot predict the rate constants of chemical elimination
In vitro data cannot predict whether linear or nonlinear kinetics will occur with specific dose of a chemical *in vivo*
Pharmacokinetic parameters (e.g., bioavailability, peak plasma concentration, and half-life) cannot be predicted based solely on *in vitro* studies
In vivo effects of chemical are due to an alteration in the higher order integration of an intact animal system, which cannot be reflected in a less complex system

weight assigned to these advantages will differ depending on the information required and how it is to be used.

Can the proper tests be selected, especially when a decision must be made between using with an existing test system or adopting a new one? What are the available options?

The division between test system models is more complex than *in vivo* and *in vitro*, of course. There is a range of options under *in vitro*, each with its own advantages and disadvantages, as shown in **Table 3**. Each of these levels will need to be considered.

It should be noted that, in addition to potential advantages, *in vitro* systems *per se* also have a number of limitations that can contribute to there not being acceptable models. Findings from an *in vitro* system that either limit their use in predicting *in vivo* events or make them totally unsuitable for the task include there being wide differences in the doses needed to produce effects or differences in the effects elicited. Some reasons for such findings are detailed in **Table 4**.

At the same time, there are substantial potential advantages in using the *in vitro* system. The scientific advantages of using cell or tissue culture in toxicological testing are isolation of test cells or organ fragments from homeostatic and hormonal control, accurate dosing, and quantitation of results. It is important to devise a suitable model system that is related to the mode of toxicity of the compound. Tissue and cell culture has the immediate potential to be used in two very different ways by industry. First, it has been used to examine a particular aspect of the toxicity of a compound in relation to its toxicity *in vivo* (i.e., mechanistic or explanatory studies). Second, it has been used as a form of rapid screening to compare the toxicity of a group of compounds for a particular response. Indeed, the pharmaceutical industry has used *in vitro* test systems in these two ways for years in the search for new potential drug entities. The extension of these approaches to safety assessment is a much more recent occurrence.

Mechanistic and explanatory studies are generally called for when a traditional test system gives a result that is either unclear or for which the relevance to the real-life human exposure situation is unclear. *In vitro* systems are particularly attractive for such cases because they can focus on very defined single aspects of a problem or pathogenic response, free of the confounding influence of the multiple responses of an intact higher-level organism.

See also: Ames Test; Analytical Toxicology; Animal Models; Dominant Lethal Tests; Dose–Response Relationship; Host-Mediated Assay; *In Vitro* Test; *In Vivo* Test; Mouse Lymphoma Assay; Toxicity, Acute; Toxicity, Chronic; Toxicity Testing, Irritation; Toxicity Testing, Modeling; Toxicity, Subchronic; Toxicity Testing, Validation.

Further Reading

European Commission, Institute for Health and Consumer Protection, European Centre for the Validation of Alternative Methods (ECVAM) (2002) Alternative (non-animal)

methods for chemical testing: Current status and future prospects. A report prepared by ECVAM and the ECVAM Working Group on Chemicals. In: Worth A and Balls M (eds.) *ATLA* 30(Suppl. 1): 1–125.
Gad SC (1993) *In Vitro Toxicology*. New York: Raven Press.
Gad SC and Kapis MB (1993) *Non-Animal Techniques in Biomedical and Behavioral Research and Testing*. Ann Arbor, MI: Lewis.
Hakkinen PJ and Green DK (2002) Alternatives to animal testing: Information resources via the internet and world wide web. *Toxicology* 173: 3–11.
Huggins J (2003) Alternatives to animal testing: Research, trends, validation, regulatory acceptance. *ALTEX (Alternativen zu Tierexperimenten)* 20(Suppl. 1): 3–61.
Meyer O (2003) Review. Testing and assessment strategies, including alternative and new approaches. *Toxicology Letters* 140–141: 21–30.

Relevant Website

http://ecvam.jrc.cec.eu.int or http://altweb.jhsph.edu – Barratt MD, Castell JV, Chamberlain M, *et al*. (1995) The Integrated Use of Alternative Approaches for Predicting Toxic Hazard. European Commission, Institute for Health and Consumer Protection, European Centre for Validation of Alternative Methods (ECVAM). The Report and Recommendations of ECVAM Workshop 8. (Reprinted with minor amendments from *ATLA* 23: 410–429, 1995).

Toxicity Testing, Aquatic

Shayne C Gad

Introduction

Freshwater and marine environments contain complex ecosystems such as ponds, rivers, lakes, and estuaries. Each of these ecosystems contains unique biota that may be represented by several thousand species. These biota, both flora and fauna, are often exposed to a variety of toxicants, including those that result from anthropogenic activities, and in some cases, toxicity and environmental damage can occur. The study of these adverse effects on freshwater and marine biota and on the ecosystems that contain them is called aquatic toxicology.

Aquatic toxicology differs from mammalian toxicology in several aspects. The primary goal of aquatic toxicology is to assess the effect of toxicants on the many diverse populations and communities of plants and animals inhabiting marine and freshwater environments. The biota are cold-blooded and the physical and chemical characteristics of the aquatic environment have a significant effect on their sensitivity to toxicants. The aquatic test species of interest, unlike in mammalian studies, can be used directly. The objective of mammalian toxicology is to assess effects on humans, whose sensitivity to toxicants is less affected by their environment than that of aquatic organisms. The dose of the toxicant used in mammalian toxicology can be measured more accurately, the mechanisms of toxic action are better understood, and the test methods are more established.

Various species of aquatic life, particularly fish, have been used in toxicity experiments for more than 130 years. One of the earliest reported studies was conducted with fish in 1863 and the first proposed standard test species was the goldfish in 1917. Toxicity tests have been conducted with increasing frequency since the 1960s due to the numerous environmental regulations that have been enacted and the increasing availability of standardized test methods, the first of which were published in 1960 for animal test species and in 1970 for algae.

Many test methods are available for aquatic toxicity testing (**Table 1**). They differ in cost, precision, complexity, and the skill needed to conduct them. Nevertheless, their objectives are similar. They are conducted to determine the relative potency among chemicals and the relative susceptibility among different species and life stages and to identify other variables that influence the overall outcome of exposure. Toxicity tests are usually conducted to meet regulatory guidelines for the use and discharge of

Table 1 Available laboratory testing methods for aquatic toxicology

Single-species tests
Trout
Fathead minnows
Daphnia
Bluegills
Algae
Multispecies tests
Experimental streams
Ponds
Microcosms
Bioconcentration tests
Effluent toxicity tests
Sediment toxicity tests
Phytotoxicity tests
Algae
Vascular plants
Duckweed

commercial chemicals such as pesticides. In addition, toxicity results are used to derive national water quality standards to protect aquatic life and to determine the environmental effects of municipal and industrial effluents.

Aquatic toxicologists do not use all the available toxicity tests for any single toxicant. Instead, a tiered approach is used to provide a systematic and comprehensive process for deriving the toxicity data needed to assess the environmental hazard of a chemical. This approach consists of conducting short-term screening tests prior to using predictive studies that are more complex and time-consuming. This sequential evaluation provides an efficient use of resources and tends to eliminate unnecessary testing. The decision points and testing phase depend on the quality and quantity of data needed for the test substance of interest. The types of toxicity tests used in the tiered approach are discussed briefly.

Single-Species Toxicity

Tests: Methodologies

There are two basic types of aquatic single-species toxicity tests: acute and chronic. Acute toxicity tests have been the 'workhorse' of aquatic toxicologists for many years. These tests are relatively simple, take little time, and are cost-effective. A large historical database exists for many chemicals and effluents. Acute toxicity tests are most often used to quickly screen toxicity or to determine the relative sensitivities of different test species. Mortality is the effect monitored during the test duration of 48 h (invertebrates) or 96 h (fish). In a typical acute toxicity test, 5–10 organisms are exposed under static conditions in glass test beakers to five test concentrations. A control is included. The experiments with test concentrations and control are conducted in triplicate. Daily observations are made on survival, and dead organisms are removed.

At test termination, the concentration that kills 50% of the test organisms (LC_{50} value) is determined using probit analysis or graphical interpolation. Unlike in chronic toxicity tests, there is no test solution renewal, the organisms are unfed, and there is no analytical verification of the test concentrations. Furthermore, cumulative, chronic, and sublethal effects of a chemical usually are not evaluated in acute toxicity tests, although frequently behavioral changes and lesions caused by a chemical can be determined.

Chronic toxicity tests are more complex and time-consuming than acute studies and for these reasons are conducted less frequently. The methodologies for these tests differ considerably, unlike for acute tests, because they are designed for the specific life histories of the various test species. Chronic toxicity tests may be for a full-life cycle (egg–egg), partial-life cycle (embryo/larval), and partial-life history (egg–death). Full-life cycle tests are uncommon with fish due to the long durations that are necessary (1 or 2 years). Partial-life cycle tests with fish can be as short as 7 days or as long as 60 days. The early life stage of fish (embryo/larva) is usually the most sensitive period in a fish's life cycle and, consequently, partial-life cycle tests are used as surrogates for the full-life cycle studies. Chronic tests may be conducted for more than one complete life cycle if algal and invertebrate species are used since their life cycles are shorter than those of fishes. Lethal and sublethal effects are monitored in chronic toxicity studies, and these effects include changes in growth, reproduction, behavior, physiology, and histology.

Toxicity tests may be static, continuous-flow, or static renewal based on the toxicant dosing technique. Static and continuous-flow procedures are more widely used in toxicity tests conducted with pure chemicals and animal test species. Chronic toxicity tests conducted with effluents are usually static renewal, and those with algae are static. There is no change or renewal of the test substance with dilution water in a static test. This design is the simplest and least expensive; however, the toxicant concentrations may decrease due to adsorption and biodegradation. The test solutions and dilution water are renewed periodically, usually daily in a static-renewal test. In a continuous-flow test, the dilution water and test substance are continuously or intermittently renewed. The exposure concentrations remain fairly constant and dose–response relationships can be well defined.

A variety of aquatic toxicity test methods have been published for single species and several have been standardized through the efforts of such organizations as the American Society for Testing and Materials, the US Environmental Protection Agency, the American Public Health Association, and the Organization for Economic Co-operation and Development. Test method development is an ongoing process, however, which continues to increase the efficiency of these methods and often results in alternative study designs.

Experimental Conditions

In general terms, toxicity tests are conducted in a laboratory or a room controlled for light and temperature. The test solutions containing the test species are monitored for pH, temperature, dissolved oxygen, and water hardness. Daily observations on lethal and

sublethal effects are made, and several calculations, such as the LC_{50} value, the highest no-observed-effect concentration (NOEC), and the lowest-observed-effect concentration (LOEC), are determined based on the most sensitive effect parameter of interest. Although toxicity tests have similarities, as discussed later, variations among test animals, instrumentation, and methods influence the outcomes and utility of the assessments.

Test Chambers

The types of test chambers used in toxicity tests depend on the test species. Various sizes of beakers, aquaria, jars, bowls, and petri dishes have been used. The test chambers usually are constructed of material such as glass, Teflon, and certain plastics that minimize leaching of toxicants and adsorption of the test substance.

Test Concentrations

Xenobiotic concentrations used in an acute toxicity test are based routinely on results obtained from a pretest or range-finding test. The test concentration range for a chronic test is based on the results of an acute test conducted prior to the chronic test. There are no standard guidelines for conducting these preliminary tests. Generally, 5–10 organisms are exposed to several test concentrations, which are usually an order of magnitude apart. The dilution water and exposure conditions (i.e., water temperature, hardness, and pH) in range-finding tests usually are similar to those in the definitive test.

The test substances used in toxicity tests have been in most cases pure chemical compounds and municipal and industrial effluents. However, toxicity tests are being conducted more frequently with dredged soil materials (prior to ocean disposal), hazardous waste leachates, and contaminated sediments due to increasing regulatory concern for their potential environmental impacts. The test organisms are exposed in the definitive test to give concentrations chosen in a geometric progression. The test concentrations and control are replicated at least threefold. The test compound is added to the dilution water, which may be well water, reconstituted water, dechlorinated tap water, uncontaminated river water, and natural or artificial seawater. The dilution water is well aerated and undesirable organisms are removed before use.

An organic solvent is used to dissolve substances with minimal water solubility. Several have been used, including triethylene glycol, dimethyl sulfoxide, acetone, and dimethyl formamide. The LC_{50} values for these solvents are between 9000 and 92 500 $mg\,l^{-1}$. The concentration of the solvent in the test water should not exceed 0.5 $ml\,l^{-1}$ or should not be more than 1/1000 of the LC_{50} value of the solvent. When an organic solvent is used, a solvent control is included in the study.

Toxicant delivery systems are used to deliver, on a once-through basis, the various test concentrations to the test chambers in continuous-flow toxicity tests. The serial proportional diluter is the most common design used to mix the dilution water with the test substance to produce the desired test concentrations. The construction materials in toxicant delivery systems, like those for the test chambers, should not be rubber, certain plastics, or metallic materials.

The test concentrations are confirmed analytically during chronic toxicity tests. Analyses are performed at least weekly for each test concentration and control for tests of 7 days' duration or longer. In tests of shorter duration, analyses usually are conducted on alternate days. Analytical verification of the test concentrations in range-finding and acute toxicity tests is seldom done, and the results from these tests generally are based on nominal concentrations.

Test Species

Historically, animal test species have been used more frequently than plant species and freshwater species more frequently than marine species. These trends can be seen in **Table 2**.

Most toxicity tests are conducted with single cultured test species such as those listed in **Table 3**. The more commonly used freshwater species, particularly

Table 2 Types of tests and test species used in deriving toxicity data for submissions under the Toxic Substance Control Act Section 4 as of 1988

Test type	*Species*[a]	*Number of tests*
Acute toxicity	Fathead minnow (F)	37
	Rainbow trout (F)	27
	Sheepshead minnow (M)	12
	Daphnids (F)	23
	Midge (F)	12
Partial-life cycle	Fathead minnow (F)	1
	Rainbow trout (F)	7
Full-life cycle	Daphnids (F)	20
	Mysid shrimp (M)	5
Phytotoxicity	Alga (F)	23
	Alga (M)	3
Bioconcentration	Bluegill (F)	1
	Fathead minnow (F)	1
	Rainbow trout (F)	12
	Mussel (M)	1
	Oyster (M)	2

[a] F, freshwater; M, marine.

in tests used for regulatory compliance, are fathead minnows (*Pimephales promelas*), several daphnid species (*Daphnia magna* and *Ceriodaphnia dubia*), and green algae (*Selenastrum capricornutum*). Common marine species are sheephead minnows (*Cyprinodon variegatus*), mysid shrimp (*Mysidopsis bahia*), and a diatom, *Skeletonema costatum*.

The species in **Table 3** were selected based on several criteria, primarily ease of culture, commercial availability, and size. The test species are acclimated for a specific period of time prior to testing to eliminate diseased organisms. Generally, a minimum of 10 animals are exposed in static and static-renewal tests and 20 in a flow-through test for each test concentration and control. The recommended loading density for the test species is between 0.5 and $0.8\,g\,l^{-1}$ in static tests and between 1 and $10\,g\,l^{-1}$ in continuous-flow through tests.

Table 3 Freshwater and marine species used in toxicity tests

Freshwater	*Saltwater*
Fish	Fish
Salmo gairdneri (rainbow trout)	*Cyprinodon variegatus* (sheepshead minnow)
Salvelinus fontinalis (brook trout)	*Fundulus heteroclitus* (mummichog)
Ictalurus punctatus (channel catfish)	*Menidia beryline* (silverside)
Pimephales promelas (fathead minnow)	*Gasterosteus aculeatus* (threespine stickleback)
Lepomis macrochirus (bluegill)	*Leiostomus santhurus* (spot)
Carassius auratus (goldfish)	Invertebrates
Invertebrates	*Acartis tonsa* (copepod)
Daphnia magna (daphnid)	*Neanthes* sp. (polychaeta)
Daphnia pulex (daphnid)	*Callinectes* sp. (crab)
Ceriodaphnia dubia (daphnid)	*Penaeus* spp. (pink shrimp)
Gammarus lacustris (amphipod)	*Palaemonetes* spp. (grass shrimp)
Chrionomus sp. (midge)	*Crassostrea virginica* (oyster)
Physa integra (snail)	*Arvacia punctulata* (sea urchin)
Chambarus sp. (crayfish)	Plants
Plants	Algae
Algae	*Skeletonema costatum* (diatom)
Selenastrum capricornutum (green)	*Thalassiosire pseudonana* (diatom)
Chlorelia vulgaris (green)	*Champia parvula* (red)
Microcystis aeruginosa (blue green)	
Navicula spp. (diatom)	
Vascular	
Lemna minor (duckweed)	
Lemna gibba (duckweed)	
Myriophyllum spicatum (water milfoil)	
Ceratophyllum demersum (coontail)	

Reference toxicants often are used to determine the 'health' of the test species. There is no widely used reference toxicant; several that have been used include dodecyl sodium sulfate (anionic surfactant), sodium chloride, sodium pentachlorophenol, and cadmium chloride.

Sensitivity is a criterion that is used in the choice of a test species. The sensitivity of the species in **Table 3** relative to one another as well as to indigenous flora and fauna in the ecosystem is a matter of contention. There is no single test species and no group of test species consistently most sensitive to toxicants or most reliable for extrapolation to all other organisms. Most toxic effects reported for a variety of test substances have been species-specific. Therefore, acute toxicity tests are conducted first with a variety of freshwater and marine test species to determine the most sensitive plant and animal. These sensitive species then are used in all subsequent chronic testing.

Calculations

The results of acute toxicity tests are reported as the LC_{50} and EC_{50} (concentration that reduces growth 50%) values and their 95% confidence intervals. Probit analysis is the most commonly used statistical method to determine LC_{50} values. Graphical interpolation can be used to estimate the LC_{50} value where the proportion of deaths versus the test concentration is plotted for each observation time.

The NOEC and the LOEC are the usual calculations reported from chronic toxicity tests. The NOEC is the highest concentration in which the measured effect is not statistically different from that of the control. The LOEC is the lowest concentration at which a statistically significant effect occurred. These concentrations are based on the most sensitive effect parameters, that is, hatchability, growth, and reproduction. The statistical procedure for these calculations combines the use of analysis of variance techniques and multiple comparison tests. In some cases, the maximum acceptable toxic concentration (MATC) is reported from chronic toxicity results. The MATC is a concentration (x) that is within the range of the NOEC and LOEC: $\text{NOEC} \geqslant x < \text{LOEC}$. The first-effect concentration can be expressed as the geometric mean of the two terms.

Variability Precision

Toxicity tests conducted with freshwater and marine species are considered relatively precise and reliable based on current information concerning

interlaboratory and intralaboratory comparisons of toxicity results. Generally, the LC_{50} values from acute toxicity tests conducted under similar experimental conditions vary less than threefold. This has been observed for metals, effluents, reference toxicants, and different organic compounds. Coefficients of variation (CV) for acute and chronic toxicity tests conducted with daphnic species and chemicals and effluents are between 27% and 39%. The CV values for several reference toxicants and acute daphnic studies ranged between 10% and 72% and from 47% to 83% for chronic toxicity tests with algae.

Multispecies Toxicity Tests

The results of the 'traditional' acute single-species toxicity tests conducted in the laboratory cannot be used alone to predict effects on natural populations, communities, and ecosystems. The cultural species in laboratory tests are different from those in most ecosystems. Conditions such as the size of the test species, its life stage, and nutritional state can have an effect on toxicity. Furthermore, the experimental conditions in laboratory tests cannot duplicate the complex interacting physical and chemical conditions of ecosystems, such as seasonal changes in water temperature, dissolved oxygen, and suspended solids. In addition to these environmental modifying factors, aquatic life is usually exposed simultaneously to numerous potential toxicants (mixtures). Although the toxicities of binary and ternary mixtures have been evaluated for some chemicals in laboratory toxicity tests, the resultant information has predictive limitations.

Because of the deficiencies of single-species toxicity tests, alternative approaches are being evolved to address the structural and functional processes of an ecosystem. Multispecies tests include the use of laboratory microcosms, outdoor ponds, experimental streams, and enclosures. There are no standardized procedures for these tests. They are conducted with plant and animal species obtained from laboratory cultures and biota collected from natural sources. They can be conducted indoors or outdoors. The toxic effects, in addition to those used for single-species tests, are determined for structural parameters, such as community similarity, diversity, and density, and for functional parameters, such as community respiration and photosynthesis. Effects on these parameters are reported as the NOEC and LOEC.

Sediment Toxicity Tests

In the past, toxicity tests have been conducted primarily with water column-dwelling or planktonic organisms, with the objective of controlling water pollution. However, it has been realized that sediments act as 'reservoirs' for chemicals that can adversely affect benthic aquatic life and, at times, also affect planktonic life. This concern has led to the development of sediment quality criteria to protect aquatic life. Test methods have been developed to support the derivation of these criteria and to support other related regulatory activities (e.g., Superfund site evaluations and ocean disposal of dredged materials).

Most sediment toxicity tests have been conducted in the laboratory with single species of freshwater and marine benthic organisms such as amphipods and midges, but in some cases planktonic species also have been used. Most tests conducted to date have been acute and have been of 10 days' duration or less. Sediment toxicity tests are conducted with the solid phase or the pore water (interstitial water). Methods have been published describing the collection and preparation techniques.

Test guidelines for marine sediment and freshwater sediment also have been reported. Standardized methods are available for freshwater invertebrates and freshwater and marine amphipods.

The availability of reliable test methods for contaminated sediments is relatively recent and the test method development process continues. A variety of issues remain to be solved before these types of studies will be considered as effective as those with planktonic species. Among the more important of these issues are validation of the single-species test results and determination of variations in species sensitivity.

Effluent Toxicity Tests

Toxicity tests are used in the National Pollutant Discharge Elimination System, permitting one to determine the toxicity effects of municipal and industrial effluents and storm water overflows on aquatic life. A summary of the experimental conditions in several of the available test methodologies appears in **Table 4**. The methodologies differ slightly from those used for pure chemicals. For example, the choice of the dilution water and the effluent collection technique are important considerations. In most cases water collected from the receiving water above the outfall is used for dilution, and composite samples of effluent are used. The test species – algae, invertebrate, and fish – are usually exposed to five effluent dilutions for 4–7 days. The tests are static renewal except those for algae, which are static. The calculations reported are the LC_{50} value, the NOEC, and the LOEC, which are expressed as percentage of effluent. The cause(s) of toxicity in the effluent – that is, specific effluent constituents – can be identified using comparative toxicity testing and chemical fractionation techniques.

Table 4 Comparison of several experimental variables in chronic toxicity tests conducted with effluents

Test type	*Duration (days)*	*Number of test concentrations*	*Test species*	*Age of the organism*	*Total test species exposed*	*Number of replicates*	*Temperature (°C)*	*Light intensity ($mE\,m^{-2}\,s^{-1}$)*
Static renewal	7	5	Fathead minnow (freshwater fish)	<24 h	30–60	3–4	25±1	10–20
Static renewal	7	5	Sheepshead minnow (marine fish)	<24 h	30–60	3–4	12±2	10–20
Static renewal	7	7	*Ceriodaphnia dubia* (freshwater invertebrate)	<24 h	10	10	25±1	10–20
Static renewal	7	5	*Mysidopsis bahia* (marine invertebrate)	<24 h	40	8	25–27	10–20
Static	4	5	*Selenastrum capricornutum* (freshwater; green alga)	4–7 days	1 × 104 (initial)	3	25±1	86±8.6
Static	4	5	*Skeletonema costatum* (marine; diatom)	4–7 days	2 × 104 (initial)	3	20±2	60±6

The freshwater invertebrate, *C. dubia*, is a test species commonly used in effluent toxicity evaluations. The *C. dubia* used in a study are obtained from a laboratory culture. Effluent collected within 72 h from the source is used after temperature acclimation. The static-renewal test usually is conducted in a laboratory located off-site from the effluent source but the tests may be conducted on-site using a mobile bioassay facility. The test is conducted at 25°C, 10–20 $mE\,m^{-2}\,s^{-1}$, and under a photoperiod of 16 h light/8 h darkness. Five test concentrations are used that include undiluted effluent (100%) and four dilutions such as 50%, 25%, 12%, and 6%. The effluent is diluted with either a high-quality laboratory water or water collected from the receiving water above the effluent outfall. The control is composed of 100% dilution water. For each test concentration and the control, ten 30 ml plastic test chambers containing 15 ml of the test solution are used. Each test chamber contains one daphnid and daily observations on mortality and production of young are made during the 7 day test. The organisms are fed daily a combination of yeast, trout chow, and algae. Surviving organisms are transferred daily to renewed test solutions. The NOEC and LOEC values are determined based on the adverse effects on survival and reproduction occurring during the 7 day test.

Phytotoxicity

The majority of aquatic toxicity tests have been conducted with animal test species since they once were thought to be more sensitive than plants. This generalization is not supported technically, based on a review of the data for most toxicants. Nevertheless, only recently have phytotoxicity tests been conducted routinely with a limited number of species of algae and vascular plants.

A variety of test methods are available to determine the phytotoxic effects of chemicals and effluents. The freshwater algal species most frequently used has been the microalga, *S. capricornutum*, for which a relatively large database exists. Marine species used include the diatom, *S. costatus*, and the red macroalga, *Champia parvula*.

Acute toxicity tests seldom are conducted with algae. The chronic toxicity tests conducted with microalgae are for 3 or 4 days' duration although exposures can be for less than 1 day if effects on photosynthesis are measured. These static exposures occur in a liquid nutrient-enriched medium under conditions of controlled pH, temperature, and light. Inhibitory and stimulatory effects on population growth are monitored during the exponential growth phase. Five test concentrations and a control are included in each study. The most common calculation reported is the 96 h EC_{50} value but algistatic (that completely stops growth) and algicidal (lethal) concentrations also have been reported. In addition, the SC_{20} (stimulatory) concentration is reported if growth stimulation is observed. The SC_{20} value represents the concentration that increases algal growth 20% above that of the algal population in the control.

Floating and rooted macrophytes are used less frequently in toxicity tests than algae. The

duckweeds, freshwater floating species, are more commonly used than most due to their small size and rapid growth. Several published methods are available describing their use, particularly *Lemna minor* and *L. gibba*. Tests with these species are usually of 4–14 days' duration, during which effects on frond number and chlorophyll content are monitored. The results are expressed as an EC_{50} value and the NOEC. The tests are conducted, as with algae, in a nutrient-enriched medium. The test chambers can be fruit jars, plastic cups, test tubes, and Erlynmeyer flasks. The key research issue that remains to be investigated before the duckweeds are more widely accepted as suitable test species is their sensitivity relative to that of other aquatic plant and animal test species.

The use of rooted macrophytes such as pondweeds (*Potamogeton* spp.), waterweeds (*Elodea* and *Hydrilla*), the water hyacinth (*Eichhornia crassipes*), coontail (*Ceratophyllum demersum*), and water milfoil (*Myriophyllum* spp.) in toxicity tests is less common than that of algae and duckweeds due to their large size and slow growth. There are no standard or commonly used test methods for these species. Consequently, there is a need for their development and validation. The experimental techniques that have been used vary considerably. Recently, seeds from aquatic macrophytic vegetation have been used to assess the toxicities of chemicals and effluents. These studies are usually of 4–7 days' duration, and the effect parameters are seed germination, root elongation, and early seedling growth. The use of whole-plant rooted macrophytes and their seeds in toxicity tests will increase in the future as sediment quality criteria to protect aquatic life and wetlands increase in regulatory importance. However, for this to occur, test method development and validation, as well as determination of species sensitivity, will be needed.

Bioconcentration

A bioconcentration study is conducted to derive information on the ability of an aquatic species to concentrate a toxicant in its tissues. This uptake and accumulation can be hazardous to the organism as well as to other aquatic life utilizing the test species as a food source. Bioconcentration tests are usually conducted with single chemicals and single species of algae, fish, and bivalve mollusks. A variety of fish have been used, including the fathead minnow, bluegill, rainbow trout, sheepshead minnow, and several species of oysters, scallops, and mussels.

There are several test designs that can be used to estimate the bioconcentration potential of a compound. Typically, one group of the test species is exposed to the toxicant for an uptake and depuration phase. A control is included in which the test species is not exposed to the toxicant. In assessing the concentration of the test chemical in the organism, the literature contains examples of measuring total residues and measuring only the parent compound, depending primarily on the methodology used. The uptake phase is usually for 28 days or until a steady state is attained. The depuration period lasts until the concentration in the test species is 10% of the steady-state concentration in the tissue. During both phases, the test water and test species are analyzed daily for the test chemical. All results from a bioconcentration study are based on measured concentrations. The uptake rate, depuration rate, and bioconcentration factor (BCF) typically are reported. The relevance of the BCF value to the survival of the organism and to ecosystem dynamics is an issue that has received and will continue to receive significant scientific attention.

See also: Analytical Toxicology; Biomarkers, Environmental; Ecotoxicology; Effluent Biomonitoring; Environmental Toxicology; Microtox; Photochemical Oxidants; Pollution, Water; Risk Assessment, Ecological.

Further Reading

Hammons AS (1981) *Methods for Ecological Testing*. Ann Arbor, MI: Ann Arbor Science.

Rand GM (1995) *Aquatic Toxicology: Effects, Environmental Fate and Risk Assessment*, 2nd edn. Washington, DC: Taylor and Francis.

Richardson M (1995) *Environmental Toxicology Assessment*. Bristol, PA: Taylor and Francis.

Toxicity Testing, Behavioral

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Introduction

Behavioral toxicology, a part of the larger domain of neurotoxicology, uses a wide range of methods to evaluate changes in behaviors of model organisms (in modern toxicology, largely rats and mice) as a means of identifying and studying adverse effects of chemicals on the nervous system. As such, behavioral toxicology tests should be considered functional testing of a complex organ system. Changes in behavior are believed to be sensitive indicators of exposures to substances that result in central nervous system (CNS) toxicity. Three important classes of behavioral neurotoxicants are metals, solvents, and pesticides.

Behavior may be defined as anything an organism does – any move an organism makes. Behavior includes all observable, recordable, or measurable activities of a living organism and reflects genetic, neurobiological, physiological, and environmental determinants and can be used in biomonitoring, the determination of no-observed-effect and lowest-observed-effect concentrations, and the prediction of hazardous chemical impacts on natural populations. The behavior of an organism at any moment is the result of the external environment, the past history of the organism, and the internal environment (e.g., biochemical or electrical processes and hormonal levels) within the organism. The behaviorist studies the functional relationship between the behavior of an organism and variables such as exposure to a chemical. An aspect of the environment that controls behavior in a functional manner is termed a stimulus. A unit of behavior, defined by the experimenter, is termed a response. There are two types of responses: respondent and operant. In addition, either type of response may be unconditioned (unlearned) or conditioned (learned).

Respondent behaviors include such actions as smooth muscle contraction, autonomic responses, glandular secretions, and elicited motor responses such as reflexes. Unlearned respondents are used frequently in observational batteries and include such measures as orienting to stimuli or reflex startle to intense stimuli. The famous experiment of Pavlov, in which dogs learned to salivate at the sound of a bell after numerous pairings of the bell with presentation of food, is an example of a conditioned response. Respondents are paired with an eliciting stimulus in a one-for-one relationship.

Operant responses, on the other hand, have no single eliciting stimulus but occur within the context of many environmental stimuli. The consequences of a certain behavior affect the probability that this behavior will be produced again. Locomotor activity is often given as an example of an unlearned operant because there is no attempt on the part of the experimenter to condition a particular type of response. Ventilatory responses are often some of the first prelethal symptoms exhibited by animals to environmental stressors. Continued, abnormal ventilatory behavior (i.e., rapid or shallow breathing, erratic breathing) can indicate physiological damage that may be irreversible. Such damage could eventually result in decreased survival, growth, or reproduction of the organism, or all of these.

An extremely powerful tool at the disposal of the behavioral toxicologist is that of operant conditioning. Operant conditioning takes advantage of the control that the immediate outcome of behavior has the subsequent frequency in determining of similar behavior. If the outcome of a particular behavior increases its frequency, it is termed a positive reinforcer (i.e., food). If it decreases the frequency, it is called negative reinforcer (i.e., shock). The great strength of this technique is that it may be used to teach a large variety of tasks with wide complexity. Questions can be asked about attention, learning, memory, sensory function, and general well being of the subject. Many techniques discussed in this chapter rely on the principles of operant conditioning. Behavioral assessment would be impossible without a thorough understanding of these principles on the part of the investigator. Behavioral changes are assessed by observational methods, operant techniques, learning and memory tasks or by a combination of these.

The National Institutes of Health have created a handbook, *Methods and Welfare Considerations in Behavioral Research with Animals*, to assist the Institutional Animal Care and Use Committee in the evaluation of protocols that employ various means to manipulate the behavior and health of laboratory animals. The report contains chapters on manipulation of access to food or fluids; experimental enclosures/physical restraint; pharmacological studies; aversive stimuli; social variables; ethological approaches; and teaching with animals. ASTM International has come up with *Standard Guide for*

Behavioral Testing in Aquatic Toxicology. Behavioral testing is often performed on fish, amphibians, and macroinvertebrates.

The behavior of organisms can be divided into motor function, sensory function, learning and memory, and performance on intermittent schedules of reinforcement. These classes are somewhat arbitrary, and virtually all behavioral tests measure more than one of these functions. For example, motor function affects almost all testing, intact sensory function is necessary for learning, performance on intermittent schedules most certainly has a learning component, and so forth. Often, different functions are not separable by one test or type of test; therefore, it is imperative to study several types of behavior to determine the function(s) that is affected. It should be pointed out that none of the procedures described in the following sections should be examined in isolation because all are part of a comprehensive investigation of the potential behavioral effects of a toxicant. Examples of changes monitored include changes in feeding, preference or avoidance, self-protection, social interactions, predation, competition, reproduction, activity level, leaning, performance, or competition.

The examples of tests presented in each section are certainly not exhaustive but were chosen because they are often used or because they promise to contribute substantially to the understanding of behavioral toxicity. The first step in a tier approach is a neurobehavioral observational screen, the tool of choice for initial identification of potentially neurotoxic chemicals. The use of such screens, other behavioral tests methods, or what are generally called clinical observations does, however, warrant one major caution or consideration. That is, short-term (within 24 h of dosing or exposure) observations are insufficient on their own to differentiate between pharmacological (reversible in the short term) and lexicological (irreversible) effects. To so differentiate, it is necessary to either use additional means of evaluation or have the period during which observations are made extended through at least 3 or 4 days.

Screening Batteries

As pointed out previously, one of the agenda that has emerged for behavioral toxicity involves the screening of new chemicals for potential neurotoxicity. The behavioral tests utilized for such purposes are often referred to as apical tests because they require the integrated function of several organ systems, including the nervous system. Such batteries typically have included two behavioral components; a functional observational battery (FOB) and an evaluation of motor activity. **Table 1** depicts the component tests of the FOB, which include an array of measures of both unconditioned operant and respondent behaviors. Such batteries have been shown to exhibit utility for screening potential neurotoxicity, that is, hazard identification and elaboration. Those components of the FOB directed to cholinergic functions exhibited sensitivity to the effects of the cholinesterase inhibitor, carbaryl, whereas few such signs of cholinergic disturbances were evident in the presence of the pesticide chlordimeform which does not inhibit cholinesterase.

Motor activity is frequently included in screening batteries both as a measure of motor function and as an apical test (see **Table 1**). Motor activity, generally considered an unconditioned behavior, exists at some baseline level and is a complex behavior that includes numerous components such as ambulation, rearing, grooming, and sniffing. As discussed previously, toxicants may alter motor activity by affecting any or all of its component behaviors.

From the standpoint of screening and hazard identification, a notable point relating to the interpretation of data from FOB and motor activity studies is whether the effects observed in response to toxicant exposure represent a direct effect of the toxicant on the nervous system or are secondary to changes in other systems since such apical tests rely on the functional integrity of multiple systems. In some circumstances, the fact that the toxic effect is ultimately expressed in behavior may minimize the importance

Table 1 Example of behavioral procedures included in a functional observation battery

Home cage and open field	*Manipulative*	*Physiological*
Posture	Ease of removal	Body temperature
Convulsions	Ease of handling	
Palpebral closure	Palpebral closure	Body weight
Lacrimation	Approach response	
Piloerection		
Salivation	Touch response	
Vocalizations	Finger-snap response	
Time to first step		
Rearing	Tail-pinch response	
Urination		
Defecation	Righting reflex	
Gait	Catalepsy	
Arousal	Hindlimb foot splay	
	Forelimb grip strength	
	Hindlimb grip strength	

of the direct versus indirect source of the effect. It also should be noted in the interpretation of toxicant-induced changes in FOBs and motor activity measures that the concurrent presence of body weight loss or decline in food or water intake does not necessarily indicate that the behavioral changes are the result of malaise or sickness as these measures may change independently of each other. In addition to those procedures mentioned in this entry a standard test battery often includes:

- a simple neurological screen for basic sensory/motor function,
- open-field test for exploratory activity and anxiety-related traits,
- light–dark exploration box for anxiety-related traits,
- an accelerating rotarod test for motor coordination and skill learning,
- prepulse inhibition for sensorimotor gating,
- acoustic startle habituation for sensorimotor adaptation,
- contextual and auditory conditioned freezing for conditioned fear learning,
- Morris water task for spatial learning and memory,
- the hot-plate test for analgesia-related responses,
- locomotor activity testing,
- elevated plus maze,
- water maze escape learning task,
- cocaine-stimulated locomotor activity,
- alcohol consumption, and
- forebrain commissures.

Motor Function Testing

Deficits in motor function are frequently produced in humans as a result of their toxic exposure to a chemical. Heavy metals such as mercury, lead, and manganese; insecticides such as chlordecone (Kepone) or organophosphorus compounds; and air pollutants such as carbon disulfide all produce changes in motor function. Gross assessment of motor function should be performed as part of an initial toxicity screen. Batteries that include observational assessment of muscle tone, body posture, equilibrium, and gross coordination have been suggested. The next level of testing includes such techniques as ability to stay on a rotating rod or quantification of hindlimb splay. The former requires an automated apparatus as well as animal training and practice in order to reduce test variability to acceptable levels. The latter technique is simpler, involving the placement of ink on the paws of rodents after which they are dropped from a specific height. Quantification of hindlimb splay does not require training of the animal and is fast and easy. Another screening procedure for assessment of neuromotor function requires a rodent grasp a bar attached to a strain gauge after which the animal is pulled on manually until it lets go. Assessment of swimming ability is also suitable for incorporation into screening tests. The rodent is placed in a pool of water and such measures as swimming movement, position in the water, and ability to keep the head above water are assessed. These analyses may reveal motor deficits that are not apparent during locomotion on land.

Little work has been focused on more sophisticated tests for assessing neuromuscular function. One promising procedure employs operant techniques to train an animal to depress a lever within a specific force band and time period, thus allowing assessment of the effects of toxic agents on fine motor control or strength.

The test that probably is used most extensively in screening for nervous system toxicity is locomotor activity, in large part because no training is required and activity can be measured rapidly. Locomotor activity represents the functional output of many systems of the body, including but certainly not exclusively motor systems. In addition, although such measurements may appear straightforward, there are many variables that must be considered. Motor activity is not a single activity but consists of many acts, such as horizontal and vertical movement, sniffing, rearing, grooming, and scratching. With some types of measuring devices, even tremor may be monitored. There are, therefore, many methods of monitoring, including scoring the classes of movement by observation, measuring horizontal movement only, measuring vertical displacement with devices that gauge force generated against the floor, and combinations of these measurements. Even within a class of automated devices, there is large variability in the configuration of each apparatus and in the method of measurement. With different kinds of apparatuses, different behaviors can be measured.

When a toxicant is introduced, activity may increase, decrease, or remain unchanged depending on choice of apparatus, age of the animal, the relative novelty and complexity of the environment, and many other variables. Although a change in an animal's activity as a result of its exposure to a toxicant indicates a change in the function of its nervous system, interpretation is not straightforward. The change can be due to the toxicant's primary effect on nervous system function or to its effect on some other system that results in a secondary effect on nervous system function. Certainly, extrapolation from activity measurements in rodents to such phenomena as 'hyperactivity' in children is unwarranted,

both because of lack of consistency in the experimental work and because such syndromes in humans do not consist exclusively, or necessarily, of increases in motor activity.

Sensory Function Testing

Sensory disturbances often result from human exposure to toxic agents, both as vague symptoms reported by the patient and as clearly demonstrable deficits in sensory function. Deficits in visual, auditory, and tactile functions have been reported for a variety of toxicants, including metals (methylmercury and lead), acrylamide, solvents, and pesticides. A variety of techniques, from very simple to extremely sophisticated, have been utilized to assess sensory function in animals exposed to toxicants. Probably the grossest of these is the orienting response, which consists of observing whether the animal turns toward a crude stimulus (e.g., click, light, or touch). Such a procedure is subjective, nonspecific, and insensitive and indicates only the possibility of gross sensory impairment. The auditory startle reflex and discrimination learning tests are often viewed as tests of sensory function. However, there are many other systems involved in these tests; therefore, sensory effects may not be discriminable from motor effects, learning and memory, and attention abilities.

An extremely promising technique for sensory system evaluation is modulation of reflex startle by presentation of a low-intensity stimulus immediately prior to a high-intensity stimulus that elicits the startle response. Such a technique may be used to estimate sensory threshold, and sensory deficits may be differentiated from nonsensory, such as motor, deficits. This technique is, therefore, specific and reasonably sensitive. It has the advantage of being inexpensive and rapid and requires no training of the animal.

Operant training of an animal allows a very detailed evaluation of sensory function. Such techniques are time-consuming and sometimes expensive, but they are useful for careful characterization of toxicant effects for which there is good evidence of sensory impairment. The species chosen for testing must have sensory function as similar to humans as possible. For visual system testing, for example, the rodent is usually not an appropriate model because its visual system differs in fundamental ways from that of humans.

Animals can be trained to report reliably and in great detail about their sensory perception. This is accomplished through 'psychophysical' techniques; that is, sensory function is determined by behavioral means. Such methodology is appropriate for determination of no-effect levels and for detailed characterization of toxic effects. Conditioned suppression is a useful technique for estimating sensory thresholds. A steady baseline rate of responding (such as a lever pressing or licking) is established by use of an intermittent schedule of reinforcement. A test stimulus is presented to an animal several times during its ongoing behavior and signals a specific latency (usually 2 or 3 min) to an unavoidable electric shock. The animal decreases its rate of response (suppresses) during the stimulus in anticipation of the shock, which indicates that the animal detects the stimulus. This technique can be employed to estimate threshold and to detect changes in threshold produced by a toxicant.

Stebbins characterized the thresholds for detection of sound over the range of frequencies normally detectable in the monkey and the effect of an ototoxic agent on these thresholds. This was done by training the monkey to keep its hand in contact with a sensor until it detected the onset of a tone and to break its contact upon detection of the tone. Intensity of the tone was then varied for each frequency tested to determine the intensity at which the monkey was unable to detect the tone. Stebbins was thus able to follow the development of hearing loss produced by an ototoxic antibiotic, from initial high-frequency loss to later low-frequency loss. These changes in hearing in the monkey were correlated with the pattern of receptor loss in the inner ear.

A psychophysical procedure was also used to determine the spatial visual function of monkeys exposed chronically to methylmercury but showed no overt signs of poisoning. In this experiment, the monkey faced two oscilloscopes, one blank and one displaying vertical bars. The monkey had access to two levers, one corresponding to each oscilloscope. The task was to respond on the lever corresponding to the scope on which the bars appeared. The oscilloscope displaying the bars varied randomly from trial to trial. The frequency and darkness of the bars was varied in a systematic manner, allowing a determination of the spatial visual function of each monkey. Monkeys exposed to methylmercury were found to have deficits of high-, but not low-frequency spatial vision. Similar behavioral techniques have been used to characterize visual and somatosensory impairment produced by acrylamide. Such studies demonstrate the power of operant techniques in detection of very subtle sensory deficits, which may be the only discernible effects of a toxicant at low-level exposure.

Learning and Memory Testing

Loss of memory and inability to concentrate are symptoms frequently reported as a result of human

exposure to toxicants such as polychlorinated biphenyls, solvents, methylmercury, and pesticides. Furthermore, developmental exposure may produce mental retardation or learning impairment. It is therefore of great value to test such abilities in animals as markers of toxic effect. There are many techniques available for assessment of learning and memory. Aside from gross screening procedures, this area has probably received the most attention from behavioral toxicologists. Techniques range in complexity from those appropriate for screening to characterization of specific deficits. A screening procedure that is often considered a test of learning is habituation, which is a progressive decrease in reactivity to repeated presentations of a stimulus. Reactivity can be measured in terms of response of the whole organism, as in startle or orienting, or in terms of habituation of a discrete reflexive response, such as blinking. Obviously, habituation must be differentiated from motor effects, fatigue, and sensory adaptation. It is a measure of gross integration of the nervous system and may not involve the higher centers. Often, an incremental repeated acquisition task is used to evaluate the effects of a potential toxicant on learning and a delayed matching to sample task to evaluate effects on memory.

A learned behavior that is obviously of adaptive advantage to an animal is its ability to avoid a substance that it ingested shortly before the onset of an illness or adverse effect. This conditional taste aversion can be used to measure toxicity, for example, by pairing a novel taste (a sugar treat, for example) with administration of a toxicant. If the animal feels ill soon afterward, it will avoid the novel substance in the future. This technique has proved to be sensitive to the effects of neurotoxic agents.

At the next level of sophistication, avoidance procedures (utilizing negative reinforcement) are frequently used. Passive avoidance procedures require the animal (rodent) to refrain from leaving a specific area in order to avoid a shock to the feet. Active avoidance requires the animal to move from a specific area at the onset of a cue in order not to be shocked. These procedures are greatly affected by the baseline level of arousal and ongoing motor activity of the animal. It may often be the case that a toxicant produces an effect on one and not on the other of these avoidance tests or affects the behavior in opposing ways, depending on whether the animal is more or less active than the control animal. These tests, therefore, are considered rather nonspecific.

Discrimination tasks have proved useful in detecting effects of toxicants on learning and memory. The procedure most often employed is termed a 'forced choice' because the animal is presented with two or more stimuli simultaneously and must indicate its choice by some operant response. These tasks are typically one of two types; spatial and nonspatial. With spatial discrimination, the animal must respond to a certain position (i.e., left) in order to be reinforced. A nonspatial task requires responding to a specific stimulus (pattern, color, or direction of a tone) regardless of position. Different operants may be utilized in discrimination testing. For rodents, mazes of various sorts are often employed, whereas for other species (as well as for rodents) operants besides locomotion are utilized.

Primates are often tested in a Wisconsin General Testing Apparatus. The monkey faces a panel on which stimuli are placed. A reinforcement, such as a raisin, is placed in a recessed well under the correct stimulus. The monkey's response consists of displacing one of these stimuli; if the choice is correct the reinforcement is collected. Automated apparatuses are used with all laboratory species. Typically, the response consists of pressing one of several available levers or push buttons in order to signal the choice. Levine developed a technique for rodents in which a photocell beam is interrupted with the nose as an operant. The technique requires no training by the investigator and may be used with young animals.

Discrimination tasks have proved to be sensitive to impairment resulting from exposure to lead. The difficulty of the task may have an important impact on the effects of a toxicant on performance.

Once the task is learned, a discrimination reversal paradigm provides additional information on the animal's learning ability. The previously correct stimulus becomes the incorrect one so that the animal is required to learn a response opposite from the one previously learned. The discrimination reversal paradigm may often be more sensitive to neurotoxicity than simply acquisition of discrimination tasks, as has been found in monkeys exposed to lead early in life.

There are several other means to test spatial orientation or memory that require little or no training of the animal. An apparatus appropriate for use with rodents is the radial arm maze. Typically, this maze consists of a central arena from which radiate a number of arms like spokes of a wheel. The end of each arm is baited with a reinforcement, and the animal simply has to find all the reinforcements within a certain period of time. The most economical strategy is to enter each arm only once. There obviously need not be a memory component to this task, depending on the strategy adopted by the animal (i.e., 'always turn left'). Similarly, motor impairment confounds this task because the number of

reinforcements collected in a specified time is the typical dependent variable. The neurotoxicant trimethyltin has been found to disrupt a rodent's ability to perform this test. A somewhat analogous task used for primates is the Hamilton Search Task. A row of boxes, each containing a reinforcement, is presented to the monkey. The monkey can collect the reinforcement from each box by lifting the lid; again, the most economical approach is to lift each lid only once. This test differs from the radial arm maze in that a delay is instituted between responses during which the boxes are withdrawn from the monkey's reach, thus making memory more likely a component of the performance. (It is possible to institute a delay in the radial arm maze as well, but this is most often not done.) Monkeys exposed to lead postnatally required more trials to learn to perform this task than did their controls.

There are several operant tasks that offer the opportunity to separate an animal's learning from its performance of a known task. Repeated acquisition is such a task and requires the animal to learn a new sequence of lever presses each session. The learning baseline may be more sensitive to disruption by a toxicant than the performance of an already acquired sequence.

A task that tests attention and short-term memory is matching to sample. Monkeys are most typically used for these tasks, although other species are also capable of learning them. In a nonspatial matching-to-sample task, for example, the animal is presented with a stimulus (color, pattern, or object) that is then withdrawn. Following this, a set of stimuli is presented, and the animal indicates which of these is identical in some dimension to the sample stimulus. Delays of various durations may be instituted between the presentation of the sample and test stimuli to test short-term memory. Such tasks have been found to be sensitive to effects produced by lead in monkeys who were exposed to it in early life.

Testing Using Intermittent Schedules of Reinforcement

Performance generated by intermittent schedules of reinforcement has played an important role in behavioral pharmacology and is proving a useful tool in behavioral toxicology. On an intermittent schedule, an animal is not reinforced for every response but for a number of responses according to certain 'rules'. Most intermittent schedules are based on reinforcing the organism as a function of the number of responses emitted, some temporal requirement for emission of responses, or a combination of these. For example, a fixed ratio (FR) schedule requires the animal to emit a fixed number of responses in order to be reinforced. A fixed interval (FI) schedule, on the other hand, requires that a certain fixed length of time elapse before a response is reinforced. Although only one response need be emitted at the end of the interval for reinforcement, the organism typically emits many responses during the interval. Interval schedules generally generate a lower rate of responding than do ratio schedules. The FI schedule generates a characteristic pattern of responding for which a variety of parameters may be analyzed. These parameters are potentially sensitive to disruption by psychoactive agents. Another schedule of some utility in behavioral toxicology is the differential reinforcement of low rate (DRL) schedule in which the animal is required to wait a specified time between responses in order to be reinforced.

Intermittent schedules may also be maintained by negative reinforcement, usually by a brief mild electric shock. The most popular of these is continuous or 'Sidman' avoidance in which each response postpones a shock by a fixed amount of time. By spacing its successive responses within this time interval, the animal may postpone shock indefinitely. This schedule is particularly useful as a comparison to behavior generated by positive reinforcement if a toxicant is suspected of producing anorexia. Simple intermittent schedules such as these have been used fairly widely in behavioral toxicology and have proved to be sensitive to the effects of a number of industrial and environmental toxicants.

Intermittent schedules of reinforcement can be combined to form more complicated schedules such as multiple schedules of reinforcement. For example, if FR and FI schedules are presented to an animal in succession during a single test session, the resulting multiple schedule is termed a multiple FR–FI schedule. Each component of the multiple schedule is independent and occurs in the presence of a different external discrimination stimulus that signals the schedule component in effect. Schedule components are typically presented in an alternating fashion, first one schedule and then the other; this allows the investigator to collect data on both types of behavior almost simultaneously. This schedule in particular has proved to be useful in detecting behavioral toxicity.

Multiple schedules offer the investigator an opportunity to study behavior controlled by different variables, which may be differentially sensitive to the effects of a toxicant. For example, toluene produced a decrease in test animals' response rate in the FR component and an increase in their response rate in the DRL component of a multiple schedule. Furthermore, the relative sensitivity of the two components was different. Similarly, the animals' response in the

FI component of a multiple FR–FI was sensitive to disruption by methyl γ-amyl ketone, whereas their response in the FR component was not. The FI component of the multiple FI–FR schedule was more sensitive to disruption in both monkeys and rodents who sustained developmental lead exposure.

Schedules of reinforcement may be used to monitor toxic effects other than or in addition to direct effects on the CNS. These may include peripheral nervous system toxicity or damage to some other organ systems resulting in general malaise or the animal's feeling 'sick'. For example, acrylamide, an organic solvent that produces a 'dying back' axonopathy, produced decreases in animals' FR response rate. The FR schedule typically produces high response rates and thus may be sensitive to unpaired motor function. Rats exposed to ozone decreased their responding on an FI schedule, which was interpreted as a decrease in their motivation as a result of the general discomfort produced by ozone.

Social Behavior Testing

Animals, particularly mammals, engage in a wide variety of social, sexual, and maternal (or paternal) behaviors that are multidimensional and extremely complex. Despite the obvious importance of social behavior in humans, very little research has been focused on the effects of toxicants on social interactions, and the utility of such interactions in behavioral toxicology is unknown. The reason for this may be the enormous number of variables, which necessitates focusing on only a few parameters to the exclusion of all others. Moreover, many of these behaviors are specific to certain species (e.g., grooming, pup retrieval, and submissive gestures), raising the question of the validity of extrapolation to human behavior.

Each of these components of behavior can be combined into an increasingly complex set of testing paradigms and, as such, then can be used to evaluate a distinct potential toxic event. An example of this is behavioral teratology.

Behavioral Teratology

Behavioral teratology is defined as a separate component of behavioral toxicology primarily in its focus on behavioral modifications resulting from toxic exposures during early development. In general, such studies track the outcome of such exposures over the postnatal and possibly into the juvenile and early adult stages of the life cycle. Outcome measures almost invariably include the development of physical landmarks and reflexes, and also generally include assessment of one or more behavioral functions. Often, attempts are made to evaluate multiple behavioral functions, such as motor function and activity, and sensory capabilities and learning in the same experiment. In addition, testing for species-specific behaviors, such as aggression, play, and vocalization, may be included.

Testing during infancy, postnatal, and juvenile periods of development sometimes requires modifications of procedures that are utilized with adults or even the development of new paradigms. In other cases, behavioral paradigms identical to those used in more mature subjects may be used, albeit with parametric modifications. One example of the former is a procedure that has been widely used in behavioral teratology studies as an assessment of olfactory and motor capabilities and is referred to as 'homing behavior', a behavior used by rat pups to locate the nest should it be displaced. In such a test, a rat pup, the typical experimental subject for most experimental behavioral teratology studies, is placed in the center of a rectangular apparatus in which one side contains clean bedding material and the other side contains bedding from the pup's home cage. The time taken for the pup to orient to or to reach the home cage bedding constitutes the dependent variable of interest. Since this performance depends on both olfactory capabilities and the development of appropriate motor skills, it represents a type of apical evaluation. It has demonstrated that olfactory discriminations can be learned by rat pups. Pairing aversive electric shock with a distinctive odor leads pups to avoid the odor.

See also: Analytical Toxicology; Behavioral Toxicology; Metals; Neurotoxicity; Ototoxicity; Organophosphates; Petroleum Distillates; Psychological Indices of Toxicity.

Further Reading

Chang LW and Stikker W (eds.) (1995) *Neurotoxicology: Approaches and Methods*. San Diego, CA: Academic Press.

Spencer PS and Schaumburg HH (eds.) (2000) *Experimental and Clinical Neurotoxicology*, 2nd edn. New York: Oxford University Press.

Relevant Website

http://books.nap.edu – Behavioral Measures of Neurotoxicity. From the Commission on Behavioral and Social Sciences and Education, National Research Council, 1990.

Toxicity Testing, Carcinogenesis

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Introduction

Carcinogenicity testing in experimental animals is typically done under conditions that maximize the potential for detecting an effect (taking into account issues related to human relevance of the effect observed and the potential for artifacts at excessively high doses) by testing at high doses, with the ultimate aim of extrapolating to human risk at much lower levels. This entry examines the assumptions involved in these undertakings, reviews the aspects of design and interpretation of animal carcinogenicity studies, takes a critical look at low dose extrapolation models and methods, and presents the framework on which risk assessment is based.

The scientific ideal is to evaluate a chemical's carcinogenic potential from human data. While controlled exposure studies for carcinogenesis are not ethical, information can sometimes be obtained from epidemiology studies, particularly occupational studies involving high exposures to a limited number of chemicals. However, it is often difficult to reach definitive conclusions from such epidemiology studies, due to inaccurate exposure measures and/or confounding from other chemical exposures. Exceptions to this general rule are possible for rare cancers (such as angiosarcoma and vinyl chloride), and consistent results in cohorts exposed to the same chemical in different industries (and thus with different confounders) can support an association. In such cases, or in the absence of useful epidemiology data, controlled experimental animal bioassays provide useful information for both hazard identification and dose–response evaluation.

Carcinogenicity studies are the longest and most expensive of the extensive battery of toxicology studies required for the registration of pharmaceutical products in the United States, and in other major countries. In addition, they are often the most controversial with respect to interpretation of their results. These studies are important because, as noted by International Agency for Research on Cancer, "in the absence of adequate data on humans, it is biologically plausible and prudent to regard agents for which there is sufficient evidence of carcinogenicity in experimental animals as if they presented a carcinogenic risk to humans."

Bioassay Design

Carcinogenicity bioassays have two primary objectives, though (as will be shown) the second is now more important and (as our understanding of carcinogenesis has increased) is increasingly crowding out the first.

The first objective is to detect possible carcinogens. Compounds are evaluated to determine if they can or cannot induce a statistically detectable increase of tumor rates over background levels, and only by happenstance is information generated which is useful in risk assessment. Most of the earlier studies had such detection as their objective. The current thought is that at least two species must be used for detection.

The second objective for a bioassay is to provide a range of dose–response information (with tumor incidence being the response) so that a risk assessment may be performed. Unlike detection, which requires only one treatment group with adequate survival times (to allow expression of tumors), dose response requires at least three treatment groups with adequate survival. The selection of dose levels for this case will be discussed later. However, given that the species is known to be responsive, only one species of animal need to be used for this objective.

To address either or both of these objectives, three major types of study designs have evolved. First type is a screening assay, such as the classical skin painting study, usually performed in mice. A single, easily detected endpoint (the formation of skin tumors) is evaluated during the course of the study. Though dose response can be evaluated in such a study (dose usually being varied by using different concentrations of test material in volatile solvent), most often detection is the objective of such a study. Though others have used different frequencies of application of test material to vary dose, there are data to suggest that this only serves to introduce an additional variable. Traditionally, both test and control groups in such a test consist of 50–100 mice of one sex (males being preferred because of their very low spontaneous tumor rate). This design is also often used in tumor initiation/promotion studies. Other screening assays include the strain A mouse lung tumor model, and transgenic mouse models that allow detection of mutagenic carcinogens with relatively short exposure periods.

The second common type of design is the original National Toxicology Program (NTP) bioassay. The announced objective of these studies was detection of moderate to strong carcinogens, although the results have also been used in attempts at risk assessment.

Both mice and rats were used in parallel studies. Each study used 50 males and 50 females at each of two dose levels (high and low) plus an equal-sized control group. The NTP has recently moved away from this design because of the recognition of its inherent limitations.

Finally, there is the standard industrial toxicology design, which uses at least two species (usually rats and mice) in groups of no fewer than 100 males and females each. Each study has three dose groups and at least one control. Frequently, additional numbers of animals are included to allow for interim terminations and histopathological evaluations. In both this and the original design, many organs and tissues are collected, processed, and examined microscopically. This design seeks to address both the detection and dose–response objectives with a moderate degree of success.

Selecting the number of animals to use for dose groups in a study requires consideration of both biological (e.g., expected survival rates and background tumor rates) and statistical factors. The prime statistical consideration is reflected in **Table 1**. It can be seen in this table that if, for example, using mice to study a compound that caused liver tumors (with a background or control incidence of 30%), 389 animals per sex per group were used, to be able to demonstrate that an incidence rate of 40% in treatment animals was significant compared to the controls at the $p = 0.05$ level.

Perhaps, the most difficult aspect of designing a good carcinogenicity study is the selection of the dose levels to be used. At the start, it is necessary to consider the first underlying assumption in the design and use of animal cancer bioassays – the need to test at the highest possible dose for the longest practical period.

The rationale behind this design is that although humans may be exposed at very low levels, detecting the resulting small increase (over background) in the incidence of tumors would require the use of an impracticably large number of test animals per group. This point is illustrated in **Table 2**, which shows, for instance, that although only 46 animals (per group) are needed to show a 10% increase over a zero background (i.e., a rarely occurring tumor type), 770 000 animals (per group) would be needed to detect a 0.1% increase above a 5% background. As the dose increases, however, the incidence of tumors (the response) will also increase until it reaches the point where a modest increase (e.g., 10%) over a reasonably small background level (e.g., 1%) could be detected using an acceptably small-sized group of test animals. There are, however, at least two real limitations to the highest dose level. First, the test rodent population must have a sufficient survival rate after receiving a lifetime (or 2 years) of regular doses to allow for meaningful statistical analysis. Typically, the survival should be at least 50% for the study design using 50 animals per sex per dose for rats at 18 months and mice at 15 months, to allow sufficient study sensitivity. Second, it is desirable for the metabolism and mechanism of action of the chemical at the highest level tested to be the same as at the low levels where human exposure would occur. Unfortunately, generally the high-dose level is selected based only on the information provided by a subchronic or range-finding study, but selection of too low a dose will make the study invalid for detection of carcinogenicity and may seriously impair the use of the results for risk assessment.

There are several solutions to this problem. One of these has been the rather simplistic approach of the INUP Bioassay Program, which is to conduct a 3 month range-finding study with sufficient dose levels to establish a level that significantly (10%) decreases the rate of body weight gain. This dose is defined as the maximum tolerated dose (MTD) and is

Table 1 Sample size required to obtain a specified sensitivity at $p < 0.05$ treatment group incidence

Background tumor incidence (%)	*p*[a]	*Required sample size*									
		Incidence rate (%)									
		0.95	*0.90*	*0.80*	*0.70*	*0.60*	*0.50*	*0.40*	*0.30*	*0.20*	*0.10*
0.30	0.90	10	12	18	31	46	102	389			
	0.50	6	6	9	12	22	32	123			
0.20	0.90	8	10	12	18	30	42	88	320		
	0.50	5	5	6	9	12	19	28	101		
0.10	0.90	6	8	10	12	17	25	33	65		
	0.50	3	3	5	6	9	11	17	31	68	
0.05	0.90	5	6	8	10	13	18	25	35	76	464
	0.50	3	3	5	6	7	9	12	19	24	147
0.01	0.90	5	5	7	8	10	13	19	27	46	114
	0.50	3	3	5	5	6	8	10	13	25	56

[a] p = power.

Table 2 Average number of animals needed to detect a significant increase in the incidence of an event (e.g., tumors and anomalies) over the background incidence (control) at several expected incidence levels using the Fisher exact probability test ($p = 0.05$)

Background incidence (%)	*Expected increase in incidence*					
	0.01	*0.1*	*1*	*3*	*5*	*10*
0	46 000 000[a]	460 000	4 600	511	164	46
0.01	46 000 000	460 000	4 600	511	164	46
0.1	47 000 000	470 000	4 700	520	168	47
1	51 000 000	510 000	5 100	570	204	51
5	77 000 000	770 000	7 700	856	304	77
10	100 000 00	1 000 000	10 000	1100	400	100
20	148 000 00	1 480 00	14 800	1644	592	148
25	160 000 000	1 600 000	16 000	1840	664	166

[a] Number of animals needed in each group – controls as well as treated.

selected as the highest dose. Two other levels, generally one-half MTD and one-quarter MTD, are selected for testing as the intermediate and low dose levels. In many early studies, only one other level was used.

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 risk assessment. 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' (i.e., the factor needed to extrapolate from a subchronic study to projected doses for a chronic study) and to study onset of pathology can be more predictive for dose setting than body weight suppression.

Also, the NTP's 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.

Selection of levels for the intermediate and lower doses for a study is easy only in comparison to the selection of the high dose. If an objective of the study is to generate dose–response data, then the optimal placement of the doses below the high is such that they cover as much of the range of the dose–response curve as possible and yet still have the lowest dose at a high enough level that one can detect and quantify a response. If the objective is detection, then having too great a distance between the highest and next highest dose creates a risk to the validity of the study. If the survival in the high dose is too low, yet the next highest dose does not show nonneoplastic results (i.e., cause other than neoplastic adverse biological effects) such as to support it being a high enough dose to have detected a strong or moderate carcinogen, the entire study may have to be rejected as inadequate to address its objective. Statistical guidelines have been proposed (for setting dose levels below the high) based on response surfaces. In so doing they suggest that the lowest dose be no less than 10% of the highest.

Although it is universally agreed that the appropriate animal model for testing a chemical for carcinogenicity would be an animal whose metabolism, pharmacokinetics, and biological responses were most similar to humans, economic considerations have largely constrained practical choices to rats and mice. The use of both sexes of both species is preferred on the grounds that it provides for a greater likelihood of utilizing the more sensitive species, in the face of a lack of understanding of which species would actually be most like humans for a particular agent. Use of the mouse as a second bioassay species is both advocated and defended on these grounds and because of the economic advantages and the species' historical utilization. There are those who believe that the use of the mouse is redundant and represents a diversion of resources while yielding little additional information, citing a 'unique contribution' for mouse data in 273 bioassays of only 13.6% of the cases (i.e., 37 cases). Others question the use of the mouse based on the belief that it gives artifactual liver carcinogenesis results. In addition, in many studies where responses in mice and rats differed, further investigation found that the rat was more pharmacokinetically similar to humans. One suggestion for the interpretation of mouse bioassays is that in those cases in which there is only an increase in liver tumors in mice (or lung tumors in strain A mice) and no supporting mutagenicity findings (a situation characteristic of some classes of chemicals), the test compound should not be considered an overt carcinogen. This last aspect, however, is even more strongly focused on the strain of mouse that is used than on the use of the species itself.

The NTP currently recommends an F1 hybrid cross between two inbred strains, the C57B1/6

female and the C3H male, the results being commonly designated as the B6C3F1. This mouse was found to be very successful in a large-scale pesticide testing program in the mid-1960s. It is a hardy animal with good survival, easy to breed, disease resistant, and has been reported to have a relatively low spontaneous tumor incidence. Usually, at least 80% of the control mice are still alive at a 24 month termination.

Unfortunately, while it was originally believed that the spontaneous liver tumor incidence in male B6C3F1 mice was 13.7%, it actually appears to be closer to 32.1%. The issue of spontaneous tumor rates and their impact on the design and interpretation of studies will be discussed more fully later. Thus, use of a cross of two inbred mouse strains is also a point of controversy. A study has presented data to support the idea that inbred strains have lower degrees of variability of biological functions and tumor rates, making them more sensitive detectors and quantitators. The study also suggests that the use of a cross from two such inbred strains allows one to more readily detect tumor incidence increases. On the other hand, it has been argued that such genetically homogeneous strains do not properly reflect the diversity of metabolic functions present in the human population (particularly functions that would serve to detoxify or act as defense mechanisms).

Study length and the frequency of treatment are design aspects that must also be considered. These are aspects in which the objective of detection and dose–response definition conflict.

For the greatest confidence in a 'negative' detection result, an agent should be administered continuously for the majority of an animal's life span. Many agencies require negative results in valid, well-conducted bioassays in two different species (typically rats and mice) for a chemical to be classified as not carcinogenic. The NTP considers 2 years to be a practical treatment period in rats and mice, although the animals currently used in such studies may survive an additional 6–12 months. The purpose for this approach is to include exposure to the test chemical for a significant percentage of the animal's lifespan, while avoiding a high incidence of early deaths, or a high incidence of background tumors which compromise study sensitivity. Study lengths of 15–18 months are considered adequate for shorter lived species such as hamsters. An acceptable exposure/observation period for dogs is considered to be 7–10 years, an age equivalent to $\sim$45–60 years in humans. For dietary treatments, continuous exposure is considered desirable and practical. With other routes, practical considerations may dictate interrupted treatments. For example, inhalation treatment for 6–8 h day^{-1}, 5 days week^{-1} is the usual practice. Regimens requiring special handling of animals, such as gavage dosing or parenteral injections, are usually on a 5 days week^{-1} basis. With some compounds intermittent exposures may be required because of toxicity, although it is preferred to choose a dose that does not require intermittent exposure. Various types of recovery can occur during exposure-free periods, which may either enhance or decrease chances of carcinogenicity. In view of the objective of assessing carcinogenicity as the initial step, intermittent exposure on a 3–5 days week^{-1} basis is considered both practical and desirable for most compounds.

Following cessation of dosing or exposure, continued observation during a nontreatment period may be required before termination of the experiment. Such a period is often considered desirable because (1) induced lesions may progress to more readily observable lesions, and (2) morphologically similar but noncarcinogenic proliferative lesions that are stress-related may regress. Neoplastic or 'neoplastic-like' lesions that persist long after removal of the stimulus are considered serious consequences from the hazard viewpoint. Many expert anatomical pathologists, however, believe they are able to diagnose and determine the biological nature of tumorous lesions existing at the time of treatment without the added benefit of a treatment-free period.

In determining the length of an observation period, several factors must be considered: period of exposure, survival pattern of both treated and control animals, nature of lesions founds in animals that have already died, tissue storage and retention of the chemical, and results of other studies that would suggest induction of late-occurring tumors. The length of a treatment-free observation period can be as long as 3 months in mice and hamsters and 6 months in rats. An alternative would be to terminate the experiment or an individual treatment group on the basis of survival (e.g., at the point at which 50% of the group with the lowest survival has died).

However, arguments exist against such prolonged treatment and maintenance. These generally revolve around the relationship between age and tumor incidence. As test animals (or humans) become older, the background ('naturally occurring') incidence of tumors increases and it becomes increasingly difficult to identify a treatment effect from the background effect. An analysis of patterns of senile lesions in mice and rats was carried out, wherein the so-called principle of biological confounding is discussed: "If a particular lesion (e.g., pituitary tumor) is part of a larger syndrome induced by the treatment, it is impossible to determine whether the treatment has 'caused' that lesion."

This could lead to a situation in which any real carcinogen would be nonidentifiable. If the usual pattern of old age lesions for a given species or strain of animals includes tumors, then almost every biologically active treatment can be expected to influence the incidence of tumors in a cluster of lesions at a sufficiently high dose.

Reconsidering the basic principles of experimental design, it is clear that one should try to design bioassays so that any carcinogenesis is a clear-cut, single event, unconfounded by the occurrence of significant numbers of lesions due to other causes (such as age). One answer to this problem is the use of interim termination groups. When an evaluation of tumor incidences in an interim sacrifice sample of animals indicates that background incidence is becoming a source of confounding data, termination plans for the study can be altered to minimize the loss of power.

A number of other possible confounding factors can enter into a bioassay unless design precludes them. These include (1) cage and litter effects, which can be avoided by proper prestudy randomization of animals and rotation of cage locations; (2) vehicle (e.g., corn oil has been found to be a promoter for liver carcinogens); and (3) the use of the potential hazard route for man (e.g., dietary inclusion instead of gastric intubation).

Bioassay Interpretation

The interpretation of the results of even the best designed carcinogenesis bioassay is a complex statistical and biological problem. In addressing the statistical aspects, some biological points which have statistical implications need to be reviewed as one proceeds.

All such bioassays are evaluated by comparison of the observed results in treatment groups with those in one or more control groups. These control groups always include at least one group that is concurrent, but because of concern about variability in background tumor rates, a historical control group is also considered in at least some manner.

The underlying problem in the use of concurrent controls alone is the belief that the selected population of animals are subject both to an inordinate degree of variability in their spontaneous tumor incidence rates and that the strains maintained at separate breeding facilities are each subject to a slow but significant degree of genetic drift. The first problem raises concern that, by chance, the animals selected to be controls for any particular study with be either 'too high' or 'too low' in their tumor incidences, leading to either a false-positive or false-negative statistical test result when test animals are compared to these controls. The second problem leads to concern that, over the years, different laboratories will be using different standards (control groups) against which to compare the outcome of their tests, making any kind of relative comparison between compounds or laboratories impossible.

See also: Analytical Toxicology; Animal Models; Carcinogenesis; Carcinogen–DNA Adduct Formation and DNA Repair; Carcinogen Classification Schemes; Dose–Response Relationship; *In Vivo* Test; International Agency for Research on Cancer; Mouse Lymphoma Assay; National Toxicology Program; Risk Assessment, Human Health; Toxicity Testing, Mutagenicity.

Further Reading

Depass L and Gad SC (eds.) (1994) *Safety Assessment for Pharmaceuticals*, pp. 167–184. New York: Van Nostrand Reinhold.

Kitchin KT (1999) *Carcinogenicity: Testing, Predicting, and Interpreting Chemical Effects*. New York: Dekker.

Waalkes MP and Ward JM (1994) *Carcinogenesis*. New York: Raven Press.

Relevant Websites

http://www.ilsi.org – Alternatives to Carcinogenicity Testing (from the International Life Sciences Institute).

http://potency.berkeley.edu – The Carcinogenic Potency Project (from the University of California, Berkeley).

http://monographs.iarc.fr – IARC Monographs Programme on the Evaluation of Carcinogenic Risks to Humans (from the International Agency for Research on Cancer).

http://cfpub2.epa.gov – US EPA (Environmental Protection Agency) (1999) Guidelines for Carcinogen Risk Assessment. Office of Research and Development, Risk Assessment Forum, Washington, DC.

Toxicity Testing, Dermal

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Virtually all man-made chemicals have the potential to contact the skin of people. In fact, many (e.g., cosmetics and shampoos) are intended to have skin contact. Also, the most common medical problems in industrial workers are skin conditions, reflecting the large extent of dermal exposure where none is intended. When a large surface area of skin is exposed to contaminated soil or water, skin absorption may be significant. It is also possible for dermal effects to arise from systemic toxicants.

Evaluation of materials for their potential to cause dermal irritation and corrosion due to acute contact has been common for industrial chemicals, cosmetics, agricultural chemicals, and consumer products since at least the 1930s (generally, pharmaceuticals are only evaluated for dermal effects if they are to be administered topically – and then by repeat exposure tests, which will not be addressed here). As with acute eye irritation tests, one of the earliest formal publications of a test method (although others were used) was that of Draize *et al*. in 1944. The methods currently used are still basically those proposed by Draize *et al*. and, to date, have changed little since 1944. These methods have more recently caught the interest of the animal welfare movement, and there are efforts under way to develop alternatives that either do not use animals or are performed in a more humane way. The data generated is somewhat difficult to extrapolate to humans; Draize type tests are also criticized because the distinction between mild and moderate irritants is difficult. The need for alternative methods of testing has become more pressing since a 1993 provision in the 6th amendment to the European Union (EU) Cosmetic Directive (93/35/EEC). This states that it will become illegal to market cosmetic products in EU countries if they contain ingredients or mixtures of ingredients that have been tested on animals, unless there are no valid alternatives to replace the animal tests.

Introduction

Among the most fundamental assessments of the safety of a product or, indeed, of any material that has the potential to be in contact with a significant number of people in our society are tests which seek to predict potential skin irritation or corrosion. Like all the other tests in what is classically called a range-finding, tier I, or acute battery, the tests used here are both among the oldest and are currently undergoing the greatest degree of scrutiny and change. Established test methods for these endpoints use several of the same animal models, but most commonly the rabbit (almost exclusively the New Zealand White) is the test subject. Other species commonly used include mice, rats, and guinea pigs. Hartley, Pirbright, or Himalayan white strains of guinea pigs of 350–400 g are considered to be good respondents. Such tests are not designed to evaluate systemic effects resulting after absorption.

Dermal Toxicity Tests

Testing is performed to evaluate the potential occurrence of two different, yet related, endpoints, irritation and sensitization. The broadest application of these is evaluation of the potential to cause skin irritation, characterized by erythema (redness) and edema (swelling). Severity of irritation is measured in terms of both the degree of these two parameters and how long they persist. Primary irritation, cutaneous sensitization, phototoxicity, and photosensitization are possible types of dermal irritation resulting from dermal application. There are three types of irritation tests, each designed to address a different concern:

1. Primary (or acute) irritation: a localized reversible dermal response resulting from a single application of, or exposure to, a chemical without the involvement of the immune system.
2. Cumulative irritation: a reversible dermal response, which results from repeated exposure to a substance (each individual exposure is not capable of causing acute primary irritation).
3. Photochemically induced irritation: a primary irritation resulting from light-induced molecular changes in the chemical to which the skin has been exposed.

Irritation is generally a localized reaction resulting from either a single exposure or multiple exposures to a physical or chemical entity at the same site. It is characterized by the presence of erythema, edema, and may or may not result in cell death. The observed signs are heat (caused by vessel dilation and the presence of large amounts of warm blood in the affected area), redness (due to capillary dilation), and pain (due to pressure on sensory nerves). The edema often observed is largely due to plasma, which

coagulates in the injured area, precipitating a fibrous network to screen off the area, thereby permitting leukocytes to destroy exogenous materials by phagocytosis. If the severity of injury is sufficient, cell death may occur, thereby negating the possibility of cellular regeneration. Necrosis is a term often used in conjunction with cell death, and it is the degeneration of the dead cell into component molecules which approach equilibrium with surrounding tissue.

There are three major objectives to be addressed by the performance of these tests:

1. Providing regulatory required baseline data: Any product now in commerce must both be labeled appropriately for shipping and be accompanied by a material safety data sheet which clearly states potential hazards associated with handling it. Department of Transportation regulations also prescribe different levels of packaging on materials found to constitute hazards as specified in the regulations. Environmental Protection Agency (EPA) (under FIFRA) also has a pesticides labeling requirement. Similar requirements exist outside the United States. These requirements demand absolute identification of severe irritants or corrosives and adherence to the basics of test methods promulgated by the regulations. False positives (type I errors) are to be avoided in these usages.
2. Hazard assessment for accidents: For most materials, dermal exposure is not intended to occur, but it will occur in cases of accidental spillage or mishandling. Here, it is important to correctly identify the hazard associated with such exposures and be equally concerned with false positives and false negatives.
3. Assessment of safety for use: The materials at issue here are the full range of products for which dermal exposure will occur in the normal course of use. These range from cosmetics and hand soaps to bleaches, laundry detergents, and paint removers. No manufacturer desires to put a product on the market, which cannot be safely used and will lead to extensive liability if placed in the marketplace. Accordingly, the desire here is to accurately predict the potential hazards in humans; that is, to have neither false positives nor false negatives.

Dermal Toxicity Test Design

Table 1 sets forth the current regulatory mandated test designs, which form the bases of all currently employed test procedures. All of these methods use the same scoring scale, the Draize scale, which is

Table 1 Primary dermal irritation test: regulatory mandated test designs for dermal irritation/corrosion

Agency	*Test material*		*Exposure time (h)*	*No. of rabbits*	*Sites per animal (intact/abraded)*	*At end of exposure*	*Occlusion*	*Scoring intervals postexposure*	*Note*
	Solid	*Liquid*							
Department of transportation	Not specified	Not specified	4	6	1/0	Skin washed w/ appropriate vehicle	Yes	4 and 48 h	Endpoint is corrosion in 2 of 6 animals
Environmental Protection Agency	Moisten	Undiluted	24	6	2/2	Skin wiped but not washed		24 and 72 h; may continue until irritation fades or is judged irreversible	Toxic Substance Control Act (TSCA); test also FIFRA
Consumer Product Safety Commission	Dissolve in appropriate vehicle	Neat	24	6	1/1	Not specified	Impervious material	24 and 72 h	Federal Hazardous Substances Act (FHSA)
OECD	Moisten	Undiluted	4	3[a]	1/0	Wash with water or solvent	Semiocclusive	30–60 min, 24, 48, 72 h or until judged irreversible	European Common Market

[a] Additional animals may be required to clarify equivocal results.

Table 2 Evaluation of skin reactions

Skin reaction	*Value*
Erythema and eschar formation	
No erythema	0
Very slight erythema	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Necrosis (death of tissue)	+N
Eschar (sloughing or scab formation)	+E
Edema formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well-defined by definite raising)	2
Moderate edema (raised ~1 mm)	3
Severe edema (raised more than 1 mm and extending beyond the area of exposure)	4
Total possible score for primary irritation	8

presented in **Table 2**. However, though the regulations prescribe these different test methods, most laboratories actually perform somewhat modified methods. New material should first be tested *in vitro* or using animal skin for short exposure times and open application sites (without occlusion). Here, two modifications (one for irritation and the other for corrosion), which reflect prior laboratory experience are recommended.

Performing *In Vivo* Dermal Toxicity Tests

Selection of Animals and Skin Testing Sites

1. A group of at least 8–12 New Zealand White rabbits are screened for the study.
2. All rabbits selected for the study must be in good health; any rabbit exhibiting sniffles, hair loss, loose stools, or apparent weight loss are rejected and replaced.
3. One day (at least 18 h) prior to application of the test substance, each rabbit is prepared by clipping the hair from the back and sides using a small animal clipper. A size No. 10 blade is used to remove long hair and then a size No. 40 blade is used to remove the remaining hair.
4. Six animals with skin sites that are free from hyperemia or abrasion (due to shaving) are selected. Skin sites that are in the telogen phase (resting stage of hair growth) are used; those skin sites that are in the anagen phase (stage of active growth, indicated by the presence of a thick undercoat of hair) are not used.

Study Procedure

1. As many as four areas of skin, two on each side of the rabbit's back, can be utilized for sites of administration.
2. Separate animals are not required for an untreated control group. Each animal serves as its own control.
3. Besides the test substance, a positive control substance (a known skin irritant – 1% sodium laurel sulfate in distilled water) and a negative control (untreated patch) are applied to the skin. When a vehicle is used for diluting, suspending, or moistening the test substance, a vehicle control patch is required, especially if the vehicle is known to cause any toxic dermal reactions or if there is insufficient information about the dermal effects of the vehicle.
4. The intact (free of abrasion) sites of administration are assigned a code number.
   a. Test substance
   b. Negative control
   c. Positive control
   d. Vehicle control

(Note that some tests, such as those required by EPA, and CPSC, see **Table 1**, require abraded sites. For these tests an area of skin should be scratched so as to open the stratum corneum, but not produce blood.)

5. Application sites should be rotated from one animal to the next to ensure that the test substance and controls are applied to each position at least once.
6. Each test or control substance is held in place with a 1 × in., 12-ply surgical gauze patch. The gauze patch is applied to the appropriate skin site and secured with 1in.-wide strips of surgical tape at the four edges, leaving the center of the gauze patch nonoccluded.
7. If the test substance is a solid or semisolid, a 0.5 g portion is weighed and placed on the gauze patch. The test substance patch is placed on the appropriate skin site and secured. The patch is subsequently moistened with 0.5 ml of physiological saline.
8. When the test substance is in flake, granule, powder, or other particulate form, the weight of the test substance that has a volume of 0.5 ml (after compacting as much as possible without crushing or altering the individual particles, such as by tapping the measuring container) is used whenever this volume weight is less than 0.5 g. When applying powders, granules, and the like, the gauze patch designated for the test sample is secured to the appropriate skin site with one of

four strips of tape at the most ventral position of the animal. With one hand, the appropriate amount of sample measuring 0.5 ml is carefully poured from a weighing paper onto the gauze patch that is held in a horizontal (level) position with the other hand. The patch containing the test sample is then carefully placed into position onto the skin and the remaining three edges are secured with tape. The patch is subsequently moistened with 0.5 ml of physiological saline.

9. If the test substance is a liquid, a patch is applied and secured to the appropriate skin site. A 1 ml tuberculin syringe is used to measure and apply 0.5 ml of test substance to the patch.
10. The negative control site is covered with an untreated 12-ply surgical gauze patch (1×1 in.).
11. The positive control substance and vehicle control substance are applied to a gauze patch in the same manner as a liquid test substance.
12. The entire trunk of the animal is covered with an impervious material (such as saran wrap) for a 24 h period of exposure. The saran wrap is secured by wrapping several long strips of athletic adhesive tape around the trunk of the animal. The impervious material aids in maintaining the position of the patches and retards evaporation of volatile test substances.
13. An Elizabethan collar is fitted and fastened around the neck of each test animal. The collar remains in place for the 24 h exposure period. The collars are utilized to prevent removal of wrappings and patches by the animals, while allowing the animals food and water *ad libitum*.
14. The wrapping is removed at the end of the 24 h exposure period. The test substance skin site is wiped to remove any test substance still remaining. When colored test substances (such as dyes) are used, it may be necessary to wash the test substance from the test site with an appropriate solvent or vehicle (one that is suitable for the substance being tested). This is done to facilitate accurate evaluation for skin irritation.
15. Immediately after removal of the patches, each 1×1 in. test or control site is outlined with an indelible marker by dotting each of the four corners. This procedure delineates the site for identification.

Observations

1. Observations are made of the test and control skin sites 1 h after removal of the patches (25 h post-initiation of application). Erythema and edema are evaluated and scored on the basis of the designated values presented in **Table 3**.
2. Observations are again performed 48 and 72 h after application and scores are recorded.
3. If necrosis is present or the dermal reaction is unusual, the reaction should be described. Severe erythema should receive the maximum score (4), and +N should be used to designate the presence of necrosis and +E the presence of eschar.
4. When a test substance produces dermal irritation that persists 72 h postapplication, daily observations of test and control sites are continued on all animals until all irritation caused by the test substance resolves or until Day 14 postapplication.

Evaluation of Results

1. A subtotal irritation value for erythema or eschar formation is determined for each rabbit by adding the values observed at 25, 48, and 72 h postapplication.
2. A subtotal irritation value for edema formation is determined for each rabbit by adding the values observed at 25, 48, and 72 h postapplication.
3. A total irritation score is calculated for each rabbit by adding the subtotal irritation value for erythema or eschar formation to the subtotal irritation value for edema formation.
4. The primary dermal irritation index is an average calculated for the test substance or control substance by dividing the sum of the total irritation scores by the number of observations (3 days $\times$ six animals = 18 observations).
5. The categories of the Primary Dermal Irritation Index (PDII) are as follows (this categorization of the dermal irritation is a modification of the original classification described by Draize *et al.*):

PDII = 0.0 nonirritant
> 0.0–0.5 negligible irritant
> 0.5–2.0 mild irritant
> 2.0–5.0 moderate irritant
> 5.0–8.0 severe irritant

Other abnormalities, such as atonia or desquamation, should be noted and recorded.

Another way of scoring is the Primary Irritation Index (PII). This is found by taking the average of the erythema scores for both abraded and nonabraded sites and adding this to the average of the edema scores both for abraded and nonabraded sites.

Photoirritation

Phototoxicity (photoirritation) is a light induced skin response, similar to an exaggerated sunburn, which can be elicited after a single exposure to a photoactive chemical (topical application, ingestion, or

Table 3 *In vitro* dermal irritation test systems

System	*Endpoint*	*Validation data?*[a]
Excised patch of perfused skin	Swelling	No
Mouse skin organ culture	Inhibition of incorporation of [^{3}H] thymidine and [^{14}C]leucine labels	No
Mouse skin organ culture	Leakage of LDH and GOT	Yes
Testskin: cultured surrogate skin patch	Morphological evaluation (?)	No
Cultured surrogate skin patch	Cytotoxicity	No
Human epidermal keratinocytes (HEKs)	Release of labeled arachidonic acid	Yes
Human polymorphonuclear cells	Migration and histamine release	Yes (surfactants)
Fibroblasts	Acid	
HEKs	Cytotoxicity	Yes
HEKs	Cytotoxicity (MIT)	Yes
HEKs, dermal fibroblasts	Cytotoxicity	Yes
HEKs	Inflammation mediator release	No
Cultured Chinese hamster ovary (CHO) cells	Increases in β-hexosamindase levels in media	No
Cultured C_3 H10T1/2 and HEK cells	Lipid metabolism inhibition	No
Cultured cells		
BHK21/C13	Cell detachment	Yes
BHK21/C13	Growth inhibition	
Primary rat thymocytes	Increased membrane permeability	
Rat peritoneal mast cells	Inflammation mediator release	Yes (surfactant)
Hen's egg	Morphological evaluation	
Skintex; protein mixture	Protein coagulation	Yes
Structure-activity relation (SAR) model	NA[b]	Yes
SAR model	NA	No

GOT: glutamic oxaloacetic transminase; MIT: metabolic inhibition test.
[a] Evaluated by comparison of predictive accuracy (in the sense used here) for a range of compounds compared with animal testing results.
[b] NA, not available.

other). Response to photoirritants includes erythema, edema, vesculation, and pigmentation, which are usually activated by the UV portion of sunlight. Phototoxic potential is not routinely tested in many industrial, household, drug, and cosmetic chemicals that are developed and marketed each year. Photomaximization tests are needed for some chemicals. Leukoderma (depigmentation) due to chemical exposure with compounds, often phenols and or thiols, is known to occur. Also, carcinogenesis and photocarcinogenesis should now be evaluated as recommended by the US National Toxicology Program.

As results vary with species (strains), lamps, detectors, doses, distances, time (exposure chemical to exposure light) chemical routes, endpoint (biological, erythema, edema, ear thickness) a somewhat structured method has been proposed to limit variation. To apply this method, at four skin sites apply 0.05 ml test chemical alone, under radiation, opaque cover, vehicle alone, and positive control at intervals of 5 min to 24 h. Irradiate all skin sites simultaneously for up to 40 min at a distance of up to 15 cm from the source. Adjust the exposure time so that 10 J cm^{-2} UVA and 0.1 J cm^{-2} UVB using:

$$T(\text{min}) = (\text{J cm}^{-2} * 1000)/(\text{mW cm}^{-2} * 60)$$

Evaluate skin at 1, 24, and 48 h and score for erythema, edema (mild, moderate, and severe) and hyperpigmentation in humans.

Testing for Sensitizers

Skin sensitization tests assess the ability of chemicals to affect the immune system, such that a second contact causes a more severe reaction than the first. The antigen involved is presumed to be formed in the bonding of the chemical to body proteins. The antibodies that form to this ligand–protein complex give rise to an allergic reaction with subsequent exposure.

QSAR, statistical, and computational methods are used to determine the possibility that a material is a sensitizer and the potential severity of sensitization. *In vivo* methods are useful to diagnose skin disorders such as drug eruptions, contact dermatitis, immediate contact reactions (contact urticaria), and more. Allergic Contact Dermatitis (ACD) is an inflammatory skin disease, marked by a delayed skin response following skin contact with an allergic chemical. Test groups must be very large to assess this effect. To test for ACD, a test article or sample(s) must be initially exposed to the same skin site/area (induction phase). After a rest period of a week or more (others say over

2 weeks) follow with a challenge exposure of the article (test sample(s)) to a virgin skin site or area. The lesions are scored on the basis of severity and the number of animals responding. Other test methods include those in which the induction phase is conducted by intradermal injection together with Freund's adjuvant and those in which the treatments are all topical and the induction phase is accompanied by intradermal injections of Freund's adjuvant.

Factors Affecting Irritation Responses and Test Outcomes

The results of local tissue irritation tests are subject to considerable variability due to relatively small differences in test design or technique. Well and Scala arranged and reported on the best known of several intralaboratory studies to clearly establish this fact. Though the methods presented previously have proven to give reproducible results in the hands of the same technicians over a period of years and contain some internal controls (the positive and vehicle controls in the PDI) to minimize large variations in results or the occurrence of either false positives or negatives, it is still essential to be aware of those factors that may systematically alter test results. These factors are summarized as follows:

1. In general, any factor that increases absorption through the stratum corneum or mucous membrane will also increase the severity of an intrinsic response. Unless this factor mirrors potential exposure conditions, it may, in turn, adversely affect the relevance of test results.
2. The physical nature of solids must be carefully considered both before testing and in interpreting results. Shape (sharp edges), size (small particles may abrade the skin due to being rubbed back and forth under the occlusive wrap), and rigidity (stiff fibers or very hard particles will be physically irritating) of solids may all enhance an irritation response.
3. Solids frequently give different results when they are tested dry than if wetted for the test. As a general rule, solids are more irritating if moistened (referring to item 1, wetting is a factor that tends to enhance absorption). Care should also be taken regarding moistening agent – some (few) batches of US Pharmacopeia physiological saline (used to simulate sweat) have proven to be mildly irritating to the skin and mucous membrane on their own. Liquids other than water or saline should not be used.
4. If the treated region on potential human patients will be a compromised skin surface barrier (e.g., if it is cut or burned) some test animals should likewise have their application sites compromised. This procedure is based on the assumption that abraded skin is uniformly more sensitive to irritation. Experiments, however, have shown that this is not necessarily true; some materials produce more irritation on abraded skin, while others produce less.
5. The degree of occlusion (in fact, the tightness of the wrap over the test site) also alters percutaneous absorption and therefore irritation. One important quality control issue in the laboratory is achieving a reproducible degree of occlusion in dermal wrappings.
6. Both the age of the test animal and the application site (saddle of the back vs. flank) can markedly alter test outcome. Both of these factors are also operative in humans, of course, but in dermal irritation tests the objective is to remove all such sources of variability. In general, as an animal ages, sensitivity to irritation decreases. For the dermal test, the skin on the middle of the back (other than directly over the spine) tends to be thicker (and therefore less sensitive to irritations) than that on the flanks.
7. The sex of the test animals can also alter study results because both regional skin thickness and surface blood flow vary between males and females.
8. The single most important (but also most frequently overlooked) factor that influences the results and outcome of these (and, in fact, most) acute studies is the training of the staff. In determining how test materials are prepared and applied and in how results are 'read' against a subjective scale, both accuracy and precision are extremely dependent on the technicians involved. To achieve the desired results, initial training must be careful and all-inclusive. Equally as important, some form of regular refresher training must be exercised, particularly in the area of scoring of results. Use of a set of color photographic standards as a training reference tool is strongly recommended; such standards should clearly demonstrate each of the grades in the Draize dermal scale.
9. It should be recognized that the dermal irritancy test is designed with a bias to preclude false negatives and, therefore, tends to exaggerate results in relation to what would happen in humans. Findings of negligible irritancy (or even in the very low mild irritant range) should therefore be of no concern unless the product under the test is to have large-scale and prolonged dermal contact with humans.

Problems in Testing (and Their Resolutions)

Some materials, by either their physicochemical or their toxicological natures, generate difficulties in the performance and evaluation of dermal irritation tests. The most commonly encountered of these problems are due to compound volatility, pigmented material, and systemic toxicity.

Compound Volatility

It is sometimes necessary or desirable to evaluate the potential irritancy of a liquid that has a boiling point between room temperature and the body temperature of the test animal. As a result, the liquid portion of the material will evaporate off before the end of the testing period. There is no real way around the problem; it is thus important to make clear in the report on the test that the traditional test requirements were not met, though an evaluation of potential irritant hazard was probably achieved (because the liquid phase would also have evaporated from a human that it was spilled on).

Pigmented Material

Some materials are strongly colored or discolor the skin at the application site. This makes the traditional scoring process difficult or impossible. One approach is to try to remove the pigmentation with a solvent; if successful, the erythema can then be evaluated. If use of a solvent fails or is unacceptable, another possibility is to (wearing thin latex gloves) feel the skin to determine if there is warmth, swelling, and/or rigidity – all secondary indicators of the irritation response.

Systemic Toxicity

On rare occasions, the dermal irritation study is begun only to have the animals die very rapidly after test material is applied.

In Vivo Study Design Alternatives and Innovations

In vivo alternative approaches to evaluating dermal toxicity are limited to one other dose site and two other species of small animals. These are the guinea pig, mouse ear, and rabbit ear tests. Gilman has previously presented a short overview of these three alternatives, but some additional information has since become available.

Guinea Pig

The response of the guinea pig has been reported as being less severe and more like that of a human, and there have been recommendations that it be the species of choice with the test being performed in the same manner as in the PDI. FIFRA guidelines, indeed, name the guinea pig as an alternative species for the PDI test. However, the rabbit is cheaper and its larger size makes multiple patching more practical than is possible in the guinea pig.

Mouse Ear

The ear of the albino mouse has been proposed as an alternative test system. As originally proposed by the author, the test was performed as follows:

- Ten microliters (liquid) or 10 mg (solid paste) is applied to the dorsal aspect of one ear; the other ear serves as a control.
- Test material is applied topically, daily on four consecutive days.
  Dermal reactions are read on Day 5 as follows:
  0: No visible blood vessels or erythema
  2: Few blood vessels, barely visible; no erythema
  4: Main blood vessels visible on lower half of ear; slight erythema over lower third or base of ear
  6: Main blood vessels more obvious; suggestion of capillary network of tips of main vessels; slight or generalized erythema
  8: Main blood vessels extended to edge of ear; more extensive capillary network between main blood vessels; possibly internal hemorrhage; erythema more pronounced; ear may begin to fold back and lose suppleness
  10: Pronounced blood vessels and extensive capillary network evident; marked erythema; possibly 'frilling' of ear margin
  12: Pronounced blood vessels and extensive capillary network extending to ear margins; severe erythema; frilling and thickening of ear margins; crusting more in evidence
  14: Pronounced blood vessels and severe erythema; obvious thickening of ear; possibly necroses; crusting may extend over whole ear surface.
- Daily differences between control and treated ears for each animal are added. A correction is given for any difference between the control and treated ears initially, divided by 5 and interpreted as follows:
  0–9: Probably not irritating to human skin
  10–15: May be slightly irritating to some users
  Over 15: Likely to prove sufficiently irritating to elicit user complaints at unacceptable levels.

Patrick utilized the mouse ear model in 1985 to evaluate dermal irritants and try to distinguish mechanisms behind irritation. Gad published a paper in

1986 in which a new method for evaluating dermal sensitization was described, but in doing so, they also presented a substantial amount of dermal irritation data arising from a mouse ear model.

Rabbit Ear

Over the years, several people have proposed a dermal irritation evaluation model based on the test material being applied to the inside surface of the rabbit ear. The advantages are that this site does not have to be shaved and the results may not over predict the toxicity as much. Seemingly no formal evaluation of a method based on this site has been performed and published.

The reader should also be aware that there are a variety of cumulative irritancy test designs available, such as the guinea pig immersion test and the 21-consecutive-day occluded patch test in rabbits.

In Vitro Alternatives

The state of development of alternative models for dermal irritation or corrosion is improving rapidly. As was noted previously, though there have been attempts to utilize other animals as models, these have not been well received nor widely adopted – nor do they seem to offer better results. Examples of *in vitro* alternatives are provided in **Table 3**. These and other such alternatives can be divided into a number of categories as can be seen in the following discussion.

Physicochemical Test Methods

Analysis of the physicochemical properties of test substances, including the pH, absorption spectra, partition coefficients, and other parameters, often can be used to assess potential dermal toxicity. According to OECD guidelines, substances with a pH <2 or >11 do not need to be tested for irritancy *in vivo*. The potential effects of acids and bases to produce irritancy have been well established.

Physicochemical analysis has evaluated the particular chemical properties of test substances, which have been identified as key structural components contributing to penetration, irritation, or sensitization. Absence of absorption in the ultraviolet (UV) range also has been used to suggest lack of photoirritant potential. Physicochemical tests are rapid, cost-effective, easily standardized, and transferable to outside laboratories.

Physiologically based pharmacokinetic modeling (PB-PK) accurately describes nonlinear biochemical and physical processes; computer hardware and software based on physiological and pharmacokinetic principles can be used for extrapolation between *in vivo* exposure conditions, doses and species.

For penetration, a partition coefficient of the test sample provides a useful guide. The size of a chemical is also indicative of potential penetration. Many of the physicochemical properties of surfactants have been found to be potential indicators of their action on skin.

Target Macromolecular and Biochemical Systems

Test methods which utilize the analysis of biochemical reactions or changes in organized macromolecules can be used to evaluate toxicity at a subcellular level. Because of their simplicity, they can be readily standardized and transferred to other laboratories to provide yardstick measurements for varying degrees of dermal toxicity.

One *in vitro* irritation prediction method that utilizes nonbiological, nonliving substances can be described as a biomembrane barrier–macromolecular matrix system. This method is known as the Skintex system. The Skintex system makes use of a two-compartment physicochemical model incorporating a keratin/collagen membrane barrier and an ordered macromolecular matrix. The effect of irritants on this membrane is detected by changes in the intact barrier membrane through the use of an indicator dye attached to the membrane. The dye is released following membrane alteration or disruption, which can occur when the synthetic membrane is exposed to an irritant. A specific amount of dye corresponding to the degree of irritation can be liberated and quantified spectrophotometrically. The second compartment within the system is a reagent macromolecular matrix that responds to toxic substances by producing turbidity. This second response provides an internal detection for materials, which disrupt organized protein conformation after passing through the membrane barrier.

Test samples can be applied directly to the barrier membrane as liquids, solids, or emulsions and inserted into the liquid reagent. The results are directly compared to the Draize dermal irritation results.

More that 5300 test samples have been studied in the Skintex system, including petrochemicals, agrochemicals, household products, and cosmetics. The reproducibility with standard deviations of 5–8% is excellent. New protocols applicable to very low irritation test samples and alkaline products have increased the applicability of this method. Skintex validation studies resulting in an 80–90% correlation to the Draize scoring have been reported by S.C. Johnson & Son and the Food and Drug and Safety Center.

Thus far, most *in vitro* irritation methods, including Skintex, have relied heavily on the vast Draize rabbit skin database for validation. As previously discussed, the discrepancies in the information generated by the Draize system raise questions about the applicability of this information to irritation reactions in man.

A new Skintex protocol called the 'human response assay' optimizes the model to predict human irritation. A collaborative study with Dr. Howard Maibach and co-workers at the University of California at San Francisco demonstrated good correlations to human response for pure chemicals with diverse mechanisms of dermal toxicity. Ongoing studies have evaluated pure chemicals, surfactants, vehicles, and fatty acids.

The Skintex test is a rapid, standardized approach with well-refined protocols and an extensive database. The results produced are contiguous with the historical *in vivo* database. However, the method cannot predict immune response, penetration, or recovery after the toxic response.

Cell Culture Techniques

In vitro cytotoxicity tests that indicate basic cell toxicity by measuring parameters such as cell viability, proliferation, membrane damage, DNA synthesis, or metabolic effects have been used as indicators of dermal toxicity.

The most commonly used approaches are the neutral red assay (cell viability and membrane damage), the Lowry (labeled proline), Coomassie blue, and Kenacid blue assays (cell proliferation and total cell protein), the MTT or tetrazolium assay (mitochondrial function), and the intracellular lactate dehydrogenase activity test (cell lysis).

In the neutral red (cell viability) and total protein (cell proliferation) assays, cells are treated with various concentrations of a test substance in petri or multiwell dishes; after a period of exposure, the substance is washed out of the medium. (An analytical reagent is added in the case of protein measurements.) Neutral red is a supravital dye, which accumulates in the lysosomes of viable, uninjured cells, and it can be washed out of cells, which have been damaged. In the protein test, Kenacid blue is added and reacts with cellular protein. Controlled cells are dark blue; killed cells are lighter colored. The IC_{50} (the concentration which inhibits by 50%) is determined; the test can be rapidly performed with automation. However, materials must be solubilized into the aqueous cell media for analysis. For many test materials this will require large dilutions which eliminate properties of the materials which cause irritation.

The MTT test assays mitochondrial function by measuring reduction of the yellow MTT tetrazolium salt to a blue insoluble product. It has been compared with the neutral red technique for testing the cytotoxicity of 28 test substances, including drugs, pesticides, caffeine, and ascorbic acid. With the mouse BALB/c 3T3 fibroblast cell line, for any given cell density the two assays ranked the test substances with a correlation coefficient of 0.939 on the basis of IC_{50} concentrations. The two assays did differ in sensitivity for a few test agents, suggesting that a combination of the two might be most effective.

Some cytotoxicity tests are likely to underestimate the toxicity of chemicals, which are metabolically activated in the body, but this problem can be overcome by the addition of liver enzymes, preferably from a human source to eliminate species differences.

Inhibition of mitogen-stimulated thymidine incorporation in human peripheral blood mononuclear cells has been reported as a method for screening for photosensitizers. Cells from at least three volunteers were used for testing each chemical.

Microorganism Studies

An important method using fungi is Daniels' test for phototoxicity, which utilizes the yeast *Candida albicans* as the test organism. A 1988 study compared favorably the results of this test with the results of photo-patch testing in volunteers for samples from six furocoumarin-containing plants. Many test materials which produce an erythemic response in the photoirritant test are not analyzed as positive in this test. A new test method, Solatex-pi, has demonstrated capability to predict the potential for photoirritation of materials in this class as well as that of other well-known photoirritants. Solatex-pi utilizes the two compartment physicochemical model of Skintex to predict the interactive effects of specific chemicals and UV radiation. Solatex-pi is being validated by Frame and the BGA (Zebet) as an *in vitro* test to predict photoirritants.

Human Tissue Equivalents

Human skin equivalents have been developed by several laboratories. One equivalent, Testskin, consists of human keratinocytes seeded onto a collagen base or collagen-glycosaminoglycan matrix containing human fibroblasts. In many respects, the epidermis which develops resembles epidermis *in vivo*. The tissue culture system survives for several weeks and may be useful in studying skin penetration. Testskin is a commercially produced skin equivalent system marketed by Organogenesis, Inc. (Cambridge, MA);

it is currently being assessed for use in skin penetration studies. Several companies launched studies of Testskin in 1990 and 1991.

Marrow-Tech, Inc. (Elmsford, NY) has also developed a human skin model. Marrow-Tech's skin equivalent consists of (1) a dermal layer of fibroblasts and naturally secreted collagen and (2) an epidermal layer of keratinocytes separated by a dermal–epidermal junction. Whereas Testskin uses bovine collagen, Marrow-Tech's skin model consists solely of human tissue.

Submerged skin co-cultures consisting of NHEK/DF cultured human skin models (neonatal foreskin, fibroblasts, and keratinocytes are grown on a 3-D nylon mesh substrate) are used in a battery of short term cytotoxicity endpoints for prediction of human skin responses to irritants *in vitro*. Several variations with many different endpoints have been developed using human skin models. These methods include, but are not limited to: NHEK/DF, using dermal fibroblasts; NHEK/NR, using neutral red in the cell viability method described previously; and NHEK/SC, which yields more accurate results when the test material is an acid, base, insoluble or neat (EpiDerm is an example of a NHEK/SC model).

All of these skin equivalent methods permit higher concentrations of test samples to be studied. However, dilutions are still necessary when, based on the physical chemistry of the test sample, the chemical structure may be responsible for irritation. Many protocols and endpoints have been evaluated as predictive of eye or skin irritation.

Isolated Tissue Methods

Skin tissues isolated from rats, rabbits, and humans have been monitored *in vitro* to predict penetration and irritation. The rat epidermal slice technique has been validated as a screen for corrosive substances. The electrical impedance changes as the integrity of the stratum corneum is altered. The use of this technique to predict irritancy is being investigated in the United Kingdom. Another method studies enzyme changes when a substance is applied to a slice whose lower surface is bathed in culture medium. Enzyme changes separate irritant and nonirritant chemicals.

Human cadaver skin has also been studied *in vitro*. Human skin shows a higher threshold of sensitivity than does rat skin. The excised or full-thickness slices are also studied in Fran 2 diffusion chambers to evaluate the diffusion or absorption characteristics of test materials. Changes in the amount of a test material at different times and different depths are monitored and are very useful in predicting penetration rates for simple solutions and solvents.

Human Volunteer Studies

Human volunteer studies are widely used to assess skin irritation, penetration, and sensitization. Much knowledge in the dermal toxicology field was previously obtained using human test panels. An advisory committee of the National Academy of Sciences in 1997 outlined a procedure for a human 24 h patch test. This procedure details use of a normal nondiseased skin area at the intrascapular region on the back (up to 10 sites) or the dorsal surface of the upper arm (up to four sites each arm). Using reference material, the procedure is to first test for 0.5–1 h without an occlusive patch. Next, for 4 h, using 1 in. gauze squares with the same amount of test material as in Draize type animal tests, express the dose in $mg\,cm^{-2}$. If using different materials and sizes of occlusive patches, different amounts of test substances are needed. Secure the patch with surgical tape, do not wrap. After a given amount of time, remove the patch, rinse the area with water and mark the test sites. Evaluate after 30 min to 1 h and at 24 h rate using the Draize scale.

Many industries regularly conduct repeat insult patch tests on human volunteers to evaluate topical irritancy. Groups of human volunteers are patched with test substance. One to five concentrations can be tested simultaneously, a wide enough range to yield results relevant to the usage. Cumulative skin irritancy is measured by applying patch applications each day for 3 weeks. Skin irritation is usually assessed visually, but blood flow and skin temperature can be measured objectively by laser Doppler flowmetry, ultrasound Doppler, heat flow disk measurement, sensitive thermocouple devices, or noncontact infrared radiative techniques. In these tests, dose–response curves can be obtained. Skin thickness can be measured with calipers as a measure of edema formation.

Human volunteers are also used in many industries in tests for allergic sensitization by cosmetic substances and formulations. The repeat insult patch test includes an induction phase (repeat applications during 3 weeks) and a 2 week rest period (incubation phase), followed by a challenge to see if sensitization has occurred. A pilot study of 20 human volunteers can be followed by more extensive testing (80–100 subjects). Positive results at more than the 10% level in the human volunteers would suggest a major problem with the formulation. User tests with the sensitized individuals and nonreactive matched control subjects can often determine the importance

of these results to end use. Such a procedure may determine whether the sensitivity is significant under normal conditions of product use. Broader tests can be carried out with 250–500 subjects.

Conclusions

Whole animal tests represent true physiological and metabolic relationships of macromolecules, cells, tissues, and organs and can be used to evaluate the reversibility of toxic effects. However, these tests are costly, time consuming, insensitive, difficult to standardize, and are sometimes poorly predictive of human *in vivo* response.

New *in vitro* test methods target the behavior of macromolecules, cells, tissues, and organs in well defined methods, which control experimental conditions and standardize experimentation. These tests provide more reproducible, rapid, and cost-effective results. In addition, more information at a basic mechanistic level can be obtained from these tests. **Table 3** provides a summary of current test systems.

The challenge of the twenty-first century will be to understand the capabilities and limitations of these methods. Combining information on new molecules obtained from structure–activity relationships with results on macromolecular alterations in Skintex that occur for undiluted molecules may provide more information on dermal toxic effects of particular chemical classes. Combining test methods can provide a greater understanding of the mechanisms of dermal toxicity. Test batteries evaluating cell cytotoxic responses at high dilutions and changes in macromolecules at low dilutions will be more informative than visual scoring of complex events *in vivo*.

See also: Analytical Toxicology; Animal Models; Eye Irritancy Testing; Hypersensitivity, Delayed Type; Organophosphates; Photoallergens; Poisoning Emergencies in Humans; Radiation Toxicology, Ionizing and Nonionizing; Skin; Tissue Repair; Toxicity Testing, Alternatives.

Further Reading

Hobson DW (1991) *Dermal and Ocular Toxicology*. Boca Raton, FL: CRC Press.

Marzulli FN and Maibach HI (2004) *Dermatoxicology*, 6th edn. Philadelphia, PA: Taylor and Francis.

McDougal JN and Boeniger MF (2002) Methods for assessing risks of dermal exposures in the workplace. *Critical Review in Toxicology* 32(4): 291–327.

Relevant Websites

http://www.epa.gov – Health Effects Test Guidelines, OPPTS 870.1200, Acute Dermal Toxicity – From the US Environmental Protection Agency.

http://www.oecd.org – OECD Guideline for Testing of Chemicals, Proposal for a New Draft Guideline 434, Acute Dermal Toxicity – Fixed Dose procedure, May 14, 2004.

Toxicity Testing, Developmental

Rochelle W Tyl

Introduction

Human concern with birth defects is as ancient as human awareness. Through the nineteenth century, the prevailing view was that; 'maternal impressions', maternal experience during the pregnancy, directly affected the newborn. Teratology, the study of monsters (terata), was essentially an observational 'art' with perceived supernatural implications. The development of basic concepts of genetics early in the twentieth century provided a scientific basis for causation of congenital defects. The recognition that environmental insult also produced birth defects in mammals inexorably followed, such as ionizing radiation (1907), sex hormones (1917), dietary deficiencies (1933), and chemicals (1948). The supposed safety of the human conceptus was refuted by German measles (rubella) epidemics in Australia in 1941 and in the United States in 1964 that resulted in thousands of children born with cataracts, deafness, and congenital heart disease from infected pregnant mothers.

The thalidomide 'epidemic' in the late 1950s and early 1960s, involving at least 8000 malformed children in 28 countries, confirmed the vulnerability of the human conceptus to environmental insult, especially in the first trimester of pregnancy. It also precipitated worldwide concern for the safety of the unborn and the role of governments to ensure testing of drugs and other chemicals in pregnant mammals.

The early term for the study of birth defects, teratology, has been supplanted by a more general term, developmental toxicology, to enable inclusion of a more diverse spectrum of adverse developmental outcomes (which may be separate and distinct in

etiology or the result of a continuum of response) and to make overt the recognition that specific results of insult in one species may not be the same in other species, including humans.

Developmental toxicity may be currently defined as any structural or functional alteration, caused by environmental insult, which interferes with normal growth, differentiation, development, and/or behavior. The targets for such insult(s) include the fertilized egg or zygote prior to implantation and the establishment of the three primary germ layers – the embryo during the period of major organ formation (i.e., organogenesis), the fetus in the postembryonic period of histogenesis, and the neonate or postnatal offspring, occurring or expressed through the postnatal period until sexual maturity. The expressions of developmental toxicity encompass death, frank structural malformations, functional defects, and/or developmental delays.

Factors Affecting the Vulnerability of the Conceptus

The vulnerability of the conceptus is viewed as due to qualitative or quantitative characteristics of both structure and function: (1) it is composed of a small number of rapidly dividing undifferentiated cells with absent or limited metabolic capabilities to alter or detoxify xenobiotics, repair lesions, etc.; (2) there is a necessity for precise temporal and spatial localization of specific cell numbers and types, as well as specific cell products, for normal differentiation, including programmed cell death; (3) sensitivities of certain cell types to certain insults may be unique to specific periods of cell movement, induction, or differentiation (i.e., transient vulnerability during the period of formation of tissues or organs); and (4) the immunosurveillance system (to provide recognition of 'self' and detection of xenobiotics or lesions) is absent or immature in the prenatal or perinatal individual.

A number of factors influence the teratogenic response. Genetic susceptibility varies among species. For example, aspirin is teratogenic in rodents but not in primates, imipramine is teratogenic in rabbits but not in humans, and thalidomide is teratogenic in primates but not in rodents. Differences also exist among strains. Inbred mouse strains differ radically in their response to many teratogenic agents, e.g., to cortisone induction of cleft palate and cadmium-induced testicular and embryotoxicity. Individuals also vary in their response to teratogenic agents in outbred strains and heterogeneous human populations. The current interpretation is that teratogens act on a susceptible genetic locus or loci which may control disposition of the agent including absorption, metabolism, transport, excretion and/or direct susceptibility of the target tissue or organ, or on genes which control the activation/inactivation of other genes. The teratogen therefore increases the incidence of previously existing malformations; its action must be viewed against the 'background noise' of spontaneous malformation rates, which also vary among species, strains, and individuals. For example, the phocomelic (seal limb) syndrome, induced by thalidomide, occurs at a low rate spontaneously in human populations; $\sim$20–80% of the human fetuses, exposed to the 'appropriate' dose of thalidomide at the 'appropriate' time, developed the malformations.

There is some specificity of agent on the teratological response, with acetazolamide causing perhaps the most specific lesion – right forelimb postaxial ectrodactyly (fourth and fifth digits); at higher doses, other structures are affected. However, there are almost always effects on other systems derived in many cases from different primary embryonic germ layers. The gestational stage of the embryo or fetus at the time of environmental insult appears to be the most critical determining factor. The predifferentiation period, from fertilization to establishment of the three primary embryonic germ layers, has been considered refractory to teratogenic agents (although there are some exceptions such as hypoxia, hypothermia, actinomycin D, and ethylene oxide). This resistance has been explained as due to the small, omnipotent cell population of the pre- and immediately postimplantation embryo. Cell damage or death is either corrected for by the surviving cells, which regulate to produce a normal albeit small term fetus, or the cell loss is so devastating that the embryo dies. Once implantation and establishment of the primary germ layers have occurred, the major period of organogenesis begins – a period of $\sim$10 days in rodents and 58 days in humans. This is the period of maximal susceptibility to teratogenic agents causing structural anomalies. Even within the organogenic period, there are differential susceptibilities of embryonic organ systems to teratogenic agents. For example, administration of an agent on gestational day (gd) 10 in the rat affects eye, brain, heart, and anterior axial skeletal development. The same agent, at the same dose, administered on gd 15, affects palate, urogenital, and posterior axial skeletal system development. These times of specific sensitivity need not correspond to the morphological appearance of the organ or organ system but rather to the time of cell biochemical commitment: the shift of cells from presumptive to determined status.

Once histogenesis has begun, defined as the differentiation of tissue-specific biochemical and morphological characteristics, the conceptus is termed a fetus and is viewed as increasingly refractory to teratogenic agents. However, this is true only of most morphological or structural manifestations. Increasing evidence indicates susceptibility of the fetus to agents causing functional deficits that presumably have a biochemical or microstructural basis. Those systems not yet complete, especially the nervous system, are most vulnerable. For example, vitamin A, lead, and methylmercury cause neurofunctional lesions when administered during this period. In addition, chemicals such as diethylstilbestrol and ethylnitrosourea act during this period to produce a system-specific tumor after a long latency in the postnatal mature animal. However, the only exposure and therefore the initiation of the later carcinogenic event occurs *in utero*, and these agents are therefore called transplacental carcinogens.

The route and duration of administration of the agent are also critical for the development of the teratogenic anomaly. Human industrial exposure is almost always by inhalation or percutaneous absorption of fumes, dusts, aerosols, or vapors. Consumer or other end-use or accidental exposure would be by more varied routes. Experimental evaluations are most useful if they duplicate the human route of exposure for experimental animal models. First-pass organ absorption and metabolism may differ if the exposure is by inhalation to the lung or orally to digestive system and liver, although subsequent transport and organ exposure may yield equivalent metabolite patterns. Most teratology studies usually employ administration of the test compound in the feed, by oral intubation, or by injection into the pregnant animal.

Timing is important. Experimental exposure before implantation or during early organogenesis may result in interference with implantation or in early embryonic death, resulting in no term fetuses to examine. Exposure before peak susceptibility or repeated exposure may induce activating and/or detoxifying enzymes in dam, placenta, and/or fetus. This may result in increased or decreased blood levels of the active metabolite in the dam and, therefore, altered exposure to the fetus. Conversely, these enzymes may be inhibited by accumulation of metabolite(s), again altering blood levels of parent compound and metabolite(s). Other effects of repeated or early exposure may be to alter liver or kidney function, for example, as well as to induce pathological changes in these organs that will affect quantity and quality of compound reaching the fetus. Saturation of protein-binding sites may also occur in the dam to alter transport of essential nutrients, vitamins, elements, hormones, etc. All of these effects may alter disposition parameters and obscure or change any teratological effects of the agent being examined.

Dose range and schedule are also critical. Three or four dose levels are usually employed: A high dose, which is toxic to the maternal organism, perhaps lethal up to 10% of dams, is used essentially to obtain an effect and to establish target organ(s); mid-dose(s), which is embryotoxic or embryolethal and possibly teratogenic; and a low dose which is comparable on a body weight basis to possible human exposure levels or small multiples thereof.

Categories of Teratogenic Agents

Many substances are known to cause malformations in one or more species of mammals. Almost all known human teratogens are drugs, with data generated by drug research companies adhering to US FDA guidelines for reproductive testing of drugs, and there is an awareness that in our drug-permissive society, women consume an average of four drugs, both by prescription and over-the-counter administration, during pregnancy. Various texts and tests have identified from 600 to 1200 drugs as teratogens in animals, only 20 of which are currently documented as human teratogens.

Human teratogenic agents have been discovered initially from anecdotal observations and then more rigorously examined in epidemiological studies and confirmed with animal studies, or they have initially been identified in animal studies with subsequent confirmation by human data. Animal model researchers have suggested that any agent positive in two or more mammalian species must be considered a suspect human teratogen.

Approximately 7% of all live-born humans bear birth defects. This value may be as high as 10% if children are evaluated to age 10 years to include subtle structural or functional deficits such as minimal brain dysfunction or attention deficit disorders. More than 560 000 lives out of $\sim$3 million births per year in the United States are lost through infant death, spontaneous abortion, stillbirths, and miscarriage due presumably to defective fetal development. The relative contributions to human teratogenesis have been estimated as follows: known germinal mutations, 20%; chromosomal and gene aberrations, 3–5%; environmental causes such as radiation, $<$1%; infections, 2% or 3%; maternal metabolic imbalance, 1% or 2%; drugs and environmental chemicals, 4% or 5%; contributions from maternal dietary deficiencies or excesses and

combinations or interactions of drugs and environmental chemicals are unknown. The contribution from unknown sources is 65–70%. The estimated 20–25% pregnancy loss due to chromosomal aberrations may be even higher due to early losses currently diagnosed as late menstrual bleeding. Recovered tissues from spontaneous abortions prior to the thirteenth week of gestation exhibit chromosomal anomalies on the order of 560 per 1000 abortions; the value at term is 5 per 1000. Of the children born alive who subsequently die in the first year of life, ~20% of the deaths are associated with or caused by birth defects, more than any other single factor.

One almost plaintive maxim, sometimes termed Karnofsky's law, states that almost any substance may be teratogenic if given in appropriate dose regimens to a genetically susceptible organism at a susceptible stage or stages of embryonic or fetal development.

Government Regulation

Soon after the worldwide thalidomide disaster in 1966, governmental regulation of the evaluation of test agents for developmental toxicity by formal testing guidelines and rules began when the US FDA established *Guidelines for Reproductive Studies for Safety Evaluation of Drugs for Human Use*. These guidelines were promulgated 'as a routine screen for the appraisal of safety of new drugs for use during pregnancy and in women of childbearing potential'. Three phases or segments were proposed: Segment I, Study of Fertility and General Reproductive Performance, provides information on breeding, fertility, nidation, parturition (birth), neonatal effects, and lactation (see Reproductive Toxicity); Segment II, Teratological Study, provides information on embryotoxicity and teratogenicity; and Segment III, Perinatal and Postnatal Study, provides information on late fetal development, labor and delivery, neonatal viability, and growth and lactation (**Figure 1**).

Segment II testing guidelines are currently followed by US FDA (since 1966), US EPA, Toxic Substances Control Act (TSCA) (since 1985), and Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (since 1982). The US EPA recently revised their reproductive and developmental toxicity testing guidelines (US EPA, OPPTS (Office of Prevention, Pesticides and Toxic Substances), 1998). International regulations also followed suit: Organization for Economic Cooperation and Development (OECD, 1981), Great Britain (1974), Japan (1984), and the European Community (1994). The International Conference on Harmonisation (ICH) also promulgated modified guidelines in 1994, adopted by the European Community, Japan, and the United States (FDA) in 1996.

Considerations for Segment II Studies

The test animals are usually rodents and nonrodents. The rodent of choice is usually the rat and, less often, the mouse. Both species satisfy the need for a small mammalian species with known (and relatively straightforward) husbandry requirements, short pregnancy, high fertility, large numbers of offspring, a low background incidence of spontaneous malformations, and a reasonably well-known embryology. The nonrodent species is usually the rabbit. The rabbit is not a rodent; mice and rats belong to the order Rodentia and rabbits to the order Lagomorpha. The requirement for the use of rabbits is predicated on the awareness that it was the only common test mammal in use in the 1960s which responded to thalidomide and (it is hoped) would have indicated the prenatal risk to humans, and on the need to distinguish between agents with specific or unique specificity (i.e., a rodent-specific teratogen) and those with more universal effects, presumably then also a greater potential risk to human development.

Prenatal development in the Rodentia and Lagomorpha differs in significant ways from that in humans. All three have a chorioallantoic placenta, but the structure differs among species. The human and rat placenta also differ functionally with different secretory patterns of placental lactogen and with the presence in primates of chorionic gonadotropin. What effect, if any, these differences have on placental transport is not fully understood. In addition, rodents and lagomorphs also form a yolk sac placenta immediately after implantation, which is the major (only) mechanism for nutrient processing and transport until gestational day (gd) 11–11.5, and persists as functional, even when the chorioallantoic placenta forms, almost to parturition. Again, what effect this has on embryo and fetal vulnerability is not yet known, although at least one rodent teratogenic agent, trypan blue, appears to act solely on the yolk sac placenta. In multifetal pregnancies, there are differences in blood flow to left and right uterine horns and to implants at ovarian versus cervical ends of the uterine horns. Different fetuses within the same dam have been shown to be at differential risk. In addition, fetal loss is handled differently in test animals: Dead implants are not expelled in a spontaneous abortion as in single-birth mammals but are resorbed *in situ*. It is not uncommon to recover healthy, viable fetuses side by side with large numbers of resorption sites. Maternal, placental, and fetal metabolism of

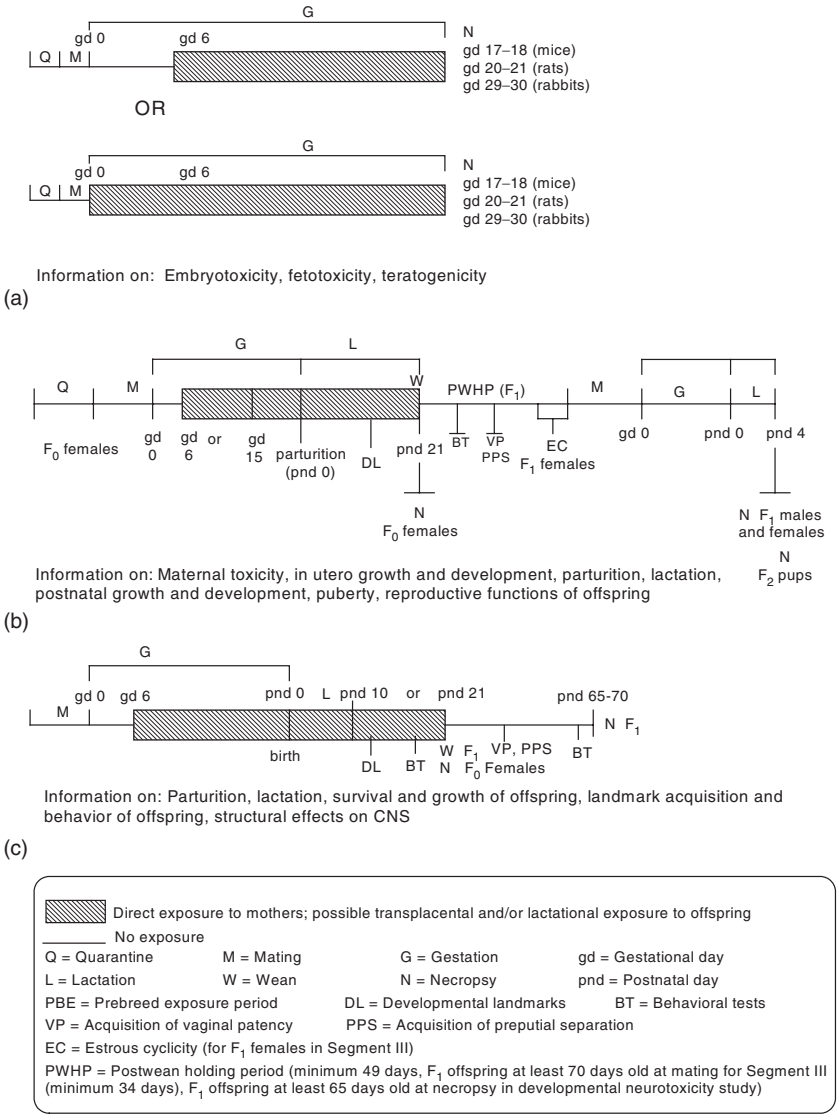

Figure 1 US governmental guidelines for study designs of developmental toxicity assessments in animal models. (a) FDA Segment II – developmental toxicity study; (b) FDA Segment III – perinatal and postnatal study; (c) EPA developmental neurotoxicity study.

xenobiotics may also differ, hence the need for prior characterization, at least, of the test organism's metabolic capabilities of the substance to be tested.

The placenta is both a transport and a metabolizing organ. Transport is accomplished by simple diffusion, facilitated diffusion, active transport across membranes, and by special processes such as pinocytosis, phagocytosis, specific transport molecules, and channels in the 'barrier'. The placenta also contains a full complement of mixed function oxidases located in the microsomal and mitochondrial subcellular fractions capable of induction and metabolism of endogenous and exogenous chemicals.

Metabolism in the test dam and/or fetus and its relevance to the pregnant human is critical. For example, the parent compound may be teratogenic and is metabolized to innocuous products as with diphenylhydantoin, an antiseizure drug used in the treatment of epilepsy. In contrast, the parent compound may be harmless and metabolized to the proximal teratogenic agent as with chlorcyclizine, an antihistamine metabolized *in vivo* to the active teratogen norchlorcyclizine. One of the current hypotheses concerning the mechanism of thalidomide-induced teratogenesis suggests that thalidomide is transmitted to the human fetus and metabolized to a more polar metabolite(s), the putative proximal teratogenic agent(s), which cannot cross the placenta back to the maternal organism for further metabolism and excretion. This sequence may be qualitatively or quantitatively different in the insensitive pregnant rodent. In contrast, imipramine, an

antidepressant, is teratogenic in rabbits in which blood levels of the parent compound stay high. In the human, imipramine is rapidly metabolized by demethylases and is not teratogenic.

Pregnancy *per se* causes many physiological changes, which may alter over the duration of the pregnancy. These changes include alterations in gastrointestinal function which may affect transport and absorption rates of chemicals in the stomach and/or intestine, and ventilatory changes which may modify pulmonary uptake, absorption, and/or elimination of chemicals with a 20–30% increase in maternal oxygen consumption and greater oxygen debt after physical activity (in humans). Changes also occur in the cardiovascular system that alter hemodynamics (there is a 30–40% increase in blood volume and a 33% decrease in erythrocytes in humans) and alter body water compartments that may influence distribution and elimination of chemicals. Plasma components with roles in chemical binding, transport, and disposition also change during pregnancy. Renal elimination is normally enhanced and hepatic metabolism may be modified during pregnancy, also affecting xenobiotic metabolism and/or elimination.

The concern for thorough evaluation of the maternal organism is based on the need to determine, when study results are interpreted, whether maternal toxicity, *per se*, is responsible for the observed embryo/fetal results. A number of fetal malformations in rodents and rabbits have been identified which are observed in the presence of maternal toxicity, regardless of the agent, route, or dose, with the clear implication that maternal toxicity is the cause of developmental toxicity, not the test agent. Mechanistic studies have also implicated the compromised status of the maternal organism as the cause for the adverse embryo/fetal outcome for many drugs. For example, elevation of endogenous corticosteroids, as a result of maternal stress irrespective of the source, or administration of exogenous corticosteroids results in cleft palate in offspring from susceptible strains of mice; hypercapnia (elevated blood CO_2) in mice has been proposed as the cause of forelimb ectrodactyly in mice exposed to acetazolamide, and bradycardia (slowed heart rate) in mice from phenytoin administration has been suggested as the cause of cleft lip/palate in offspring. Renal toxicity from mercuric chloride exposure to the mother may be the cause of hydrocephalus in the offspring. If maternal toxicity *per se*, including even 'stress' from restraint, for example, is the cause of developmental toxicity, then the classification of the test agent as a teratogen may be erroneous.

In addition, information on maternal toxicokinetics and metabolism of the test agent is essential to characterize the conditions under which toxicity to dam or conceptuses is observed; these conditions include evidence of systemic exposure, blood levels of parent compound and/or metabolite(s), identification of metabolites, bioavailability, half-life, and evidence for or against bioaccumulation. This information is necessary to extrapolate results from one route to another, from one species to another, and for human risk assessment. That is, the handling of the test agent by the test species must be characterized so that one can say that, for a specific test agent, maternal and/or developmental toxicity occurs in the presence of the parent compound or identified metabolite(s) at specific blood levels for a specified duration, with the expectation that another species that produces the same metabolite(s) at comparable levels for comparable duration will exhibit the same or similar toxicities. In the absence of metabolic information, one cannot assess whether the test animal is an appropriate surrogate for humans for specific test agents. Histopathology of target organ(s) and organ function tests may also be appropriate.

Standard Segment II Testing Protocol

In brief, the current US EPA and OECD guidelines for the Segment II study consists of exposure of a pregnant rodent species (rats or mice) and a nonrodent species (usually rabbits) to the test agent during organogenesis and fetogenesis, sacrifice of the maternal animals 1 or 2 days prior to the date of expected parturition, Caesarean delivery of the gravid uterus, and thorough evaluation of the fetuses by examination of external, visceral (including craniofacial), and skeletal structures.

The current guidelines call for at least 20 rodent and rabbit litters per group (although the previous guidelines called for 12 rabbit litters and 20 rodent litters) and at least four dose groups (three agent-exposed groups and a concurrent vehicle control group). On gd 0, mated animals are placed on study and randomly assigned or assigned by a randomization procedure (stratified by body weight) into test groups.

The current testing guidelines specify dosing from implantation to term with no postdosing recovery period. Previous testing guidelines specified the period of exposure of the maternal organism to the test agent only during the period of major organogenesis. This corresponds to gd 6–15 for rodents and gd 6 or 7 through 18 or 19 for rabbits. This period of dosing was specifically chosen to preclude efforts on implantation so there would be conceptuses to evaluate and to maximize the chances of inducing and detecting structural changes in the conceptuses

(beginning dosing at fertilization (gd 0) is recommended only if there is evidence that there are no effects on the preimplantation conceptus). The possible effects of the test agent on the reproductive and developmental processes prior to organogenesis are evaluated in Segment I or multigeneration studies. The start of dosing for the previous and current testing guidelines precludes induction of maternal metabolizing enzymes prior to the presence of implanted conceptuses. The previous testing guidelines also allowed for a postexposure recovery period prior to scheduled sacrifice close to term. Variations in the exposure period in the current guidelines include exposure during the entire gestational period (gd 0 to term sacrifice), or exposure beginning prior to gd 0. These latter extended exposure periods may be useful and appropriate if the test agent, or route of administration, results in slow and/or limited systemic absorption and therefore delayed attainment of steady state or maximal blood levels in the maternal organism. In these circumstances, the usual dosing period could result in the conceptuses exposed to less than maximum levels during some or most of organogenesis and the misleading conclusion of little or no developmental toxicity. Extended exposure periods may also be called for if bioaccumulation of or cumulative toxicity from parent compound or metabolites is an important aspect of known or potential human risk.

The guidelines specify the preferred route of administration as gavage (orogastric intubation) to deliver the largest possible bolus dose in order to maximize the potential of the test agent to cause maternal and developmental toxicity, i.e., 'worst case scenario', and to control the delivered dose to the maternal animal. Use of other routes to simulate possible human exposure situations is becoming increasingly popular and is acceptable if scientifically defensible. These alternative routes include dosed feed, dosed water, inhalation by whole body or nose-only exposure, cutaneous application, injection by intravenous, subcutaneous, intraperitoneal, or intramuscular routes, or subcutaneous insertion (for implants or for minipumps for continuous infusion).

Maternal data to be collected from the current Segment II studies include maternal mortality; pregnancy rate; maternal body weights on gd 0 and throughout the dosing period, and at sacrifice; sacrifice weight corrected for the weight of the gravid uterus; body weight changes through gestation, and during the treatment period; feed and/or water consumption when body weights are recorded (water consumption if the test material is administered in the drinking water or is known to affect the kidneys); clinical observations; gravid uterine weight; and weight of other organs (absolute and relative to sacrifice body weight). Additional evaluations of morphological, physiological, and/or biochemical status are suggested, such as histopathology of target organs, more detailed behavioral evaluation, clinical pathology such as hematology, clinical chemistry, and urinalysis, etc.; these could be performed on maternal animals during and after the treatment period or at necropsy. These tests may duplicate those performed in other studies, but pregnant animals may respond quantitatively or qualitatively differently (vide supra), and these data will be critical in interpretation of any observed developmental toxicity.

Reproductive and embryo/fetal data to be collected from Segment II studies at sacrifice include number of ovarian corpora lutea (number of eggs ovulated); number of total, nonviable (resorptions and dead fetuses) and live uterine implantations; and calculation of pre- and postimplantation loss. For litters with live fetuses, data collected include number, sex, and individual fetal body weights, sometimes crown – rump length, anogenital distance, individual fetal external, visceral and skeletal and total malformations, and variations reported by fetus, by sex by litter, and per fetus per litter (male and female fetuses differ in body weights, with males significantly heavier). These procedures thoroughly assess two of the four embryo/fetal end points – death and structural malformations – and also assess developmental delays, but only in terms of delays in growth such as reduced body weight, reduced crown – rump length, and delays in structural development, such as reduced ossification relative to concurrent and historical control fetuses (usually designated as variations), especially in those skeletal districts that ossify late in prenatal development. The current US EPA and OECD guidelines require visualization of both ossified and cartilaginous bone, with the method left up to the performing laboratory. Almost all laboratories perform double-staining of fetuses with alizarin red S for ossified bone and alcian blue for cartilaginous bone to provide information on the status of bones not yet ossified. These techniques aid in the interpretation of a finding as a skeletal malformation or permanent skeletal variation (when there is no cartilage in a short bone or for a missing bone, so no subsequent growth, ossification, or correction would be anticipated) versus a variation of transient delay in ossification (where there is cartilage with anticipated subsequent growth, ossification, and possible correction).

There is apparent potential for extensive remodeling of the skeletal system in the postnatal period; extra ribs become vertebral arches and fused ribs and other skeletal malformations disappear prior to

sexual maturity. This plasticity of the skeletal system may result in revision of the current classification of morphological findings in term fetuses as malformations or variations. The current definition of a malformation specifies a permanent morphological change that is incompatible with or detrimental to postnatal survival, normal growth, and development. Short ribs, extra ribs, fused ribs, alterations in sternebrae (which fuse to form the sternum), alterations in vertebral centra, and arches are currently designated malformations or variations depending on the laboratory; if these changes do not persist, their designation could change. The reverse situation is also true; that is, findings commonly designated as variations, usually delays, in term fetuses may, in fact, sometimes develop into findings designated as malformations in postnatal life. For example, a dilated renal pelvis (reduced renal papilla) may or may not be the precursor of hydronephrosis, and dilated lateral ventricles of the fetal cerebral hemispheres may or may not be the precursor to hydrocephaly in the postnatal organism. With structural evaluations of term fetuses 'frozen in time' in a Segment II study, there is no way to project the postnatal consequences of the initial findings or to identify postnatal consequences of *in utero* exposure. In addition, the term evaluation is based on structure. If the lungs or kidneys, etc., are in the right location and are the right size, shape, and color under a dissecting microscope, they are designated as normal; there is no assessment of microscopic integrity or of function. Additional evaluations of term fetuses should perhaps include biochemical assessment of organ function and histological examination of structure. However, the most important drawback in a Segment II study is the lack of postnatal assessment of the reversibility of detected structural lesions and of the structural and functional sequelae of the prenatal insult; other testing guidelines do evaluate this (see sections Standard Segment III Testing Protocol and Developmental Neurotoxicity Test).

Statistical Analyses of Maternal and Developmental Toxicity Data

As part of protocol development, the choice of statistical analyses should be made *a priori* although specific additional analyses may be appropriate once the data are collected. The unit of comparison is the pregnant female or the litter and not individual fetuses as only the dams are independently and randomly sorted into dose groups. The fetus is not an independent unit and cannot be randomly distributed to groups. Intralitter interactions are common for a number of parameters, for example, fetal weight or malformation incidence. Two types of data are collected: ordinal/discrete data, which are essentially present or absent (yes or no) such as incidence of maternal deaths, abortions, early deliveries, clinical signs, and incidence of fetal malformations or variations; and continuous data such as maternal body weights, weight changes, food and/or water consumption, organ weights (absolute or relative to body or brain weight), and fetal body weights per litter. For both kinds of data, three types of statistical analyses are performed. Tests for trends are available and appropriate to identify treatment-related changes in the direction of the data (increases or decreases), overall tests are performed for detecting significance among groups, and specific pairwise comparison tests (when the overall test is significant) to the concurrent vehicle control group values are the critical end point to identify statistically significant effects at a given dose relative to the concurrent vehicle control group. Continuous data are designated parametric (distributed along a bell-shaped curve) or nonparametric (skewed distribution), with different specific tests employed for the three types of statistical analyses depending on whether the data are parametric or nonparametric.

Risk Assessment

The US government's new approach (2000) to health assessment of agents involves the iterative interaction of four major components: basic scientific research (hazard identification), science-based toxicity/risk assessment (dose–response assessment), exposure assessment, and risk characterization (**Figure 2**). This section relies heavily on the US EPA guidelines for the health assessment of suspect developmental toxicants which describe how the government uses, and plans to use, developmental toxicity data as part of their 'weight-of-evidence' approach to both the hazard identification and the dose–response assessment components of risk assessment.

Standard developmental toxicity studies are performed, under the appropriate governmental toxicity guidelines, for a drug early in the drug discovery period (FDA), for a pesticide prior to registration (as required by EPA FIFRA), or for an industrial chemical (performed on a case-by-case basis under EPA TSCA). These studies provide information on the intrinsic capacity of the test agent to cause developmental toxicity under conditions to maximize the opportunity, that is, hazard identification, and the dosage or dosages at which the developmental toxicity (death, malformation, delays, and/or deficits) is observed, that is, dose–response assessment. Of the three dosage levels employed, the highest dose should

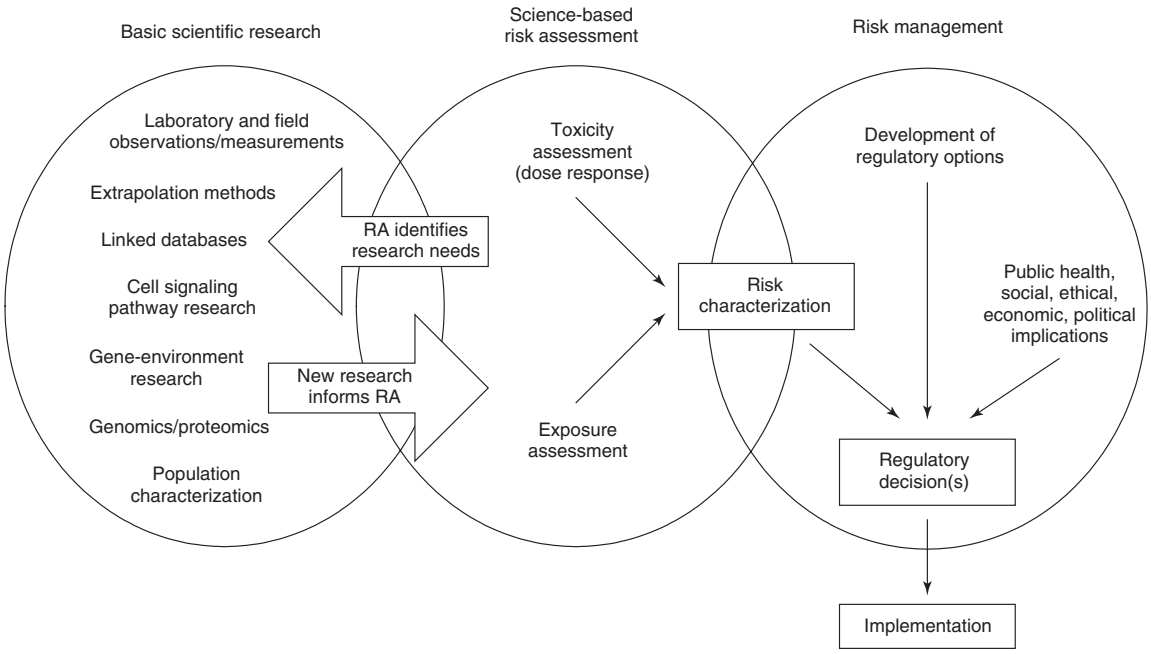

Figure 2 US National Research Council, new risk assessment/risk management paradigm. (Modified from NRC (2000) *Scientific Frontiers in Developmental Toxicity and Risk Assessment.* Washington, DC: National Academy Press.)

result in overt maternal toxicity, including significantly reduced body weight, weight gain, and specific organ toxicity, with maternal mortality up to 10% viewed as acceptable. This dose level should characterize embryo/fetal outcome in a compromised dam/doe and should represent a 'worst-case scenario' for hazard identification. However, the presence of maternal toxicity *per se* confounds the interpretation of observed developmental toxicity since these effects may be due to the status of the dam and not to the test agent *per se* (see previous discussion for maternal toxicity data).

The low dose should be a no-observed-adverse-effect level (NOAEL) for both dams and conceptuses. The NOAEL is defined as the highest dose (or exposure concentration) at which no statistically significant and/or biologically relevant adverse effects are observed in 'any adequate developmental toxicity study'. The middle dose may or may not result in maternal and/or developmental toxicity and should be a lowest-observed-adverse-effect level (LOAEL), defined as the lowest dose or exposure concentration at which a statistically significant and/or biologically relevant adverse effect is observed in 'any adequate developmental toxicity study'. The characteristics of the NOAEL (or the LOAEL) are that (1) it is obviously experimentally derived and therefore dependent on the statistical power of the study (which is in turn dependent on the number of animals employed); (2) it is dependent on the number and sensitivity of the parameters examined; and (3) its presence implies a 'threshold', that is, a dose below which adverse effects would not be observed, again with the same experimental caveats. The attainment of a NOAEL (or LOAEL) is critical for subsequent risk assessment processes since it is used to ultimately extrapolate to human exposure limits. However, the NOAEL is not a characteristic of the population (all rats, all mice, etc.) but only of the group under test and, in a real sense, specific to the species, strain, laboratory, staff, specific time of performance, source and purity of test material, identity of any vehicle, parameters evaluated, etc. The NOAEL also does not provide information on the slope of the dose–response curve (steep or shallow), although it is obviously at the low end of the dose–response continuum. These characteristics are very important since regulators are usually extrapolating from (relatively) high dose levels in animal studies to (relatively) low exposure levels for humans, and the presence and location of the threshold is crucial to risk assessment.

Once a NOAEL (or LOAEL) is provided by the experimental data, the proposed next step by risk assessors is to define a reference dose for developmental toxicity (RfD_{DT}) according to the following equation:

$$RfD_{DT} = \frac{NOAFL/LOAEL}{UF}$$

where UF is uncertainty factors. The RfD_{DT} is defined by the US EPA as an estimate of the daily

human exposure that is likely to be without appreciable risk of adverse developmental effect and is characterized by the use of NOAEL or LOAEL (if NOAEL is unavailable) of most sensitive indicators for most appropriate (if known) and/or most sensitive mammalian species. If the NOAEL is used,

$$\mathrm{RfD_{DT}} = \frac{\text{NOAEL of most sensitive indicator}}{\underline{\text{inter}}\text{species variability (UF; 10)} \times \underline{\text{intra}}\text{species variability (UF; 10)}}$$

The $\mathrm{RfD_{DT}}$ is assumed to be below the threshold for an increase in adverse developmental effects in humans and is used for risk characterization along with human exposure assessments.

A second use for NOAELs (or LOAELs) is in the calculation of a proposed margin of exposure (MOE) for developmental toxicity to be used in risk characterization. The MOE is defined as the ratio of the NOAEL from the most sensitive or appropriate species to the estimated human exposure level from all potential sources. If the MOE is very high relative to the estimated human exposure level, then risk to the human population would be considered low.

The proposed US EPA weight-of-evidence (WOE) scheme for suspect developmental toxicants defines three levels of confidence for data used to identify developmental hazards and to assess the risk of human developmental toxicity: (1) definitive evidence for human developmental toxicity or for no apparent human developmental toxicity, (2) adequate evidence for potential human developmental toxicity or no apparent potential human developmental toxicity, and (3) inadequate evidence for determining potential human developmental toxicity. The scheme may require scientific judgment based on experience to weigh the implications of study design, statistical analyses, and biological significance of the data.

Standard Segment III Testing Protocol

There is growing concern about postnatal sequelae to *in utero* structural and/or functional insult as well as a recognition that exposure to a developing system may result in qualitatively or quantitatively different effects than an exposure to an adult system. In brief, the Segment III study consists of exposure of pregnant rats to the test agent starting at the end of the organogenesis period (gd 15), through the histogenesis period (concepti are termed fetuses), through parturition, and through the lactational period until the offspring are weaned (postnatal day (pnd) 21). The offspring are 'exposed' only from possible transplacental and/or translactational (via the milk) routes (**Figure 1**). There are usually three test material groups and a vehicle control group, with at least 20 litters per group; exposure is by gavage to the dam (to minimize disruption of the mother and her litter). During gestation, the dam is weighed periodically and feed consumption is measured. Dams and pups are weighed, sexed, and examined externally, and feed consumption is measured at birth (pnd 0) and repeatedly during the lactation period (e.g., on pnd 0, 4, 7, 14, and 21). Litters are culled to eight pups on pnd 4. The time of acquisition of developmental landmarks is recorded, such as surface righting reflex, pinna (external ear) detachment, incisor eruption, eye opening (pups are born blind with eyes shut), auditory startle (pups are born deaf with the external ear canal closed), mid-air righting reflex, and testes descent. If the pups are maintained after weaning, then vaginal patency (opening of the vaginal canal) and/or preputial separation (separation of the foreskin from the penile shaft) are monitored as well as motor activity (initial exploratory behavior as well as habituated behavior); learning and memory may also be assessed. This test provides information on the last 'trimester' of pregnancy; delivery; maternal–pup interactions and behaviors, such as pup retrieval, nursing, grooming, nest building, etc.; and on pup postnatal growth and development. At weaning, the dam is sacrificed and the number of uterine implantation scars are counted to obtain information on prenatal loss; pups can be necropsied at wean or beyond, with target tissues examined histologically.

Developmental Neurotoxicity Test

The nervous system – with its long developmental phase, involving proliferation, migration, and differentiation of cells and regions at different gestational and perinatal ages, and its complexity – is one for which there is special concern for postnatal consequences of *in utero* exposure to developing systems. In response, the US EPA has developed a 'standalone' standardized developmental neurotoxicity test to assess 'potential functional and morphologic hazards to the nervous system which may arise in the offspring from exposure of the mother during pregnancy and lactation' (**Figure 1**). When this study design would be employed, that is, the 'triggers' for its requirement, is still not fully established and will (and should) probably be decided on a case-by-case basis. Agents which should be candidates for developmental neurotoxicity or behavioral teratology testing include those that cause central nervous system (CNS) malformations, that are psychoactive,

adult neurotoxicants, hormonally active, and peptides or amino acids. The last agents might be antagonists or agonists of endogenous CNS chemical signalers and could easily cross the blood–brain barrier. Such testing protocols should assess sensory and motor function, neuromotor development, learning and memory, reactivity and/or habituation, reproductive behavior, and other functions such as social or aggressive behaviors.

The study design for the developmental neurotoxicity screen, as currently mandated by the US EPA (OPPTS, 1998), involves performance in rats, at least three agent-exposed groups and one vehicle control group, and at least 20 usable litters in each group. The route of administration should be 'orally by intubation'; other routes of administration are acceptable, on a case-by-case basis, with appropriate justification. If the agent has been previously shown to be developmentally toxic, 'the highest dose for this study shall be the highest dose which will not result in perinatal deaths or malformations sufficient to preclude a meaningful evaluation of neurotoxicity'. If there are no developmental toxicity study data, 'the highest dose shall result in overt maternal toxicity, with weight gain depression not to exceed 20% during gestation and lactation'. The lowest dose should not result in either overt maternal or developmental neurotoxicity, while the intermediate dose(s) must be equally spaced between the highest and lowest doses. With gd 0 designated as the day of copulation, the dosing period extends from gd 6 through pnd 10. Maternal animals are evaluated for body weights and weight gain, functional observational battery (FOB), and clinical signs of toxicity from gd 0 through pnd 21. Live pups are counted and weighed at birth and throughout the lactation and post-wean periods. On pnd 4, litters are culled to yield eight pups (four/sex). Pre- and post-wean developmental landmarks assessed on all appropriate pups include age of vaginal opening and testes descent and/or preputial separation. Motor activity is monitored at multiple pre- and post-weaning times. The period of evaluation for motor activity will include the exploratory phase and the habituation phase. Auditory startle test, including magnitude and habituation of response, and tests to evaluate learning and memory are performed at weaning and at 60 days of age. Necropsy and histopathology requirements include perfusion of pups with fixative *in situ* and specified central and peripheral nervous system tissues examined histologically with 'qualitative, semiquantitative, and simple morphometric analysis'. Additional animals are decapitated, the brains are removed, and regional brain weights are obtained. Current discussions on this testing guideline between the US EPA, pesticide manufacturers, and performing laboratories, especially as used for pesticide registration (now required, rather than triggered, along with an adult neurotoxicity test) center around: (1) direct dosing of offspring pups (if there is no proof that the test chemical and/or metabolites is transferred to the offspring in the milk from the dosed dam, or if there is proof that there is no lactational transfer), including start of direct dosing of pups (and cessation of direct maternal dosing); and (2) timing of neuropathologic evaluations of the brain in pups (current guideline specifies on pnd 11 and at study termination on pnd 60). The assumption by the US EPA is that all brain structures are present by gd 10, but there is evidence that various regions of the brain go through proliferation, differentiation, and scheduled cell death with different timing, so that evaluation on pnd 11 is too early and the natural intra- and inter-litter biological variability in regional brain weights, differentiation, etc., will obscure many treatment-related effects.

This test is perceived as useful in the risk estimation process, to identify specific agents, or classes of agents, for which acceptable exposures in the adult may not be acceptable to the developing organism, to elucidate long-term consequences of pre- and perinatal exposures and results, to determine the relationship of lowest effective (or highest no effect) dose for behavioral effects versus the dose for overt or general toxicity effects, and to identify, for human exposures, those effects which may be important to monitor.

Although the developing nervous system has received the most attention from researchers and governmental regulators, there are many other systems with continuing proliferation and differentiation in the postnatal period. Evaluation of the postnatal sequelae of prenatal exposure has been done for three of these: the renal system, gastrointestinal tract, and immunosurveillance system. Transplacental carcinogenesis, expressed in the adult from late gestational *in utero* exposure, is also well documented in test animal species; diethylstilbestrol is currently the only documented transplacental carcinogen in humans.

Male-Mediated Developmental Toxicity

All of the previously described approaches focus on the maternal–placental–fetal unit as the subject of testing and the object of concern. However, increasing evidence has implicated the male as the cause of any of the classic four end points of developmental toxicity. Human male exposure, such as operating room personnel, to waste anesthetic gases results in

increased incidences of spontaneous abortions, stillbirths, and congenital defects. Male production worker exposure to Oryzalin has been implicated in congenital heart defects in their children. The pesticide DBCP (1,2-dibromo-3-chloropropane) is a human male sterilant. Elevated caffeine consumption in men has been reported to result in spontaneous abortions, stillbirths, and premature births. In animal studies, exposure of the male to methadone, thalidomide, lead, narcotics, alcohol, and caffeine results in malformations in the offspring. Possible mechanisms of male-mediated developmental toxicity include genetic or epigenetic damage to the sperm, the presence of the agent or its metabolite(s) in the semen which may affect the conceptus directly or act on the gravid uterus, or indirect or more systemic actions on the male affecting the hormonal milieu and perhaps libido.

Developmental Toxicity Screening Protocols

Over 80 000 chemicals are listed in the TSCA registry, with 1500–2500 new chemicals added each year; 20 000 chemicals are commonly found in the workplace (NIOSH list) with $<1\%$ tested for reproductive and developmental hazard potential. It is therefore necessary and appropriate to develop fast, inexpensive, sensitive, and accurate methods to prescreen the plethora of chemicals and concentrate resources on those identified by the screening test(s) as potential human health hazards. However, the mechanisms of action of developmental toxicants appear numerous and frustratingly difficult to identify (see section Mechanisms).

A number of approaches have been taken to develop screening protocols, herein arbitrarily classified into *in vivo*, *in vivo/in vitro*, and *in vitro* categories. *In vivo* screens include developmental toxicity range-finding studies, which can also be used to identify (or prioritize) agents which produce developmental toxicity for more rigorous testing, and the so-called Chernoff–Kavlock assay. The assay employs a block design of one dose (the maternal minimally toxic dose; MTD) per chemical for a number of chemicals and a concurrent control group, with 24–50 timed-pregnant animals, usually mice, per group. Dosing is on gd 8–12, with the date of a vaginal plug being designated gd 1. The earliest version of this study design collected maternal weights at the beginning and end of the treatment period and also weight change, with dams allowed to litter. Litters were counted, sexed, weighed, and examined externally on pnd 1 (date of birth) and 3 and discarded. This protocol does not require extensive or intensive technical training in pup visceral or skeletal examinations and assumes that the pups will be their own assay system, that is, if the pups survive and thrive, then they do not exhibit significant toxicity at a dose which is minimally toxic to the dam (the MTD) and they do not bear malformations or variations which preclude or affect normal early postnatal growth and development. Chernoff and Kavlock set up three levels of concern: If there is pre- or postnatal mortality and/or malformations of the offspring, then the test agent has the 'highest priority' for further classic developmental toxicity testing; if the pups exhibit reduced weight gain, then the agent has a 'lower' priority for further testing; and if there is no evidence of developmental toxicity, pre- or postnatally, then the agent has the 'lowest priority' for further testing. The block design described previously provides comparisons among the test agents in the block, all at the MTD, for relative potency with regard to developmental toxicity.

Modifications to the initial protocol include multiple dose levels, dosing during the entire period of major organogenesis, and more thorough evaluation of pups on pnd 3 (including visceral and skeletal examinations) so that this protocol resembles more closely the classic Segment II protocol but with a postnatal component to assess viability and growth.

One *in vivo/in vitro* screening protocol involves administration of the test agent to pregnant rodents, removal (on gd 10 after one or more daily doses to the dam), explantation and culture of embryos for 24–48 h, and evaluation of toxicity and teratogenicity. This protocol allows for the full mammalian complement of metabolizing enzymes in the dam to act on the conceptuses *in utero* and for evidence of early expression of developmental toxicity (limited) to be detected in the explanted embryos *in vitro*. The next step is one whereby explanted rat headfold embryos are cultured for 48 h in human, monkey, or rodent serum after the serum donor had been exposed to the test agent. This protocol utilizes serum containing whatever metabolites, etc. are produced by and transported in the blood of the donor mammal – a condition duplicating the embryonic exposure *in utero*. In a fascinating offshoot of this work, serum from women who were chronic aborters has been used in the embryo culture system to identify missing nutrients and the women were supplemented prior to and during subsequent pregnancies with some early apparent success.

There are a number of fully *in vitro* screens as well, employing mammalian, lower vertebrate, and invertebrate species. Materials used include explanted rat or mouse embryos cultured in rodent serum to which is added the test agent or known metabolites, and

portions of rodents, as intact organs or as dissociated cells (e.g., limb buds, dissected midbrain cells, and palatal cells), explanted and cultured in medium containing test agents and/or metabolites. When explanted embryos (or parts thereof) are exposed to the test agent in culture, they are exposed only to the added test agent since metabolic capability is minimal or absent, so this study paradigm may expose embryos to situations they would not encounter *in utero* and therefore result in false-positive or (worse) false-negative study results. Cloned totipotent stem cell lines from murine embryonal teratocarcinoma or pluripotent lines from neuroblastoma are cultured, exposed to test agents (including those which are 'proteratogens' requiring metabolic activation), and the cultures scored for effects on differentiation. Both tumor lines are capable of extensive differentiation in culture; restriction of this capability is presumed indicative of potential developmental toxicity *in vivo*.

In a novel approach to examine a fundamental property of differentiating cells, cell-to-cell communication, Chinese hamster lung cells or normal embryonic palatal mesenchymal cells in culture are exposed to the test agent and evaluated for disruption of cell-to-cell communication. Cell attachment is another presumed universal cell function during development and therefore a basis for a screen. Ascites or dissociated solid tumor cells are grown in culture in the presence of the test agent and scored for attachment (or inhibition of attachment) to surfaces as a measure of potential developmental toxicity.

Explanted chick embryos at presomite or multiple somite stages or chick embryonic parts are cultured with the test agent incorporated into the culture medium and evaluated for growth and differentiation.

Amphibians are also proposed for use in screening protocols. The FETAX system (Frog Embryo Teratogenesis Assay: Xenopus) involves exposure of early *Xenopus laevis* (African clawed frog) embryos at the notochord stage and/or as late premetamorphic larvae to test agents in the water. A teratogenic index (TI) is proposed to compare relative potencies of test agents and to identify any agents which affect development at doses below which general toxicity is observed; the TI is defined as LC_{50}/ED_{50} (the concentration lethal to 50% of the animals divided by the concentration producing effects in 50% of the animals).

Drosophila melanogaster (the fruit fly) is used in two ways: Larvae are grown on feed containing the test agent, are allowed to pupate, and emerging adults are scored for viability (toxicity) and malformations from alterations in imaginal discs present in the larvae and used to form adult structures; or early primary embryonic cell cultures are grown in medium containing the test agent and are scored for differentiation of embryonic cell types.

Synchronous cultures of *Artemia* sp. (brine shrimp) in seawater or rodent or human serum have also been suggested as a screen, with scoring for survival, growth, and morphological and molecular differentiation after exposure directly to agents or to serum from agent-exposed individuals.

Hydra attenuata (a coelenterate) is the source of the 'artificial embryo' assay. The adult Hydra can be dissociated and the cells pelleted by centrifugation. The cells of the pellet will sort and reaggregate by cell type and redifferentiate into an adult Hydra. The assay consists of pellets (artificial embryos) and adult Hydra exposed to the test agent to determine the lowest effect concentration (or the highest no-effect concentration) of the developing 'embryo', as measured by inhibition of redifferentiation or abnormal differentiation, and of the adult, as measured by mortality or overt damage to adult structures. An A/D ratio is calculated: that is, the ratio of the adult toxicity lowest effect (or highest no-effect) concentration to the developmental toxicity lowest effect (or highest no-effect) concentration. The developers and users of this assay suggest that an A/D ratio ≥ 3 indicates a unique or greater susceptibility of the developing organism relative to that of the adult and therefore a potential of the test agent for mammalian developmental toxicity. They also claim that the A/D ratio is fairly consistent across widely divergent species and therefore predictive of relative risk, although this latter claim has been contested by data from other workers in the field.

The consensus on screening assays appears to be that the *in vivo* protocols are appropriate and useful to prioritize chemicals for subsequent testing, to decide early in the chemical/drug development phase whether to pursue a particular formulation, to evaluate what effect changes in chemical structure have on toxicity, and to 'fill in the blanks' on a chemical series, all relative to the potential for developmental toxicity, including teratogenicity. The *in vivo/in vitro* assay requires the same number of maternal animals to do fully *in vivo* studies, requires sophisticated technical procedures for culturing embryos, and provides for only a limited number of embryological end points due to the limitations on the length of time embryos can be maintained in culture. There does not appear to be an advantage in using these assays as screens.

The *in vitro* assays with mammalian embryos or tissues have two critical limitations. First, the metabolic capabilities of the embryo are very limited and only the embryo is cultured. Any metabolic changes to the parent compound by the maternal organism

and therefore the metabolites to which the embryo would be exposed *in vivo* are totally missing in the explant system. Currently, attempts are being made to provide metabolic capability by coculturing embryos with adult liver cells or cell fractions, which are the major source of metabolism of xenobiotics to obviate the first limitation. Second, the duration of sustained normal growth and development of embryos appears very limited (24–48 h) so that the numbers of structures differentiating and the extent of differentiation are similarly limited. A two-system approach, for example, midbrain plus limb bud micromass culture assay, is an attempt to increase the number of systems evaluated, but it is still very limited relative to the tremendous range of systems developing which may be vulnerable. The *in vitro* assays are very useful in answering research-oriented questions since the age of the embryos (as judged by somite number or other specific morphological signposts) can be precisely controlled, identity and concentration of the test agent are precisely controlled, and early responses can be observed and characterized. They can be used to identify the proximate teratogen by exposing the explanted embryos to specific metabolites which they cannot further transform and to elucidate mechanisms of action of known teratogens at the organ, tissue, cellular, subcellular, or molecular levels early in the toxic response, prior to cell death or demise of the embryo. The utility of nonmammalian (nonvertebrate) assays as predictors of potential mammalian developmental toxicity appears unclear at this time, although the concept of phylogenetically conserved universal processes in embryonic development is attractive and compelling.

Mechanisms

There is no mechanism fully understood for any developmental toxicant causing fetal malformations. Although in many cases the proximate teratogen is known and maternal and/or developmental toxicity is well characterized, what is not known is how the observed effects result in malformation(s). The site(s) of action may be intranuclear, intracellular, at the cell membrane, extracellular, outside of the conceptus, in the placenta, or in the maternal organism. The mode(s) of action may be general or specific, biochemical, physiological, or microstructural. It is also likely that the mechanism(s) will vary from agent to agent. The two extremes in mechanisms, from very specific to very general, may be exemplified by those proposed for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), valproic acid, and ethylene glycol (EG). TCDD produces cleft palate (and hydronephrosis at higher doses) in susceptible mouse strains. The putative mechanism for the induced cleft palate is that TCDD binds to certain epidermal growth factor (EGF) receptors and prevents the normal reduction in expression of certain EGFs in the medial epithelial cells of the palatal shelves just prior to fusion. Therefore, with TCDD, abnormally high levels of certain EGFs apparently continue to stimulate proliferation and differentiation of the cells normally destined to die, and the shelves do not fuse. The suggested mechanism for induction of cleft palate may also explain the induction of hydronephrosis since EGF, TGF-α, Ah, and ARNT play a role in embryonic cell proliferation during normal palatal and urinary tract development and are altered in both the palate and urethral buds in culture from TCDD exposure. Valproic acid causes neural tube defects, including exencephaly (in mice) and spina bifida (in humans). Valproic acid and other weak acid teratogens (of which there are many) reach the mammalian embryo and lower the intracellular pH of the embryonic cells. (The embryonic intracellular pH is more basic than the maternal intracellular pH, especially early in development, and changes over time.) The specificity of the effect probably lies in the sensitivity of the target neural tube. EG causes major malformations, predominantly skeletal, in rats and mice at high oral doses, but not in rabbits and not by nose only, cutaneous, or dosed feed exposures. EG is metabolized to acidic intermediates and forms oxalic acid (oxalate crystals in the kidneys). At high oral bolus doses, the major intermediary metabolite in rodents is glycolic acid (rabbits do not make glycolic acid but do form oxalate crystals in the kidneys). Co-administration of sodium bicarbonate prevents maternal acidosis and ameliorates but does not prevent fetal malformations. Oral administration of glycolic acid in rats produces the same malformations as EG. In embryo culture, either acidosis or sodium glycolate (the salt of the acid) causes malformations *in vitro*. It appears that glycolic acid and maternal metabolic acidosis are both responsible for the teratogenesis produced by large oral bolus doses of EG. The suggested mechanism for valproic acid (and other teratogens which are weak acids) does not explain the specificity and susceptibility of the targets since other weak acid teratogens do not affect the neural tube and many weak acids are not teratogens. Perhaps the most important barrier to understanding the mechanism(s) of abnormal development is that we do not know enough about the mechanism(s) of normal development.

Studies performed initially on *Drosophila* embryos indicated that sequential activation of a hierarchy of regulatory genes occurs during the development of multicellular organisms. These genes regulate the

transcription and translation of genetic information into structures (and functions) by orchestrating a precise temporal and spatial expression of structural genes, which in turn control differentiation, that is, establishment of cell types and organ formation. Many of these genes also appear to play a role in pattern formation during or after gastrulation in vertebrates. The mechanisms of these regulatory genes include genetic and epigenetic control. Genetic mechanisms include the role of genes in establishing the basic embryonic axes (cephalocaudal and dorsoventral), specifying specific embryonic regions, controlling the transition of cells from presumptive to determined in the establishment of the fate of diverse cell types and ultimately specifying directly the differentiated patterns of gene expression, including inter- and intracellular molecules, structure, and functions. Epigenetic mechanisms include the interactions between cells, between cell types, and between cells and the products of other cells. The genetic and epigenetic roles are linked and integrated by so-called second messengers, which translate molecular signals by individual cells into commands to produce specific effects in other cells on cell growth and patterns of gene activity.

Abnormal expression of genes from this regulatory class results in abnormalities in development, which also provide information on normal development and suggest a mechanism(s) of action of xenobiotics. It is clear that cell division, cell migration, and differentiation are directed by regulatory gene classes that control which genes are expressed in which tissues at which times in development. The molecular approach to identifying these fundamental controlling factors of mammalian development may be the most fruitful in the long run in elucidating mechanisms of normal and abnormal development and providing mechanisms of action of developmental toxicants.

See also: Ames Test; Carcinogen–DNA Adduct Formation and DNA–Repair; Chromosome Aberrations; Developmental Toxicology; Dominant Lethal Tests; Dose–Response Relationship; Environmental Hormone Disruptors; Epidemiology; Host-Mediated Assay; Levels of Effect in Toxicological Assessment; Molecular Toxicology–Recombinant DNA Technology; Mouse Lymphoma Assay; Reproductive System, Female; Reproductive System, Male; Risk Assessment, Human Health; Sister Chromatid Exchanges; Toxicity Testing, Reproductive; Toxicology, History of.

Further Reading

Tyl RW (2000) Chapter 53, Developmental toxicology. In: Ballantyne B, Marrs T, and Syversen T (eds.) *Textbook of General and Applied Toxicology*, vol. 2, pp. 1167–1201. Basingstoke, UK: Macmillan Press, Ltd.

Tyl RW and Marr MC (1997) Chapter 7, Developmental toxicology methodology. In: Hood RD (ed.) *Handbook of Developmental Toxicology*, pp. 175–225. Boca Raton, FL: CRC Press.

Tyl RW and Marr MC (2005) Chapter 7, Developmental toxicity testing – methodology. In: Hood RD (ed.) *Handbook of Developmental Toxicology*. Boca Raton, FL: CRC Press.

Toxicity Testing, Inhalation

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Introduction

Inhalation is, in many senses, simply a particular route of administration in toxicology studies in general and in acute toxicology studies in particular. As will be reviewed here, animal inhalation studies are difficult and complex to perform correctly. The complexity and cost of such studies firmly dictates that they be performed only when there is the substantial likelihood that humans will be exposed to the substance of interest. Human inhalation occurs in three types of settings. In order of decreasing occurrence or importance, these are (1) occupational settings, (2) environmental settings, or (3) therapeutic settings. The toxicity due to human inhalation exposures is often expressed in terms of the inhalation reference concentration (RfC), which is an estimate of a continuous inhalation exposure to the human population that is likely to be without appreciable risk of deleterious noncancer lifetime health effects. Since this estimate includes sensitive population subgroups, it may be considered a conservative estimate.

All inhalation studies can be classified either by the pattern of exposure or by the physical nature of the contaminant. Both of these classifications are important because they dictate equipment, animal selection, and details of study design.

Exposure Pattern

Pattern refers to how (or how much of) a test animal is exposed to the contaminated atmosphere of

interest. In practice, there are only very limited situations in which exposure to a toxicant is purely by inhalation (these are therapeutics cases when a material is administered directly into the nasal or oral cavity of an organism and in an inhalation test system, where nose-only exposure is truly achieved). Rather, both in the real world and in the laboratory, inhalation exposure is accompanied by dermal and oral exposures. How concerned one is with the possible confounding effects of such other routes of exposure on the evaluation of biological outcome dictates selection of the exposure pattern.

The three categories of exposure patterns are nose only, head only, and whole body. There are also minor patterns (lung only and partial lung) that will not be discussed here as they are used only in special research settings for precise delivery of doses of test material directly to the lungs.

In 'nose-only' exposure, which is the least commonly used pattern (particularly in acute tests), the test animal is situated in such a way that only its nasal region (or, for dogs and primates, where a mask is used to administer test compound, only the mouth and nasal region) is exposed to a test atmosphere. This can be achieved by having the animal restrained with only its nose poking through an elastic barrier or with a breathing mask fitted over the nose and mouth region. There is still some small amount of oral exposure in such a system because animals will swallow any material deposited on the surfaces of their mouths or 'cleared' from the nasal region or lungs back into the trachea.

In 'head-only' exposure, the entire animal is inserted into a chamber into which a test atmosphere is introduced. The head is sealed off, using a collar or membrane, so that only the head is exposed. There are a wide variety of chamber designs available such that all common laboratory species can be exposed using this methodology. The head-only approach is especially favored for pharmaceuticals.

There is extensive dermal and oral exposure in animals exposed 'whole body' (particularly oral exposure in rodents and rabbits which carefully 'preen' themselves after an exposure). For gases and vapors, of course, such considerations have minimal impact. The advantages and disadvantages associated with each of these exposure patterns are summarized in **Table 1**.

Physical Nature of the Contaminant

The second method of classifying inhalation studies is in terms of the exposure 'media' – that is, the physical nature of the contaminant atmosphere that is being evaluated. Though these media can be subdivided in a variety of ways, for our purposes the types of possible test media are gases, aerosols, and dusts.

Gases are generally the easiest type of media to use because the contaminants in the test atmosphere are in the gaseous phase. Technically, aerosols include any liquid- or solid-phase material that forms a stable suspension in air. Dusts are solid-phase (contaminant) particles suspended into a gaseous (atmosphere) phase. They are generally the most difficult to use when conducting a study.

Performing Inhalation Exposure Studies

Basic Steps

A technically good inhalation exposure study can be broken into four major parts or basic steps. The following are the four basic steps:

1. generation of a test atmosphere;
2. containment, mixing, and movement of test atmosphere and animals (both before and after exposure);
3. measurement and characterization of what animals have been exposed to; and
4. cleanup and disposal of resulting 'wastes' (gaseous, solid, and liquid).

Mechanics of Exposure

The mechanics of performing acute inhalation exposures to state-of-the-art standards can be complex in their entirety but the individual technical components of the problem are rather simple.

There are four sequential components to an exposure system. These are generation systems, exposure chambers, systems for measuring exposure, and systems for cleaning the effluent air stream.

Generation Systems Optimal generation systems have four major desirable features:

1. uniform rate of sample delivery,
2. uniform character of sample delivered,
3. ability to deliver in desired range of concentrations, and
4. safety of operations.

For each of the types of exposure (vapor, aerosol, and dust), there are a multitude of systems available.

Vapor Generation Vapor generation systems are based on the principle of maximizing the surface area of the liquid, and the temperature (within the limits of chemical stability) and airflow across the surface of the liquid as a means of increasing efficiency.

Table 1 Advantages, disadvantages, and considerations associated with patterns of inhalation exposure

Mode of exposure	*Advantages*	*Disadvantages*	*Design consideration*
Whole body	Variety and number of animals	Messy	Cleaning effluent air
	Chronic studies possible	Multiple routes of exposure: skin, eyes, oral	Losses of test material
	Minimum stress and labor	Variability of 'dose'	Noise, vibration
	Minimum restraint	Difficulties with dispersion and measurement techniques	Even distribution of space
	Controllable environment		Loading
	Large historical database		Sampling and observation
Head only	Good for repeated exposure	Stress to animal	Even distribution
	Limited routes of exposure	Labor in loading/unloading	Pressure fluctuations
	More efficient dose delivery	Seal around neck	Sampling and losses
		Losses can be large	Animal restraint
Nose/mouth only	Exposure limited to mouth and respiratory tract	Stress to animal	Pressure fluctuations
	Efficient use of material	Seal about face	Sampling
	Can pulse the exposure	Effort to expose large numbers	Airlocking
			Losses in plumbing/masks
Lung only	Precision of dose	Technically difficult	Stress to the animal
	One route of exposure	Anesthesia or tracheostomy	Physiologic support
	Efficient use of material	Limited to small numbers	
	Can pulse the exposure	Bypasses nose	
Partial lung	Precision and localization of total dose	Anesthesia	Stress to animal
	Can achieve very high local doses	Placement of dose	Physiologic support
	Unexposed control tissues from same animal	Possible redistribution of material within lung	
		Difficult in interpretation of results	
		Technically difficult	

There are four common generation systems:

- *Tube generators*. Liquid flows along the inside surface of a tube while air is forced over this surface.
- *Wick generators*. A liquid phase is passed up a porous wick while air is forced over it.
- *Bubble generators*. The air is passed through the liquid phase.
- *Special instrument generators*. A turning tube with ridges or sections to increase surface area is warmed while air is forced through it.

Aerosol Generation Liquid aerosols may be difficult to generate; if they are extremely volatile, one may actually end up generating a vapor. Second, denser or more viscous liquids require greater energy to overcome surface tension and form droplets of the desired size. There are four widely used aerosol generation systems:

- spray nozzle,
- ultrasonic generation (uses sound to provide energy to disrupt liquid into droplets),
- spinning disks, and
- nebulizers.

After a stream of test material is generated into airflow, it is mixed (usually in a dueling system of sufficient length, with some turbulence) and then introduced into the exposure chamber system in which test animals are or will be contained.

Dust Generation The following factors need to be considered in dust generation:

- particle size (and size distribution),
- particle shape,
- density of material, and
- concentrations needed (necessary capacity).

Exposure Chambers Technically, chamber exposures can be dynamic or static. In a static chamber exposure, there is no airflow through the system; animals are entered into a closed system that

contains an atmosphere 'precharged' with the desired test material. Static systems are inadequate for anything other than some minor short-term hazard-type assessments. In any other circumstance the animals should be tested with inhalation equipment designed to sustain a dynamic airflow that exceeds at least twice the respiration ventilation volume of all animals in the inhalation device or at least 10 air changes per hour, preferable 12–15 for whole-body chambers, and an oxygen content of at least 19%, with uniform conditions throughout. Maintenance of slight negative pressure inside the chamber will prevent leakage of the test substance into the surrounding areas. It is normally not necessary to measure chamber oxygen concentration if airflow is adequate. Food should be withheld during exposure. Water may also be withheld in certain circumstances.

Measurement Systems While animals are being exposed in a chamber, certain characteristics of the measuring system that are critical include:

- accuracy and precision across the range of concentrations (or characteristics) to be measured;
- reproducibility;
- direct measurement of the variable interest (not inference or calculation based on an indirect measurement); and
- automatic measurement at a number of discrete locations in the exposure chamber.

For gases and vapors chemical analysis of the chamber concentration is mandatory and concentrations should be monitored continuously or intermittently depending on the method of analysis. Whenever the test substance is a formulation, the analytical concentration must be reported for the total formulation and not just for the active ingredient. The actual concentrations of the test substance should be measured in the breathing zone. The particle-size distribution of the test aerosol should be determined at least twice during each 4 h exposure to establish the stability of aerosol concentrations. The MMAD particle-size range should be between 1 and 4 μm in the animal's breathing zone and must be calculated. The rate of airflow should be monitored continuously, but recorded at least three times during the exposure. The particular problems, concerns, and instruments associated with each of the separate types of exposure (dust, aerosol, and vapor or gas) are very different. Overcoming all of these problems and concerns in practice is generally impossible. Rather, a number of acceptable compromises are made. For example, the mean of the test atmosphere samples is allowed to vary within $\pm 25\%$ of the concentration tested.

There are a variety of analytical techniques for determining inhalation chamber concentrations and they depend on the nature of the contaminant. These techniques are summarized below:

For vapors

- gas chromatography (direct sampling, extracted samples),
- infrared spectroscopy,
- ion-selective electrodes, and
- ultraviolet–visible spectrophotometers.

For aerosols

- Concentration (gravimetric, forward-scatter detectors, back-scatter detectors, J-attenuation detector, quartz crystal microbalance detector).

For particles (sizing)

- cascade impactors,
- microscopy (fiber morphology), and
- laser/Doppler type.

The most commonly used analytical techniques for analyzing gases and vapors are gas chromatography (GC) and infrared spectroscopy. GC is the most versatile and frequently used analytical technique for monitoring gases and vapors. GC offers chemical separation of components for specific analysis, low detection limits, and rapid turnover of data for feedback control. Infrared spectroscopy works well since most gases and vapors give reasonably intense and unique spectra. The Miran portable gas analyzer is particularly useful for continuous monitoring. Other techniques shown to be useful include the use of ion-specific electrodes, ultraviolet–visible spectrophotometers, and scrubbing colorimeters. As is always the case, frequent calibration of analytical instruments is essential.

Aerosols present a special case in that the investigator needs to measure the mass concentration of the chemical, the chemical composition as a function of particulate size, and the particle-size distribution of the aerosol. No continuous sampling instruments are available to measure both particle-size and chemical concentration. Particle detection can be accomplished using both forward- and back-scatter detectors. A typical back-scatter allows for non-invasive determinations over a range from 6 to $10\,000\,\mathrm{mg\,m^{-3}}$. In the test, the aerosol is drawn through an orifice and articles impact on a surface positioned between a source and a counter.

For particle sizing, many varieties of cascade impactors perform well, although care must be taken to avoid errors introduced by sampling (such as collection in sampling lines). The laser/Doppler-type particle-size device can be used to measure aerodynamic size and low concentrations with a rapid readout. In a system described by Cook, a powerful pulsed laser using temporal analysis of back-scattered light can be used to measure the spatial distribution of particles.

Cleanup Methods The last step or phase in the process of properly conducting an inhalation exposure is cleaning up the air-stream leaving the exposure system before releasing it into the atmosphere, and, of course, then properly disposing of the collected waste products that result from such a cleanup. For acute studies, one has much more flexibility in applying these methods than for longer-term studies in which logistics limit choices. Depending on the nature of the chemical being evaluated, one or a combination of three types of equipment may be utilized. These are filters, incinerators, or scrubbers.

Design of Inhalation Studies

All available information on the test substance should be considered by the testing laboratory prior to conducting the study. Three concentration levels should be used and spaced appropriately to produce a concentration–response curve and permit an estimation of the median lethal concentration. Range-finding studies using single animals may help to estimate the positioning of the test groups so that no more than three concentration levels will be necessary. An acceptable option for pesticide products would be to set the dose levels in correlation with the Environmental Protection Agency Office of Pesticide Programs (OPP) toxicity categories (bracketing). In these cases, the determination of an LD_{50} may not be necessary.

Exposure methods vary in the length of duration with acute (1–4 h exposure), subacute (13 week exposure), subchronic (3–12 month exposure), and/or chronic (multiple years exposure). The observation period should be at least 14 days with an examination at least once each day. Observations should include, but not be limited to, evaluation of skin and fur, eyes and mucous membranes, respiratory and circulatory effects, autonomic effects such as salivation, central nervous system effects, including tremors and convulsions, changes in the level of activity, gait and posture, reactivity to handling or sensory stimuli, altered strength, and stereotypes or bizarre behavior (e.g., self-mutilation, walking backwards), changes in weight and time of death. Care should be taken when conducting examinations for clinical signs of toxicity that initial poor appearance and transient respiratory changes, resulting from the exposure procedure, are not mistaken for treatment-related effects.

Study Guidelines

EPA's Office of Prevention, Pesticides, and Toxic Substances (OPPTS) provides Health Effects Test Guidelines for use in testing of pesticides and toxic substances and the development of test data that must be submitted to the agency for review under Federal Regulations. The European Organization for Economic Cooperation and Development (OECD) also has published guidelines for inhalation studies.

OPPTS Health Effects Test Guidelines Revised June 1996

	Number	OECD
Acute inhalation toxicity	870.1300	403
Subchronic inhalation toxicity	870.3465	413
Repeated dose inhalation toxicity: 28/14 day		412
Proposed: Acute inhalation toxicity-fixed Concentration Procedure		433

Attempts are being made to have a worldwide testing and classification system for inhalation toxicity testing. At present, however, this has not been achieved. The following table shows the correspondence between the Globally Harmonized Classification System and the EU classification system. Conversion of Classifications for LC_{50} by inhalation using the Globally Harmonized Classification System for Chemical Substances and Mixtures (GHS) and EU classification systems are as follows:

	Classify EU category		
GHS classification	*Vapors*	*Dusts/mists*	*Gases*
Class 1	T+	T+	T+
Class 2	T	T+	T
Class 3	H	T	T
Class 4	H	H	H
Class 5 (unclassified)	U	U	U

T+ = very toxic; T = toxic; H = harmful; U = unclassified.

Acute Studies

Acute studies are generally conducted at a relatively high concentration and are useful in determining the approximate range of toxicity of a chemical. Acute studies can be used as a starting point in the determination of dose levels for longer-term tests. The clinical signs evoked at three high exposures often

allow determination of the nature of the toxic effect induced. The two most common numerical values derived from an acute study are the approximately lethal concentration (ALC) and the LC_{50}. The ALC is defined as the lowest concentration that produces death in at least one of a group of exposed animals, while the LC_{50} is the calculated concentration at which half of the exposed population would be expected to die. Generally, the exposures are conducted for a single 4 or 6 h period and the animals are observed for 14 days after treatment.

OECD Guideline 403 suggests the use of data from substantially similar mixtures to minimize the need for animal testing. In certain cases, it may be possible to get enough information from these data to make preliminary hazard evaluations that may reduce the need for further animal testing. The primary endpoint for Guideline 403 is mortality. Several groups of male and female animals are exposed for at least 4 h to graduated concentrations of the test substance, one concentration being used per group. Subsequently, observations of effects and deaths are made. In practice, many studies are limit tests at the maximum concentration and use only one group, but for full studies exposure concentrations should be sufficient in number, at least three, and spaced appropriately to produce test groups with a range of toxic effects and mortality rates. From such studies, a concentration mortality curve can be generated and an LC_{50} value calculated. Where a vehicle is used to help generate an appropriate concentration of the substance in the atmosphere, a vehicle control group may be used.

Recently, the OECD proposed a new guideline for inhalation testing, Guideline 433: Acute Inhalation Toxicity – Fixed Concentration Procedure. Development of the proposed Inhalation Fixed Concentration Procedure (FCP) will allow the use of a series of fixed concentrations for the determination of acute inhalation toxicity in only one sex (usually females). This will reduce suffering and distress by the animals and, to the extent feasible, reduce the number of animals used. Underpinning the FCP is a belief that the toxic profile of a substance can be characterized with sufficient reliability for most regulatory situations without the need for the identification of a lethal concentration. The primary endpoint for Guideline 433 is the observation of clear clinical signs of toxicity termed 'evident toxicity'. Evident toxicity is a general term describing clear clinical signs of toxicity following exposure to the test substance, such that an increase to the next highest fixed concentration would be expected to result in the development of severe toxic signs and probably mortality.

According to the Guideline, concentrations that are expected to cause marked pain and distress, due to corrosive, class 1 or severely irritant actions, should not be administered. Moribund animals or animals obviously in pain or showing signs of severe and enduring distress shall be humanely killed, and are considered in the interpretation of the test results in the same way as animals that died on test.

Limit Tests

The highest concentration should result in toxic effects but not produce an incidence of fatalities that would prevent a meaningful evaluation. The intermediate concentrations should be spaced to produce a gradation of toxic effects. The lowest concentration should produce no evidence of toxicity. In the case of potentially explosive test substances, care should be taken to avoid generating explosive concentrations. The limit test is an efficient way to characterize substances of low toxicity, when there is sufficient information available, indicating that the toxic concentration is higher than the limit concentration. Guideline 433 provides a limit test suitable for the design of the main study ($20\,mg\,l^{-1}$, $5\,mg\,l^{-1}$, or 5000 ppm for vapors, dusts/mists, and gases, respectively). A prespecified fixed concentration of $5\,mg\,l^{-1}$ (actual concentration of respirable substance, or where this is not possible due to physical or chemical properties of the test substance, the maximum attainable concentration) is used for a limit test conducted according to Guideline 403. Also when data on structurally related chemicals are inadequate, a limit test may be considered. In the limit test, a single group of five males and five females is exposed to $2\,mg\,l^{-1}$ for 4 h, or where this is not possible due to physical or chemical properties of the test substance, the maximum attainable concentration. If no lethality is demonstrated, no further testing for acute inhalation toxicity is needed. If compound-related mortality is produced, further study may need to be considered. The head/nose-only exposure method allows testing of high concentrations as required for limit tests without the need for large quantities of test material.

Procedures for Acute Tests

Groups of female animals are exposed for at least 4 h to graduated concentrations of the test substance, one concentration being used per group. A sighting study is included in the proposed Guideline 433 in order to choose an appropriate starting concentration for a main study and to minimize the number of animals used (see **Table 2**). Prespecified fixed

Table 2 Flowchart for the sighting study – vapors

Sighting study starting concentration: 0.5 mg l^{-1}											
	START										
	1 animal			1 animal			1 animal			1 animal	
	0.5 mg l^{-1}			2 mg l^{-1}			10 mg l^{-1}			20 mg l^{-1}	
A	B	C	A	B	C	A	B	C	A	B	C
Classify GHS class 1[a] main study starting (concentration mg l^{-1}):											
	0.5		0.5	2		2	10		10	20	20
Sighting study starting concentration: 2 mg l^{-1}											
				START							
	1 animal			1 animal			1 animal			1 animal	
	0.5 mg l^{-1}			2 mg l^{-1}			10 mg l^{-1}			20 mg l^{-1}	
A	B	C	A	B	C	A	B	C	A	B	C
Classify GHS class 1[a] main study starting (concentration mg l^{-1}):											
	0.5	0.5		2	2		10		10	20	20
Sighting study starting concentration: 10 mg l^{-1}											
							START				
	1 animal			1 animal			1 animal			1 animal	
	0.5 mg l^{-1}			2 mg l^{-1}			10 mg l^{-1}			20 mg l^{-1}	
A	B	C	A	B	C	A	B	C	A	B	C
Classify GHS class 1[a] main study starting (concentration mg l^{-1}):											
	0.5	0.5		2	2		10		10	20	20
Sighting study starting concentration: 20 mg l^{-1}											
										START	
	1 animal			1 animal			1 animal			1 animal	
	0.5 mg l^{-1}			2 mg l^{-1}			10 mg l^{-1}			20 mg l^{-1}	
A	B	C	A	B	C	A	B	C	A	B	C
Classify GHS class 1[a] main study starting (concentration mg l^{-1}):											
	0.5	0.5		2	2		10	10		20	20

Outcome: A, death; B, evident, C, toxicity; no toxicity.
[a] For outcome at 0.5 mg l^{-1} there is an optional supplementary procedure to confirm the GHS classification.

concentrations of 0.5, 2, 10, and 20 mg l^{-1} for vapors, 0.05, 0.5, 1, and 5 mg l^{-1} for dusts/mists, and 100, 500, 2500, and 5000 ppm for gases are used both in the sighting study and the main study. Groups of animals are exposed in a stepwise procedure, with the initial concentration being selected as that expected to produce some signs of evident toxicity. Further groups of animals may be exposed at higher or lower fixed concentrations, depending on the presence of signs of evident toxicity, until the study objective is achieved; that is, the classification of the test substance based on the concentration(s) causing evident toxicity, except when there are no effects at the highest fixed concentration (**Table 3**). It may be necessary to conduct another full acute inhalation toxicity study in the second sex.

Sighting Study

The test substance is administered to single animals in a sequential manner following the flowcharts in **Table 2** for a period of at least 4 h. The sighting study is completed when a decision on the starting concentration for the main study can be made, based on signs of evident toxicity or if a death is seen at the lowest fixed concentration. In the absence of evidence from *in vivo* and *in vitro* data from the same chemical and from structurally related chemicals, the starting concentration will be 0.5 mg l^{-1}, 1 mg l^{-1}, or 2500 ppm for vapors, dusts/mists, and gases, respectively. A period of at least 24 h will be allowed between the testing of each animal. All animals should normally be observed for at least 1 week.

In cases where an animal tested at the lowest fixed concentration level in the sighting study dies or exhibits clear clinical signs of toxicity, the normal procedure is to terminate the study and assign the substance to GHS class 1. If further confirmation of the classification is required, a second animal is tested at the lowest fixed concentration. If this second animal dies, then GHS class 1 will be confirmed and the study will be immediately terminated. If the second animal survives, then a maximum of three additional animals will be tested at this concentration. Because there will be a high risk of mortality, these animals should be tested in a sequential manner with a sufficient time interval between animals to protect animal welfare. If a second death occurs, the testing

Table 3 Flowchart for the main study – vapors

Starting concentration: 0.5 mg l^{-1}											
	START										
	5 animals		5 animals			5 animals			5 animals		
	0.5 mg l^{-1}			2 mg l^{-1} [a]			10 mg l^{-1}			20 mg l^{-1}	
A	B	C	A	B	C	A	B	C	A	B	C
Classify GHS class											
1	2		2	3		3	4		4	5	5/Unclassified
Starting concentration: 2 mg l^{-1}											
	START										
	5 animals		5 animals			5 animals			5 animals		
	0.5 mg l^{-1}			2 mg l^{-1}			10 mg l^{-1}			20 mg l^{-1}	
A	B	C	A	B	C	A	B	C	A	B	C
Classify GHS class											
1	2	2		3		3	4		4	5	5/Unclassified
Starting concentration: 10 mg l^{-1}											
	START										
	5 animals		5 animals			5 animals			5 animals		
	0.5 mg l^{-1}			2 mg l^{-1} [a]			10 mg l^{-1}			20 mg l^{-1} [a]	
A	B	C	A	B	C	A	B	C	A	B	C
Classify GHS class											
1	2	2		3	3		4	4		5	5/Unclassified
Starting concentration: 20 mg l^{-1}											
	START										
	5 animals		5 animals			5 animals			5 animals		
	0.5 mg l^{-1}			2 mg l^{-1} [a]			10 mg l^{-1}			20 mg l^{-1}	
A	B	C	A	B	C	A	B	C	A	B	C
Classify GHS class main study starting (concentration mg l^{-1}):											
1	2	2		3	3		4	4		5	5/Unclassified

Outcome: A > 2 deaths; B > 1 with evident toxicity and/or 1 death; C, no evident toxicity and no deaths.
[a] Animal welfare override, if this concentration caused death in the sighting study, then no further animals will be tested. Go directly to outcome A.

sequence will be immediately terminated and no further animals will be tested. The classification will be as shown in **Table 2**: class 1 if there are two or more deaths (outcome A), or class 2 if there is one death (outcome B).

Main Study

A total of five animals of one sex will normally be used for each concentration level investigated, in addition to the single animal used in the sighting study. The time interval between exposures at each level is determined by the onset, duration, and severity of toxic signs. Treatment of animals at the next concentration should be delayed (initially 3–4 days is recommended) until there is confidence in the survival of the previously tested animals.

The action to be taken following testing at the starting concentration level is indicated by the flowcharts. One of three actions will be required: stop testing and assign the appropriate hazard classification class, test at a higher fixed concentration, or test at a lower fixed concentration. However, a concentration level, which caused death in the sighting study, will not be revisited in the main study. Experience has shown that the most likely outcome at the starting concentration level will be that the substance can be classified and no further testing will be necessary.

Procedures for Subchronic Studies

Subchronic studies generally precede lifetime studies and are conducted to determine what the target organ or organ system might be and what exposure regimen (concentration × time) is required to produce this change. For this purpose, it is common to expose groups ($n = 10$) of male rats to three test concentrations. The highest concentration tested is set at one-fifth the ALC (or the LC_{50} depending on the steepness of the mortality dose–response curve) and the lower two would be one-fifteenth and one-fiftieth of ALC. It is desirable to have not only a concentration–response relationship but also a no-observed-exposure limit and a range of toxic effects. In subchronic tests a concurrent control group is required in addition to a vehicle control group.

Animals should be exposed to the test substance for 6 h day^{-1} on a 7 day per week basis for a period of at least 90 days. Another acceptable metho d is one in which rats are exposed 6 h a day for 5 days, given a 2 day rest period, and are again exposed for 5 days, and this is repeated 10 times. *In vivo* observations, including body weight measurements, are made daily. Following exposure, all rats are subjected to hematological, clinical blood chemistry, and urine analysis evaluations. Half of the rats are sacrificed at that time and complete pathological examinations including histological evaluations are conducted. The remaining rats are held without additional exposures for 2–4 and the parameters altered in rats sacrificed immediately following exposure are evaluated to determine the reversibility of the change(s).

Design of Subchronic Inhalation Study

Test species	Rat
Sex	Male
Number of test groups	3(1/5, 1/15, 1/50 ALC)
Number per group	10
Exposures	6 h day^{-1}, 5 days week^{-}, 2 weeks
Animal sacrifice	Five per group after 10th exposure Five per group after 14 day recovery period

Parameters measured: Growth and *in vivo* responses, clinical pathology, urine analyses, gross pathology with organ weights, microscopic pathology, and chemical index of exposure (where possible).

A variation of this design uses an increasing exposure regimen that continues until severe biologic effects are observed. This provides target organ toxicity data using fewer animals (only one group is treated), but the quantitative aspects can be masked in cases in which chemical buildup in the body occurs or change occurs only after some protective function in the body has been depleted. In both of the subchronic studies, the importance of adequate concurrent control animals needs to be underscored.

In this variation, a satellite group of 20 animals (10 animals per sex) may be treated at the high concentration level for 90 days and observed for reversibility, persistence, or delayed occurrence of toxic effects for a posttreatment period of appropriate length, normally not less than 28 days. In addition, a control group of 20 animals (10 animals of each sex) should be added to the satellite study. Animals in the satellite group (if used) scheduled for followup observations should be kept for at least 28 days further without treatment to assess reversibility.

Chronic Studies

Chronic studies are conducted to determine effects of long-term exposures at levels where acute toxicity is not obvious. Chronic exposure patterns generally follow those encountered in the workplace – animals are exposed 6 h day^{-1}, 5 days week^{-1} for their lifetime. For environmental contaminants, continuous exposures of 23 h day^{-1} (allowing 1 h day^{-1} to feed the animals and clean the exposure chambers) for 7 days week^{-1} might be considered more appropriate.

In both chronic study types, exposures are designed to be as constant as possible with minimal deviation from the target or design concentrations.

Dose Quantitation and Effects

In practice, the relationship between dose and lethality for most chemicals is approximately lognormal, but skewed toward hypersensitivity. However, when this frequency population is transformed to a logarithmic abscissa, a normal distribution generally results. When exposure is by inhalation, calculating dose is not straightforward. It is based on knowledge of two variables: How long an animal was exposed and the concentration of material in the atmosphere? What volume of the atmosphere was inhaled by the animal or how much of the material in the inhaled volume was absorbed are not known. As a result, the traditional approach has been to express exposures in terms of concentrations and times of exposure. For most acute exposures, Haber's rule generally holds. That is,

$$ct = K$$

where c is the concentration, t is the time of exposure, and K is the constant specific for that material.

However, in a dynamic exposure situation, the initial concentration in a chamber is clearly lower than the final concentration. It takes time for the atmosphere to equilibrate to a concentration at or very near the desired target concentration. This equilibration time can be calculated as

$$C = (w/b)[1 - \exp(bt/a)]$$

where C is the desired chamber concentration, w is the weight of material introduced per unit time, b is the total airflow through chamber, t is the time, and a is the chamber volume.

A second complication to expressing concentration is that, for gases and vapors, it is properly expressed as parts per million (ppm). Interconversions can be calculated with the formulas

$$\text{mg l}^{-1} = \text{g m}^{-3} \quad \text{mg m}^{-3} = (\text{ppm})\ (\text{MW})/24.5$$

where MW is the molecular weight.

One consideration in model selection is the comparability of doses received to those likely in humans. In the special case of the inhalation route, doses received must be calculated (rather than measured) in a manner somewhat specific to the animal models being employed.

Calculated inhalation dosimetry models, thought not extremely accurate, do have some utility in the cases of (1) comparing toxicity via the inhalation route with toxicity via other routes, (2) risk assessment models and calculations, and (3) interspecies calculations and extrapolations.

These calculations are performed using the formula

$$E = [\mathrm{RF} \times \mathrm{TV} \times C \times 60 \times T]/1000$$

where E is the total maximum possible exposure, RF is the respiratory frequency (per minute), TV is the tidal volume in milliliter, C is the concentration of test agent in milligrams per liter, and T is the daily exposure time in hours. Note that this formula can also be used to compare total doses received over different lengths of exposure. If exposure is repeated over a period of several days, the result of the previous calculation is also multiplied by the number of days of exposure.

Values to be used in this equation for the laboratory species commonly used in inhalation studies and humans are as follows:

Species	*RF*	*TV*	*Hourly exposure*
Rat	85.5	0.86	4.4118 liters × *C*
Mouse	109	0.18	1.1772 liters × *C*
Guinea pig	90	1.8	9.720 liters × *C*
Rabbit	49	15.8	46.452 liters × *C*
Human	11.7	750.0	526.5 liters × *C*

Using this model, the values obtained will be the maximum average limits for the dose received. A number of factors that are not included will affect the actual values, generally serving to reduce the actual values of doses received. These factors include the following:

1. There will be variations in individual animals (and in the same animal at different ages, weights, and states of exercise) in RF and TV.
2. If the material is a particulate or is water insoluble, the degree of deposition and clearance (respectively) in and from the lungs will vary.
3. The degree of absorption from the lungs into the body will vary from compound to compound.

Additional Methods

Intratracheal instillation of materials is a popular alternative to inhalation exposure of animals for studying substances absorbed through the lungs. The advantages of this type of exposure include: very small amounts of test agent are needed (a safety feature in terms of handling and containment of the chemical), extensive chambers are not required, and the complex technical support needed to generate and maintain experimental exposure conditions is avoided. These factors make this type of study very inexpensive to conduct. Furthermore, the dose can be delivered very precisely to the respiratory tract tissues. However, dose distribution to the respiratory tract tissues does not accurately simulate an inhaled dose and, hence, does not reflect the real-life response very closely. Inhalation of airborne toxins generally results in a relatively well-distributed dose throughout the respiratory system. Intratracheal instillation tends to lead to a less uniform deposition and to favor the lower portions of the lung due to gravimetric settling of material. Rats and hamsters were exposed to radioactive particles and the distributions following both inhalation and instillation were examined. The resulting distributions were strikingly different with instillation producing heavy deposits in the medium-sized bronchi. Instilled materials seldom reached the alveoli, whereas inhalation led to considerable deposition in the small airways. High local concentrations following instillation can lead to localized tissue damage which would not be seen following more uniform deposition. The use of this technique then is basically limited to situations in which tissue reactions (both of an acute (inflammation) and chronic (neoplasia and fibrosis) nature) to a variety of materials are to be compared side by side.

Other inhalation toxicology test methods that are less invasive or potentially harmful include:

- noninvasive lung function tests in spontaneously breathing animals (now mandatory under ICH S7A for new pharmaceuticals before they go into humans);
- bronchoalveolar lavage (BAL);
- cell and/or organ cultures of nose, trachea, and lungs; and
- lung slices of different species including man.

See also: Analytical Toxicology; LD_{50}/LC_{50} (Lethal Dosage 50/Lethal Concentration 50); Levels of Effect in Toxicological Assessment; Occupational Toxicology; Pollution, Air; Respiratory Tract; Toxicity, Acute; Toxicity, Chronic; Toxicity, Subchronic.

Further Reading

Pallone TJ and Papademas K (1995) The need for *in vitro* testing to assess inhalation toxicity of consumer spray

products. The Johns Hopkins University 1997–2003. *CAAT Newsletter* 12(2) Winter.
Pauluhn J (2003) Issues of dosimetry in inhalation toxicity. *Toxicology Letters* 140–141: 229–238.
Pauluhn J (2003) Overview of testing methods used in inhalation toxicity: From facts to artifacts. *Toxicology Letters* 140–141: 183–193.
Salem H (ed.) (2004) *Inhalation Toxicology*, 2nd edn. New York: Dekker.

Relevant Website

http://www.oecd.org – OECD Environment, Health and Safety Publications Series on Testing and Assessment No. 39. See also OECD Guideline for Testing of Chemicals: Proposal for a New Guideline: 433; Acute Inhalation Toxicity – Fixed Concentration Procedure.

Toxicity Testing, Irritation

Pertti J Hakkinen

Several entries in this encyclopedia address eye and skin toxicology, and the testing approaches used to assess irritation as an endpoint. The most progress in the development of nonanimal procedures has been for the assessment of local toxicity, for example, eye and skin corrosion and irritation, and many alternatives to animal testing are now available to assess the eye and skin corrosion and irritation potentials of chemicals. Work on the development and validation of additional alternatives to animal testing methods is continuing internationally.

The European Centre for Validation of Alternative Methods (ECVAM) and other organizations have developed and/or recommended tiered testing approaches and strategies to assess eye and skin corrosion and irritation potential. These tiered approaches use *in vitro* methods and other nonanimal approaches, for example, structure–activity relationship (SAR) models. A tiered testing strategy is now

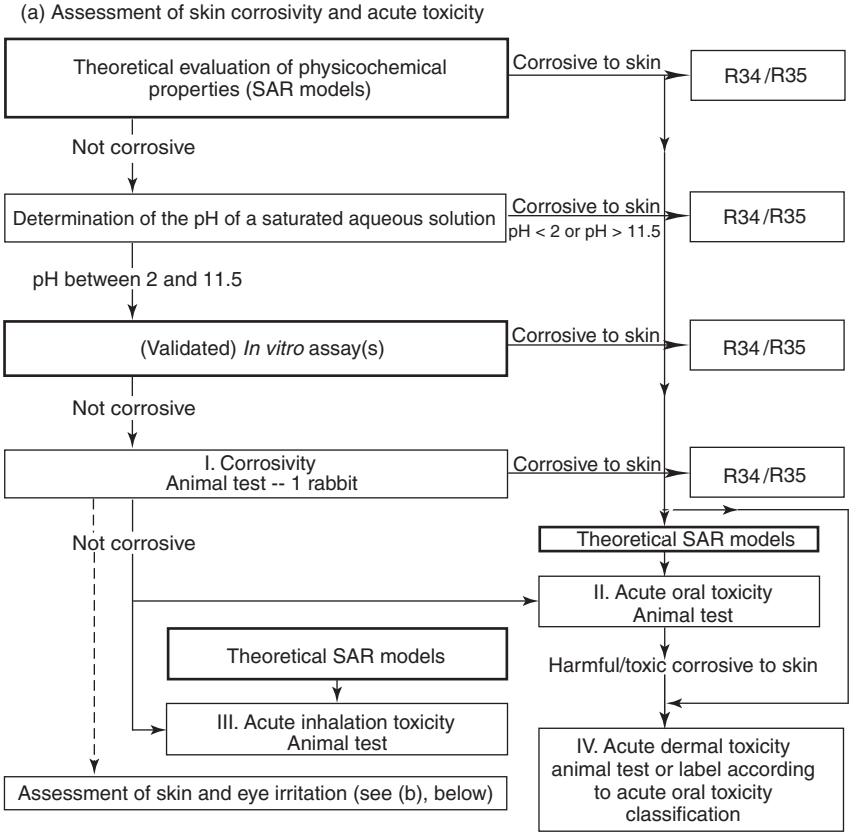
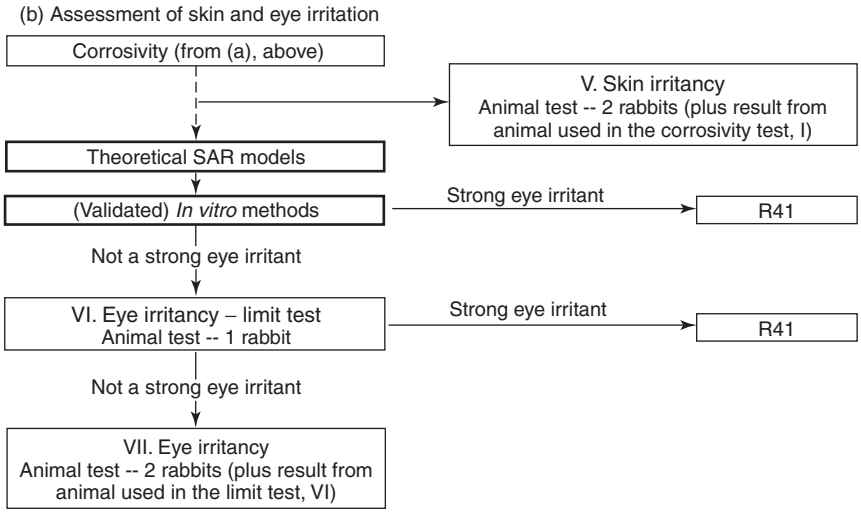


Figure 1 Example of a testing strategy for acute toxicity, corrosivity, and irritancy. (From the European Commission, Institute for Health and Consumer Protection, European Centre for Validation of Alternative Methods (ECVAM) (1995). The Integrated Use of Alternative Approaches for Predicting Toxic Hazard. The Report and Recommendations of ECVAM Workshop 8.)



Strategy for the intoduction of alternative methods (SAR models, *in vitro* tests) into acute toxicity, corrositivity, and local irritancy testing, developed following evaluation of data submitted to the Chemicals Department of the German Federal Institute for Health Protection of Consumers (I. Gerner, BgVV, Germany).
I–VII: Animal tests conducted according to EU/OECD test guidelines.
EU risk phrases: R34 = causes burns; R35 = causes severe burns; and R41 = risk of serious damage to eyes.
= *alternative approaches*

Figure 1 Continued

recommended by the Organisation for Economic Cooperation and Development (OECD), and the European Union (EU) has also adopted this approach. For confirmation of absence of local irritating effects, limited animal testing, however, may still be necessary to obtain the required certainty for the classification and labeling process and to demonstrate the absence of irritating effects. Further, some human eye and skin testing may be very useful in the safety evaluation of cosmetics, and for other consumer products and materials that come in contact with the skin or eyes.

Examples of these tiered testing approaches and strategies are shown in **Figure 1**.

In addition to the tiered testing strategies noted above for assessment of eye and skin effects, a tiered testing and assessment strategy for respiratory toxicity testing *in vitro* was proposed by ECVAM in 1996. This approach and strategy included assessment of irritation potential and other endpoints of cell injury. This included checking the existing data available for the test material itself, or on related substances, followed by acquiring knowledge on the physicochemical properties of the test material, followed by the use of computer modeling techniques (if available) to try to predict the likely toxic effects and target sites. 'First-phase' *in vitro* tests could then follow to identify likely target cells, using tracheal rings, lung slices, alveolar macrophages, or other types of cells. Cell morphology should be determined and crude assessments of the cellular energy status could be undertaken – the results may make it possible to do (semi)quantitative ranking studies of toxic potency. A second phase of *in vitro* tests could then be conducted on the basis of results obtained in the first phase of *in vitro* testing, choosing from tests using airway epithelial ciliated or nonciliated cells, Clara cells; Type II cells, alveolar macrophages; or other type(s) of cells.

See also: Animal Models; European Union and Its European Commission; Eye Irritancy Testing; In Vitro Test; In Vivo Test; Organisation for Economic Cooperation and Development; Respiratory Tract; Safety Testing, Clinical Studies; Toxicity Testing, Modeling; Toxicity Testing, Alternatives; Toxicity Testing, Dermal; Toxicity Testing, Inhalation.

Further Reading

Barratt MD, Castell JV, Chamberlain M *et al*. (1995) The Integrated Use of Alternative Approaches for Predicting Toxic Hazard. The Report and Recommendations of ECVAM Workshop 8. European Commission, Institute for Health and Consumer Protection, European Centre for Validation of Alternative Methods (ECVAM).

Fentem JH and Botham PA (2002) ECVAM's activities in validating alternative tests for skin corrosion and irritation. *Alternatives to Laboratory Animals* 30(Suppl. 2): 61–67.

Gerner I and Schlede E (2002) Introduction of *in vitro* data into local irritation/corrosion testing strategies by means of SAR considerations: Assessment of chemicals. *Toxicology Letters* 127: 169–175.

Hakkinen PJ and Green DK (2002) Alternatives to animal testing: Information resources via the Internet and World Wide Web. *Toxicology* 173: 3–11.

Huggins J (2003) Alternatives to animal testing: Research, trends, validation, regulatory acceptance. *Alternativen zu Tierexperimenten* 20(Suppl. 1): 3–61.

Lambré CR, Aufderheide M, Bolton RE *et al*. (1996) *In Vitro* Tests for Respiratory Toxicity. The Report and Recommendations of ECVAM Workshop 18. European Commission, Institute for Health and Consumer Protection, European Centre for Validation of Alternative Methods (ECVAM).

Meyer O (2003) Testing and assessment strategies, including alternative and new approaches. *Toxicology Letters* 140–141: 21–30.

Roggeband R, York M, Pericoi M, and Braun W (2000) Eye irritation responses in rabbit and man after single applications of equal volumes of undiluted model liquid detergent products. *Food and Chemical Toxicology* 38: 727–734.

Rogier V, Balls M, Basketter D, *et al*. (1999) The Potential Use of Non-invasive Methods in the Safety Assessment of Cosmetic Products. The Report and Recommendations of ECVAM/EEMCO (The European Group for Efficacy Measurements on Cosmetics and Other Topical Products) Workshop 36. European Commission, Institute for Health and Consumer Protection, European Centre for Validation of Alternative Methods (ECVAM).

Van de Sandt J, Roguet R, Cohen C, *et al*. (1999) The Use of Human Keratinocytes and Human Skin Models for Predicting Skin Irritation. The Report and Recommendations of ECVAM Workshop 38. European Commission, Institute for Health and Consumer Protection, European Centre for Validation of Alternative Methods (ECVAM).

Worth A and Balls M (eds.) (2002) Alternative (non-animal) methods for chemical testing: Current status and future prospects. A report prepared by ECVAM and the ECVAM Working Group on Chemicals. European Commission, Institute for Health and Consumer Protection, European Centre for the Validation of Alternative Methods (ECVAM). *Alternatives to Laboratory Animals* **30**(Suppl. 1): 1–125.

Relevant Websites

http://ecvam.jrc.cec.eu.int – European Centre for Validation of Alternative Methods (ECVAM) website. European Commission, Institute for Health and Consumer Protection.

http://altweb.jhsph.edu – Under the management of the Johns Hopkins Center for Alternatives to Animal Testing (CAAT), a diverse group of organizations serve on the Altweb Project Team, many of which maintain their own websites that provide key links from and to AltWeb. The intent of Altweb is to be "the online clearing house for resources, information, and news about alternatives to animal testing" and to serve as the most comprehensive resource on animal alternatives for scientists, educators, veterinarians, and individuals throughout the world.

Toxicity Testing, Modeling

Charles A Pittinger, Andrew Worth, Joanna Jaworska, and Joanne Shatkin

Structure–activity relationships (SARs) and quantitative structure–activity relationships (QSARs), are theoretical models that can be used to predict the physicochemical, biological, and environmental properties of substances. An SAR is an (qualitative) association between a chemical substructure and the potential of a chemical containing the substructure to exhibit a certain biological property or effect. A QSAR is a mathematical model that quantitatively relates a quantitative numerical measure of chemical structure (e.g., a physico-chemical property) to a physical property or to a biological effect (e.g., a toxicological endpoint).

QSARs are a tool used in the absence of available data for prioritization, classification and screening level risk assessment. In addition, QSARs are rapid, inexpensive, can offer a consistent approach, and help to focus on priorities in data gathering. A broad range of QSAR models can readily fill data gaps for assessing chemicals, particularly for fundamental physical – chemical properties, in an expedient and cost-effective manner. QSAR-based evaluations can provide a systematic and consistent approach to chemical evaluations involving large numbers of chemicals. Certain (Q)SARs and other types of theoretical models have gained broad acceptance by regulatory institutions and the private sector. Predictive models can reduce costs, time, and concerns related to conducting toxicity bioassays, for example, for animal welfare reasons, there is considerable pressure to minimize the reliance on animal testing to obtain the information on chemical effects. Input data requirements are generally modest and increasingly available through public and computerized databases.

The Broad Universe of QSARs

An SAR model qualitatively compares structurally similar chemicals for which a measured toxicological or environmental property or endpoint ('the

activity') is available to estimate the same property/endpoint for an analogous, untested chemical. In QSAR models, the endpoint is quantitatively related to a series of structurally similar chemicals (which are often related). The relationship may be continuous or categorical, and is typically developed by regression methods, classification methods (e.g., discriminant analyses and decision trees), or neural networks.

The broadest array of QSAR models is available for endpoints related to physical and chemical properties of chemicals, such as solubility, hydrophobicity, adsorptivity, and volatilization. Fewer are available for biological processes such as biodegradability and toxicity to nonmammalian organisms. Still fewer are QSARs for predicting mammalian and/or human health effects. This is due to several factors. Prediction of physical – chemical parameters often relies upon relatively well-characterized physical and chemical principles and processes. These may require less complex mechanistic understanding than biological processes involving enzyme kinetics and physiological interactions among multiple organs and organ systems (e.g., adsorption, distribution, metabolism and elimination (ADME) pathways). In addition, physical – chemical endpoints typically have a longer history of study, such that more homogeneous data sets are available for a broader range of chemicals.

QSAR Applications for Chemical Screening, Prioritization, and Regulatory and Corporate Decision-Making

QSAR predictions are used by regulatory authorities and private corporations and institutions in three major contexts: priority-setting, hazard classification and labeling, and screening for health and ecological risks of chemicals. Regulatory uses of QSARS include: (1) supporting priority setting of chemicals; (2) guiding experimental design of regulatory tests or testing strategies; (3) providing mechanistic information; (4) grouping of chemicals into categories based on similarity; (5) filling a data gap needed for classification and labeling; and (6) filling a data gap needed for risk assessment. Each application carries unique considerations for QSAR, with the most stringent considerations placed upon QSARs used for 'high regulatory impact', for example, risk assessments under mandated regulatory programs.

QSAR Reliability and Validity

The reliability of QSAR results estimates varies both with application and endpoint. Environmental fate endpoints based on predictions of physical/chemical parameters are the most common and best validated uses of QSAR, and results are relatively well accepted. Systemic toxicity endpoints are more complex, as toxicity is the net expression of multiple biological processes including ADME. Reliability of QSAR predictions for human health endpoints is generally regarded as less than those for non-mammalian (e.g., fish and invertebrates) toxicity endpoints, partly due to the limited availability of high quality data availability. (Nonmammalian species such as fish and aquatic invertebrates (e.g., *Daphnia, Ceriodaphnia*) and algae are typically less expensive to test, and the bioassays carry fewer legal and ethical concerns.)

In order to be considered for regulatory use, it is widely agreed that QSARs need to be assessed for scientific validity. Importantly, QSARs are generally not used as the sole information source upon which to base regulatory decisions. Empirical data are considered first, if available, and have greater reliability than QSAR predictions, unless there are explicit reasons to consider the data erroneous. Because of the inherent uncertainty in QSAR predictions and the need for conservatism in screening level management decisions, they are not recommended in decisions to support reduced concerns, to demote priorities, or to remove a chemical from a regulatory list of chemicals of concern. QSAR predictions are considered in the context of the weight of evidence from multiple sources (e.g., empirical bioassay data, monitoring, epidemiology, etc.). The collective evidence is often weighed on a case-by-case basis by trained experts, applying the best professional judgment.

Risk assessments with 'high regulatory impact' (e.g., enforceable standards such as new chemical registrations or litigation over contaminated sites) must be legally defensible, transparent and unbiased. Many commercial QSAR packages (e.g., TOPKAT, DEREK, MCASE) maintain proprietary and confidential training sets, algorithms, and software. As such, they can be challenged as legally indefensible in a court of law, due to lack of transparency. QSAR packages developed by public organizations, such as the US Environmental Protection Agency's (EPA's) EFAST and EPISUITE models, are typically more transparent. In many cases, they apply the same or similar peer-reviewed databases and algorithms used in commercial QSAR packages. There is currently a need for well-validated, public domain QSAR models for broad regulatory applications and decision support systems.

A number of principles for judging the validity of QSARs were proposed at an international workshop in Portugal in 2002, and hence have come to be

known as the 'Setubal Principles for QSAR Validity'. They include:

- *Endpoint Criteria*. QSARs should be associated with a well-defined endpoint with clear relevance to priority setting, risk assessment, or classification.
- *Descriptor Transparency Criteria*. QSARs should be associated with unambiguous structural descriptors and algorithms supported by available databases. Input data for some QSARs can be generated using outputs of other QSARs, thus propagating additional uncertainty in results. Risk management decisions based on model outputs may be perceived as sound, when in fact, key underlying assumptions may be flawed.
- *Mechanism Criteria*. QSARs should ideally have a physico-chemical or biological basis and toxicological pathway. Because mechanisms of chemical metabolism and intoxication in mammals are not known for the majority of chemicals, the validity feasibility of assessing toxicity endpoints is limited for many chemicals. Many experts contend that health endpoint QSARs should ideally only be applied to chemicals with a mechanism of action consistent with the domain of the training set. Interpolation within the domain is the best use; extrapolation beyond it may lead to spurious, indefensible results. Some models (e.g., TOPKAT) provide cautionary indications for predictions of chemical activity beyond the domain of the training set.
- *Applicability Domain Criteria*. The applicability domain of a (Q)SAR is the physico-chemical, structural, or biological space, knowledge or information on which the training set of the model has been developed, and for which it is applicable to make predictions for new compounds. Ideally, QSARs should only be used to make predictions within the applicability domain of the training set (i.e., interpolation versus extrapolation). Many chemicals and chemical classes do not conform to current QSAR models, as they extend beyond the domain inherent in the training data sets. These include polymers, reaction products, mixtures and inorganics.
- *Validation Criteria*. QSARs should include a measure of the goodness of fit as well as results from external validation of the QSAR, using independent data beyond the training set. The uncertainty and variability of underlying test data limits the precision, accuracy, and reliability of many QSAR predictions. Hence, large uncertainty factors are commonly applied to QSAR predictions requiring comparisons to protective human health benchmarks.
- *Transparency Criteria*. QSARs should be accompanied with full access to the data sets as well as the methods and quality assurance used to generate the data. The apparent sophistication, user-friendliness and flexibility of many publicly available computer-based models may at times convey a false sense of accuracy and a broader range of applicability than the underlying databases and algorithms would justify. Even well validated, technically robust assessment models can be subject to misuse.

QSARs for Predicting Physical – Chemical Properties of Chemicals

Physical – chemical QSAR models are available to predict a range of chemical properties including: melting point, boiling point, water solubility, biodegradability, vapor pressure, Henry's law constant, sediment adsorptivity, octanol–water partition coefficient, and half-life in the environment. These and other parameters can be readily predicted by EPISUITE (see Relevant Websites section), and enables batch data entry based on Chemical Abstract Service (CAS) numbers or SMILES notations.

QSARs for Predicting Environmental Fate and Transport

The physical – chemical parameters predicted in the QSAR models above are often used to estimate fate and transport of chemicals in the environment, a critical aspect of exposure analysis in chemical risk assessments. QSAR model predictions of physical and chemical properties of chemicals, together with empirical data, can be used as inputs to more sophisticated environmental fate models to predict chemical concentrations in source waters and drinking waters. An Organisation for Economic Co-operation and Development (OECD) website (see Relevant Websites section) lists predictive models for environmental fate and exposure pathways, including human health routes of exposure. Models such as EFAST (e.g., the Exposure and Fate Assessment Screening Tool, at the EPA website) are capable of incorporating multiple parameters in predicting fate and transport processes including wastewater treatment from point-source emissions, fate in the environment, concentration at drinking water intakes, atmospheric deposition, land runoff, soil leaching, groundwater migration, etc.

In addition, a wide range of QSAR models to predict biodegradability or persistence are summarized at the OECD website. QSARs models for

biodegradation are more limited in scope and accuracy than packages that predict physical – chemical parameters. The 'PBT Profiler' (Persistent, Bioaccumulative and Toxic) is a public domain QSAR package developed through the US EPA. EPISUITE and the PBT Profiler include estimates of biodegradability based on chemical similarity. More sophisticated but narrower models such as CATABOL (see Relevant Websites section) require mechanistic understanding of enzyme-mediated processes, similar to those available for predicting toxicity. CATABOL is an expert system software that predicts microbial biodegradation pathways and mineralization extent – key factors to determine environmental exposure of chemicals. Features of CATABOL are: (1) capability to predict aerobic biodegradation pathway and assess persistence of metabolites; (2) probabilistic assessment of the extent of biodegradation based on the entire pathway (not, as with other models, the parent structure alone); and (3) online documentation of $\sim$900 microbial transformations.

QSARs for Predicting Ecological Effects

QSARs for the prediction of toxicity to aquatic organisms, including fish, invertebrates and algae, are relatively well developed for a broad range of chemical classes. More than 100 SARs for 55 chemical classes are available in a free, downloadable model called ECOSAR from the EPA website, based on test data and assumptions from test data. Aquatic toxicity endpoints include; reproduction, growth and mortality, such as acute toxicity to fish, invertebrates, and algae. The PBT Profiler also estimates chronic toxicity to fish by means of the ECOSAR model; it compares the fish chronic value to maximum water solubility, in order to estimate potential for aquatic risk.

QSARs for Predicting Human Health Effects

A variety of QSAR models have been developed for human health endpoints and 'packaged' into user-friendly commercial or public-use programs. Human health hazard endpoints commonly predicted by QSAR models include: mutagenicity, carcinogenicity, teratogenicity, neurotoxicity, reproductive and developmental toxicity, skin/eye sensitization and irritation, and systemic toxicity. The more popular commercial QSAR packages for human health include The Open Practical Knowledge Acquisition Toolkit (TOPKAT), Multicase (MCASE), and the Deductive Estimation of Risk from Existing Knowledge (DEREK). Two general types of models can be distinguished: statistically based models such as TOPKAT, and rule-based models such as MCASE. Concise characterizations of these and other QSAR packages appear on the OECD website.

International Uses of QSARs by Regulatory Authorities

United States

A number of US regulatory agencies currently employ QSARs broadly in chemical screening, prioritization and decision-making. The New Chemicals Program in the US EPA's Office of Pollution Prevention and Toxic Substances (OPPTS) uses a variety of methods to make predictions that include QSAR, nearest analog analysis, chemical class analogy, mechanisms of toxicity, chemical industry survey data, and professional judgment. The models are used to identify possible chemicals of concern, for which additional experimental data may be requested from the notifying company through the OPPTS Sustainable Futures Program, new chemical submitters may 'fast track' the review process by use of the Pollution Prevention Framework, incorporating The PBT Profiler. EPA's Office of Pesticides Programs (OPP) is exploring the use of QSARs for nonactive (i.e., 'inert') components of pesticide formulations. The EPA has used QSAR on a discretionary basis under the Hazardous Waste Identification Rule to screen and prioritize some 4000 chemicals for PBT characteristics.

The TSCA Interagency Testing Committee (ITC) uses QSAR predictions, in combination with empirical data and professional judgment, to maintain a 'Priority Testing List'. Finally, ongoing investigative programs in EPA's Office of Research and Development (ORD) are in place to develop, validate and apply QSARs for various health, ecological, and exposure-related endpoints. ORD's National Center for Environmental Assessment is using TOPKAT to predict health effects associated with drinking water disinfection by products, among others. ORD's National Health and Environmental Effects Research Laboratory is developing receptor-binding QSAR models to predict endocrine disruption. ORD's National Center for Environmental Research Star Grant Program supports academic research in QSAR development.

European Union

The European Union (EU), like the US EPA, supports the use of QSARs for screening new chemicals. The EU's Technical Guidance Document provides extensive guidance for the use of QSARs (see Further

Reading section). Under the current EU legislation for New and Existing Chemicals, the use of QSARs is limited, probably because there has been disagreement in the scientific and regulatory communities over the applications of QSARs, and the extent to which QSAR estimates can be relied upon. However, under the future REACH (Registration, Evaluation and Authorisation of CHemicals) system, proposed by the Commission's White Paper on a Future Chemicals Policy, it is anticipated that QSARs will be used more extensively, in the interests of time- and cost-effectiveness and animal welfare.

The EU's Global Harmonised System of Classification and Labeling similarly recognizes the need for QSAR predictions of chemical properties. Within the EU, the Danish EPA has applied 'validated QSAR models' (i.e., TOPKAT, MCASE, and EPIWIN) to screen some 47 000 discrete organic chemicals included in the European Inventory of Existing Chemical Substances (EINECS) Directory of 100 116 chemicals. They identified 20 624 substances deemed to require classification for one or more of these 'dangerous properties': acute oral toxicity, dermal sensitization, mutagenicity, carcinogenicity, and danger to the aquatic environment. They have also developed a physical – chemical properties prediction database for some 166 000 chemicals.

Further, The European Commission's Joint Research Centre (JRC), Institute for Health and Consumer Protection in Ispra, Italy is partnering in several initiatives to establish an international framework for the development, validation, and implementation of QSAR models that are useful for regulatory purposes. Within the JRC, the work involves the European Chemicals Bureau (ECB) and the European Centre for the Validation of Alternative Methods (ECVAM, in addition to external partners, such as the OECD.

Canada

The Canadian Environmental Protection Act, 1999 (CEPA 1999) requires the Ministers of the Environment and Health to 'categorize' the substances on the Canadian Domestic Substances List (DSL). The DSL contains ~23 000 substances that are subject to categorization (i.e., prioritization). Generally the data selection process involves a search of the scientific literature and databases for quality experimental data for persistence, bioaccumulation potential and 'inherent toxicity' to humans and nonhuman species. If acceptable data are not found, QSARs or other models are used to estimate the persistence, bioaccumulation, and aquatic toxicity of substances based on structure and physical – chemical properties.

Other Countries

Other countries such as the Netherlands and the United Kingdom are also developing adopting and using developing QSAR models for the screening and assessment of new and/or existing chemicals. Models in use by these countries can be identified on the OECD website.

See also: Toxicity Testing, Alternatives.

Further Reading

Cronin MTD, Dearden JC, Walker JD, and Worth AP (2003) Quantitative structure–activity relationships for human health effects: Commonalities with other endpoints. *Environmental Toxicology and Chemistry* 22: 1829–1843.

Cronin MTD, Jaworska JS, Walker JD, *et al.* (2003) Use of quantitative structure–activity relationships in international decision-making frameworks to predict health effects of chemical substances. *Environmental Health Perspectives* 22: 1391–1401.

Cronin MTD, Walker JD, Jaworska JS, *et al.* (2003) Use of quantitative structure–activity relationships in international decision-making frameworks to predict ecological effects and environmental fate of chemical substances. *Environmental Health Perspectives* 22: 1376–1390.

Danish Environmental Protection Agency (2001) Report on the Advisory List of Self-Classification of Dangerous Substances, Environmental Project No. 636, Miljoprojekt, Miljostyrelsen.

Eriksson L, Jaworska JS, Worth AP, *et al.* (2003) Methods for reliability, uncertainty assessment, and applicability evaluations of classification and regression based QSARs. *Environmental Health Perspectives* 22: 1361–1375.

European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) (2003) QSARs: Evaluation of the commercially available software for human health and environmental endpoints with respect to chemical management applications. ECETOC Technical Report No. 89.

MacDonald D, Breton R, Sutcliffe J, and Walker J (2002) Uses and limitations of QSARs to categorize substances on the Canadian Domestic Substance List as persistent, and/or bioaccumulative, and inherently toxic to nonhuman organisms. *SAR and QSAR in Environmental Research* 13: 43–55.

Nabholz JV, Clements RG, Zeeman MG, Osborn KC, Wedge R (1993). Validation of structure activity relationships used by the Office of Pollution Prevention and Toxics for the environmental hazard assessment of industrial chemicals, environmental toxicology and risk assessment. In: Gorsuch JW, Dwyer FJ, Ingersoll CG and La Point TW (eds.) *ASTM STP 1216*, vol. 2, pp. 571–590. Philadelphia, PA: American Society for Testing and Materials.

Swanson MB and Socha AC (1997) Chemical ranking and scoring: Guidelines for relative assessments of chemicals. In: Sandestin FL (ed.) *Proceedings of the Pellston Workshop on Chemical Ranking and Scoring*, February

12–16, 1995. Pensacola. FL: Society of Environmental Toxicology and Chemistry (SETAC).
Walker JD and Carlsen L (2002) QSARs for identifying and prioritizing substances with persistence and bioconcentration potential. *SAR and QSAR in Environmental Research* 13: 713–725.
Wedebye EB and Niemela JR (2000) Database with QSAR estimates for more than 166 000 chemicals, QSAR 2000, *Ninth International Workshop on Quantitative Structure–Activity Relationships in Environmental Sciences*, September 16–20, Bourgas, Bulgaria. Abstracts, PV.2.
Worth AP, Hartung T, and van Leeuwen CJ (2004) The role of the European Centre for the Validation of Alternative Methods (ECVAM) in the Validation of (Q)SARS. *SAR and QSAR in Environmental Research* 15: 331–343.
Worth AP, van Leeuwen CJ, and Hartung T (2004) The prospects for using (Q)SARS in a changing political environment – High expectations and a key role for the Commission's Joint Research Centre. *SAR and QSAR in Environmental Research* 15: 345–358.

Relevant Websites

http://www.oecd.org – Organisation for Economic Cooperation and Development (OECD). Database on Chemical Risk Assessment Models.
http://www.epa.gov – Environmental Protection Agency (EPA) website. Freely distributed EPISUITE QSAR program. SARs for 55 chemical classes are available in a free, downloadable model called ECOSAR. The New Chemicals Program in the US EPA's Office of Pollution Prevention and Toxic Substances (OPPTS).
http://www.oasis-lmc.org – Website for PBT Profiler, a public domain QSAR package.
http://btu6.btu.bg – CATABOL: An expert system software that predicts microbial biodegradation pathways and mineralization extent.
http://www.accelrys.com – The Open Practical Knowledge Acquisition Toolkit (TOPKAT): A popular commercial QSAR package for human health.
http://www.multicase.com – Multicase website. Provides MCASE, a QSAR package.
http://www.chem.leeds.ac.uk – University of Leeds website. Provides Deductive Estimation of Risk from Existing Knowledge (DEREK), a popular QSAR package
http://ecb.jrc.it – The European Union's Technical Guidance Document.
http://www.unece.org – United Nations Economic Commission for Europe. The European Union's Global Harmonized System of Classification and Labeling of Chemicals.

Toxicity Testing, Mutagenicity

Robin C Guy

Background Information

Genotoxicity studies are used to aid in the detection of compounds that may lead to genetic damage and frequently to cancer. A significant proportion of chemical carcinogens have been shown to cause DNA damage. There are, however, other carcinogens, such as hormones, some metals, inert physical agents, and some other chemicals, that are believed to cause cancer by mechanisms other than interaction with DNA. Such carcinogens are not usually detected in tests for genotoxicity.

There have been numerous advances in genetic toxicology research that have led to assays to identify compounds that may cause genetic changes. Genetic damage is often classified into three groups, with different types of genotoxicity tests detecting different types of damage. The first group consists of gene mutations, including deletions or insertions of a few base pairs. The second group consists of chromosomal rearrangements, deletions or breaks (clastogenicity), as well as loss or gain of whole chromosomes (aneuploidy) or chromosomal segments. The third group consists of premutagenic damage, such as DNA adducts or DNA strand breaks, or changes reflecting cellular responses to damage, such as unscheduled DNA synthesis. The first two types of damage result in a permanent genetic change that can be transmitted to daughter cells after cell division, while the damage in the third group may be repaired prior to cell division (or the assay may measure evidence of that repair). The term genotoxicity refers to both mutation induction and DNA damage, while mutagenicity refers specifically to mutation induction at the gene and chromosome levels. Many of these assays have been widely used and a large amount of historical data exist both for individual laboratories and in the published literature.

The selection of the most appropriate assay to meet a specific requirement is dependent on a number of factors. These include the following:

- type of genetic alteration that is essential to detect;
- metabolic capability of the test system in relation to the structure of the chemical to be tested;
- proposed use of the test material and the anticipated level of exposure and distribution;

- predictive value of the assay in terms of mutagenicity and carcinogenicity;
- available expertise and facilities; and
- regulatory requirements, when appropriate.

Genetic Toxicology Testing Battery

Genetic toxicology tests are applied so that data are generated on the activity of a compound until a point is reached where an assessment of the probable mutagenic and possible carcinogenic hazard can be made with an acceptable degree of confidence. Since no single assay has proved capable of detecting mammalian mutagens and carcinogens with an acceptable level of precision and reproducibility, it is common practice to perform the assays in a battery, or specific series, of tests.

The International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use and the US Food and Drug Administration (FDA), Center for Food Safety & Applied Nutrition ('Toxicological Principles', *Redbook*, 2000) have published recommendations for a genetic toxicology battery and detailed information on how to conduct those studies. For specific guidance on the conduct of genetic toxicology tests, consult the Organization for Economic Cooperation and Development, US Environmental Protection Agency (EPA), ICH S2B, and US FDA *Redbook* guidelines.

ICH, FDA, and other organizations, such as the US EPA, have developed similar recommendations for the standard battery of genetic toxicity tests, although there are some differences regarding the second test. The battery includes testing for gene mutations in bacteria, testing for gene mutations in mammalian cells *in vitro* using a system that can also detect structural chromosome aberrations, and an *in vivo* test for chromosome aberrations. Note that, due to the specificity of testing methods, it is essential that the battery includes assays that detect both gene mutations and chromosomal aberrations.

The recommendations are following:

- a test for gene mutations in bacteria. This assay detects frameshift and point mutations that involve substitution, addition, or deletion of one or a few DNA base pairs; and
- an *in vitro* test with cytogenetic evaluation of chromosomal damage using mammalian cells. This test detects structural chromosomal aberrations; or
- an *in vitro* mouse lymphoma thymidine kinase with or without gene mutation assay. This assay detects point mutations, as well as large deletions, translocations, mitotic recombination/gene conversion, and aneuploidy. Because it can detect both point mutations and chromosome aberrations, the mouse lymphoma assay is preferred by ICH and FDA over an *in vitro* cytogenetics test, and is listed as the second component of the battery by EPA; and
- an *in vivo* test for chromosomal damage using mammalian hematopoietic cells. These *in vivo* tests detect structural chromosomal aberrations in the case of the mammalian bone marrow chromosome aberration test, and structural damage to chromosomes or damage to the mitotic apparatus in the case of the mammalian erythrocyte micronucleus test.

The assays that comprise the primary test battery are further described in the rest of this section. Other assays may be utilized to elucidate any observed effects, and are described in the next section.

The assay for gene mutations in bacteria is also known as the Ames bacterial reverse mutation test. This assay is relatively simple to perform, reproducible, and gives reliable data on the ability of a chemical to interact with DNA and produce mutations. Because of the ease of use, this assay is often used as an initial screening evaluation. Strains of *Salmonella typhimurium* and *Escherichia coli* are used that require supplementation with amino acids (histidine or tryptophan, respectively) for growth, due to specific mutations in the genes for the synthesis of these amino acids. Defined reverse mutations restore the ability of the bacteria to synthesize the specific amino acid. Different tester strains require different point mutations or frameshift mutations to revert to the wild-type phenotype (which is able to synthesize the amino acid), allowing the researcher to identify the type of mutation caused by the chemical. Procaryotes are very simple organisms, and a positive result in a bacterial assay does not necessarily indicate that the compound will induce similar effects in eukaryote cells. Similarly, a negative result does not invariably mean that the compound lacks mutagenic activity in eukaryotic cells or in intact mammals.

The mouse lymphoma assay is the preferred mammalian mutation assay, as it measures heritable genetic damage *in vitro* arising by means of several mechanisms in living cells and is capable of detecting chemicals that induce either gene mutations or heritable chromosomal events, including genetic events associated with carcinogenesis. (Note that the term 'heritable' mutations refers to mutations that can be inherited by the next generation of cells,

not necessarily mutations in germ cells.) The cell line used is the L5178Y mouse lymphoma 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 semidefined medium (Fischer's medium). In the early 1970s, Clive *et al*. developed the mouse lymphoma forward mutation assay, which screens for mutations conferring resistance to the pyrimidine analog trifluorothymidine. Resistant mutants are visible as colonies in soft agar, with large colonies being due to gene mutations and small colonies reflecting chromosome deletions or other large chromosomal changes.

Either of the two common tests for chromosomal damage (clastogenicity) *in vivo* can be part of the standard testing battery. *In vivo* assays have a clear advantage over *in vitro* assays, since they include an evaluation of the effects of metabolism and other physiological functions and interactions. The chromosome aberration assay involves a direct observation of structural changes in chromosomes analyzed at the first mitotic division after exposure. This assay requires an experienced cytogeneticist to microscopically evaluate metaphase chromosome spreads, typically of bone marrow cells. The micronucleus assay is an *in vivo* or *in vitro* assay for the detection of chromosome damage using mammalian hematopoietic cells. Micronuclei are formed in polychromatic erythrocytes 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. The micronucleus assay has the advantage of requiring less time and specialized skill than the metaphase analysis involved in evaluating chromosome aberrations.

Table 1 General genetic toxicology assays

Assays for gene mutations
Salmonella typhimurium reverse mutation
Escherichia coli reverse mutation
Gene mutation in mammalian cells in culture, including evaluation of mutations at
Hypoxanthine-guanine phosphoribosyl transferase (*hprt*)
A transgene of xanthine–guanine phosphoribosyl transferase (*xprt*)
Thymidine kinase (*tk*)
Drosophila sex-linked recessive lethal
Gene mutation in *Saccharomyces cerevisiae*
Mouse spot test
Assays for chromosomal aberrations
In vitro cytogenetic
In vivo cytogenetic
Micronucleus
Dominant lethal
Heritable translocation
Mammalian germ cell cytogenetic
Assays for DNA effects
In vitro DNA damage and repair, including DNA adducts, single-strand breaks
Unscheduled DNA synthesis
Mitotic recombination in *Saccharomyces cerevisiae*
Sister chromatid exchange

Additional Genetic Toxicology Assays

Although a great deal of effort was put into battery harmonization, there is no universal agreement on the best combination of tests for all purposes. There are other mutagenicity tests in use that may also be useful (**Table 1**). The choice of additional test(s) or protocol modification(s) depends on various factors. Compounds that contain structural alerts for genotoxicity are usually detectable in the standard test battery. However, compounds bearing structural alerts that have given negative results in the standard test battery may require some additional testing. When the standard test battery produces negative results with a chemical that falls within a class known to require special test conditions, then additional testing with appropriate test modifications should be performed. There are compounds for which standard *in vivo* tests do not provide additional useful information. This includes compounds that toxicokinetic or pharmacokinetic data indicate are not systemically absorbed and therefore are not available for the target tissues in standard *in vivo* genotoxicity tests. In cases where sufficient target tissue exposure cannot be achieved, it may be appropriate to base the evaluation only on *in vitro* testing. Alternatively, *in vivo* genotoxicity studies are often conducted using intraperitoneal injection to avoid complications related to poor absorption. Additional genotoxicity testing may be conducted to help determine the mechanism of action if the material was clearly negative in the standard test battery but was positive in carcinogenicity bioassay(s). Additional testing can include modified conditions for metabolic activation in *in vitro* tests or can include *in vivo* tests measuring genetic damage in target organs of tumor induction.

In addition to the mouse lymphoma assay discussed above, several other assay systems are available for evaluating gene mutations in eukaryotic

cells. These systems include gene mutation assays in a variety of mammalian cell systems, gene mutation assays in yeast, the sex-linked recessive lethal test in *Drosophila*, and *in vivo* gene mutation tests, such as the mouse spot test (a somatic cell-specific locus test) and assays in transgenic animals.

Cell lines used for *in vitro* mammalian cell gene mutation assays include the CHO, AS52, and V79 lines of Chinese hamster cells and TK6 human lymphoblastoid cells. In these cell lines, different spectra of genetic events are detected. The most commonly used genetic end points measure mutation at the genes for hypoxanthine–guanine phosphoribosyl transferase (*hprt*), a transgene of xanthine–guanine phosphoribosyl transferase (*xprt*), or thymidine kinase (*tk*). The *tk* and *xprt* genes are autosomal and appear to allow for the detection of genetic events (e.g., chromosomal exchange events) that are not detected at the *hprt* locus, which is located on the X-chromosome. This is because genetic damage that involves vital genes adjoining the *hprt* locus on the X-chromosome is likely to be lethal to the cell, while damage to vital genes in an autosomal cell will be compensated for by intact genes on the homologous chromosome (which lack functional *tk* or *xprt*). Also, the lack of a homologous chromosome in the case of the *hprt* gene may preclude mutations that arise via homologous recombination.

In vivo tests for gene mutations have historically been limited to evaluation of visible mutations (e.g., coat color) or evaluation of specific biochemical changes. However, the development of transgenic mice and rats has revolutionized the field of *in vivo* mutagenesis assays, making it theoretically possible to evaluate mutations in any tissue. The transgenic rodents contain a foreign DNA sequence, commonly the *E. coli lac* genes. After the animal is exposed to the chemical, the transgene is recovered from the animal tissue, and mutants are evaluated. For example, the *lac* genes are readily packaged into phage lambda, and mutant plaques can be identified based on color phenotype on appropriate media.

The same chromosome aberration assay described above can be used to detect numerical chromosome changes (i.e., aneuploidy) in bone marrow cells. Chemicals that cause chromosome damage in germ cells can be detected using *in vivo* assays, either the dominant lethal assay, a test for structural chromosome aberrations in spermatogonia, or a more preferred test, the mouse heritable translocation assay.

A number of assays are available that can detect premutagenic DNA damage and cellular responses to effects on DNA. The initiation of enzymatic repair of the damage involves degradation of the damaged part of the DNA and subsequent synthesis of a new, short strand of DNA to replace the degraded area. This synthesis is termed unscheduled DNA synthesis (UDS). The UDS assay may be conducted as an *in vitro* assay in primary or cultured mammalian cells or as an *in vivo/in vitro* assay. The sister chromatid exchange assay (SCE) detects reciprocal exchanges, at homologus loci, of DNA between two sister chromatids of a duplicating chromosome. Although this is a cytogenetic effect, SCEs are considered to be general indicators of mutagen exposure, analogous to DNA damage and repair assays, due to the uncertainties about the mechanism of their formation. Single-strand breaks in DNA are also indicative of DNA damage, and may be formed by chemical exposure or by the cell's response to the chemical. Mitotic crossing over (the exchange of segments of DNA between genes or between a gene and its centromere) and mitotic gene conversion (transfer of segments of DNA within a gene) can be investigated in the yeast, *Saccharomyces cerevisiae*. While these two end points occur at a low level in untreated cells, they occur at an increased level in yeast cells exposed to DNA-damaging agents, partially as part of the DNA repair response. DNA adducts provide direct evidence that a chemical interacts directly with DNA, but they are not proof of mutagenicity, since an adduct may be repaired prior to the formation of a mutation. Different adducts may have different rates of repair, so the most common adduct produced by a chemical may not be the most important for prediction of mutagenesis or carcinogenesis.

Factors to Consider in the Conduct of Assays

To ensure that the results of an assay are valid, specific criteria have been determined. Care must be taken to follow the published procedures to ensure that the genetic structure of the test organisms meets the requirements of the particular assay. The test material must be able to reach the molecular target (e.g., DNA) in the cell in its reactive form.

Many mutagenic materials are not able to interact with DNA until they have undergone some degree of enzyme-mediated biotransformation. 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 an exogenous metabolic activation system (S9), a rat liver homogenate prepared from the livers of rodents treated with enzyme-inducing agents such as Aroclor 1254. Concurrent testing should be done with negative (solvent) and appropriate positive controls both in the presence and in the absence of S9.

For all studies, 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 the test article), extended exposure to S9, or high levels of cytotoxicity.

Cultures of established cell lines or cell strains should be used. Mammalian cell lines should be determined to be mycoplasm-free and should be karyotyped. This is to ensure that they will respond in the expected fashion in the experimental system being used.

Regulatory agencies and testing laboratories have developed standard criteria for accepting many of the established genotoxicity assays. Negative controls should exhibit some minimal level of viability (e.g., plating efficiency for cell lines), and mutagenicity in the negative control should be within accepted limits. Appropriate positive controls should be used to test both the activity of the S9 and the specific cell line or strain, and should produce a response above some specified minimum level. The test chemical should be evaluated up to sufficiently high doses, or limits of solubility or cytotoxicity, taking into account the caveat about excessive cytotoxicity mentioned above. The sample size and number of replicates should be sufficient for adequate sensitivity, and, for *in vivo* studies, it is desirable to show that the test compound reaches the target tissue.

Once the overall assay conduct has been determined to be acceptable, the assay is evaluated for a positive response. A variety of aspects of the response to the test chemical are considered in evaluating the response. These include the magnitude of the response, the statistical and biological significance, reproducibility (both in replicates within the assay and in repeated independent assays), and the presence or absence of a dose–response. Based on these considerations, an overall evaluation is made.

Interpretation

The carcinogenic process is an extremely complex system. It is apparent from the relative simplicity of short-term assays that they cannot copy all of the stages in the carcinogenic process and are frequently assumed to detect only the event leading to the initiation phase, that is, the ability to induce a mutagenic or clastogenic DNA lesion. Although the short-term assays provide useful qualitative information, considerable caution is required in their interpretation in terms of carcinogenic activity. Results of the mutagenicity tests should be considered together with data from other toxicity tests and pharmacokinetic studies.

See also: Chromosome Aberrations; Environmental Protection Agency, US; Federal Insecticide, Fungicide, and Rodenticide Act, US; Food and Drug Administration, US; Genetic Toxicology; Good Laboratory Practices (GLP); Harmonization; Host-Mediated Assay; Immune System; International Conference on Harmonisation; Micronucleus Assay; Mouse Lymphoma Assay; National Institutes of Health; National Toxicology Program; Organisation for Economic Cooperation and Development; Redbook; Sister Chromatid Exchanges; Toxicity Testing, Alternatives.

Further Reading

Adler ID, Shelby MD, Bootman J, *et al.* (1994) Summary report of the working group on mammalian germ cell tests. *Mutation Research* 312: 313–318.

Auletta AE, Dearfield KL, and Cimino MC (1993) Mutagenicity test schemes and guidelines: US EPA Office of Pollution Prevention and Toxics and Office of Pesticide Programs. *Environmental and Molecular Mutagenesis* 21(1): 38–45.

Braman J (2001) *In vitro Mutagenesis Protocols*, 2nd edn. Totowa, NJ: Humana Press.

Food & Drug Administration (FDA) (2001) Toxicological principles for the safety of food ingredients. *Redbook 2000*. US FDA, Center for Food Safety & Applied Nutrition.

Kirkland DJ (ed.) (1990) *Basic Mutagenicity Tests: UKEMS Recommended Procedures*. New York: Cambridge University Press.

Kirkland DJ and Muller L (2000) Interpretation of the biological relevance of genotoxicity test results: The importance of thresholds. *Mutational Research* 464(1): 137–147.

Madle S, Dean SW, Andrae U, *et al.* (1994) Recommendations for the performance of UDS tests *in vitro* and *in vivo*. *Mutation Research* 312: 263–285.

Pratt IS and Barron T (2003) Regulatory recognition of indirect genotoxicity mechanisms in the European union. *Toxicology Letters* 140–141: 53–62.

Tice RR, Hayashi M, MacGregor JT, *et al.* (1994) Report from the working group on the *in vivo* mammalian bone marrow chromosomal aberration test. *Mutation Research* 312: 305–312.

Relevant Websites

http://www.fda.gov – Guidance for Industry: S2B Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals.

http://www.bgvv.de – Working Group on Genetic Toxicology Documents (from the German Federal Institute for Risk Assessment).

Toxicity Testing, 'Read Across Analysis'

Pertti J Hakkinen

Testing requirements often mandate that certain sets of toxicological information have to be provided for new substances (e.g., 'base sets' or 'Screening Information Data Sets'). While the data typically come from animal studies and *in vitro* alternatives to animal testing, they can also come from use of modeling, and from human clinical or epidemiological data. A further source of information can be derived from 'read across' evaluations or analyses of the data sets available for structurally similar substances. The 'read across' approach has been accepted by some regulatory authorities, and is based on the understanding that substances with similar physicochemical property profiles will generally have similar toxicity profiles.

The focus of the read across evaluation approach is on interpolation rather than extrapolation, and the rationale and data sources for the read across evaluation should be documented. For example, a read across table of data could have the related chemicals as columns, and the various types of toxicology tests and their results as the rows under each substance. Reading across the columns will highlight the amount and types of data for the group of substances. The read across evaluation will also find any gaps in the data set for a specific chemical that might be judged by the reviewer(s) to be filled by data relevant to those data gaps for the similar substances.

The read across evaluation can reduce the extent of testing required for substances within the group. There is, of course, expert judgment involved. In addition, thoroughness and skill are needed in making sure all relevant substances and their available data sets have been identified for the read across. Another issue is that the read across data should be developed and presented according to the guidelines of the relevant regulatory or other organization. Further, it should be indicated whether the data for each study for each substance were developed by current, established testing protocols and, if so, which guideline (e.g., those from the Organisation for Economic Cooperation and Development)? If established testing protocols were not used in some instances, the protocols used to conduct the studies should be at least described to the extent that they can be reviewed to understand their design, strengths, and limitations as the read across evaluation is performed.

A number of issues should be considered when assessing the toxicological properties of a new substance and similar substances by read across evaluation. These issues include assessing the similarity of the purity and impurity profiles of the new substance and the similar substances. This is important since there should be no toxicologically meaningful differences in the purities or impurities on a scale that would be likely to influence the overall toxicity. Further, the physicochemical properties of the new substance should be compared with the similar substances. This includes the physical form, molecular mass, water solubility, partition coefficient, and vapor pressure. In addition, the likely toxicokinetics of the substances, including the possibility of different metabolic pathways, should be considered.

An example of a read across table of information is shown below. Substances X_1, X_2, X_3, X_4, X_5, X_6, X_7, and X_8 are structurally similar substances. For this example, the main structure could be CH_3–C_x–CH_3, with the only difference being the length of the C_x section of the molecule. X_1 would be one carbon, X_2 would be two carbons, etc., and thus X_2 and X_4 would be the closest in structure to X_3, and X_4 and X_6 would be the closest in structure to X_5. Note that acute oral toxicity data have been identified for X_3 and X_4, while X_5 is supported by read across from X_4 to X_6, with data also identified for X_1 and X_8. For the *in vivo* genetic toxicity (i.e., micronucleus test), no data are available for X_3, X_4, and X_5; however, these substances are supported by read across from X_2 to X_6, with data also available for X_1 and X_8. Analysis of these data sets might lead to a judgment that a basic level of acute, genetic, repeat dose, and reproductive toxicity information is available for all of these substances, either directly or by read across to the other structurally similar substances.

Example of a Read Across table of available studies

Type of toxicity study	*Substance X_3*	*Substance X_4*	*Substance X_5*	*Substances similar in structure to X_3, X_4, and X_5*
Acute toxicity				
Oral	✓	✓	RA	✓ (for substances X_1, X_6, and X_8)
Dermal	RA	RA	RA	✓ (for substances X_1, X_2, X_6, and X_7)
Inhalation	✓	✓	✓	✓ (for substances X_1, X_2, X_6, and X_8)
In vitro genetic toxicity				
Bacterial	✓	✓	RA	✓ (for substances X_1, X_6, and X_8)
Cytogenetics	✓	✓	✓	✓ (for substances X_1 and X_8)
In vivo genetic toxicity				
Micronucleus	RA	RA	RA	✓ (for substances X_1, X_2, X_6, and X_8)
Repeat dose toxicity	✓	✓	RA	✓ (for substances X_1, X_6, X_7, and X_8)
Reproductive toxicity screen	✓	✓	RA	✓ (for substance X_6)

✓ = One or more studies available
RA = Read Across

See also: High Production Volume (HPV) Chemicals; Toxicity Testing, Alternatives; Toxicity Testing, Modeling.

Further Reading

Barratt MD (2000) Principles of toxicity prediction from chemical structure. In: Balls M, van Zeller A-M, and Halder ME (eds.) *Progress in Reduction, Refinement and Replacement in Animal Experimentation*, pp. 449–456. Amsterdam, The Netherlands: Elsevier.

Combes R, Barratt M, and Balls M (2003) An overall strategy for the testing of chemicals for human hazard and risk assessment under the EU REACH system. *Alternatives to Laboratory Animals; ATLA* 31: 7–19.

Hanway RH and Evans PF (2000) Read-across of toxicological data in the notification of new chemicals. *Toxicology Letters* 116(Suppl. 1): 61.

Indans I (2002) The use and interpretation of *in vitro* data in regulatory toxicology: Cosmetics, toiletries and household products. *Toxicology Letters* 127: 177–182.

Worth AP and Balls M (eds.) (2002) Alternative (non-animal) methods for chemicals testing: Current status and future prospects. Chapter 3: The scientific basis of chemical risk assessment. *Alternatives to Laboratory Animals: ATLA* 30(Suppl. 1): 21–25.

Relevant Website

http://www.epa.gov – Miller MJ (no date but accessed in 2004). Use of (Q)SAR and Read-Across in the HPV Challenge. US Environmental Protection Agency (EPA). EPA's Chemical Right-to-Know website.

Toxicity Testing, Reproductive

Rochelle W Tyl

Introduction

It is currently estimated that ~15% of couples are clinically infertile (no conception after 1 year of unprotected intercourse); ~30% of the infertility is attributable to the male partner, 20% to the female partner, 20% to a combination of problems in both partners, and another 30% is not explained by diagnosis of adverse conditions in either partner. Once conception has occurred, up to 80% of human pregnancies may be lost, most in the first trimester. Reproductive toxicity may be defined as an adverse effect on any aspect of male or female reproductive structures or functions, on the developing offspring, or on lactation, which would interfere with the development of normal offspring through sexual maturity, in turn capable of normal reproduction. This definition includes aspects of developmental toxicity, including teratogenesis, and developmental neurotoxicity. This entry focuses on the male and female mammalian reproductive systems, chemicals that affect the status and functions of these systems, and tests currently utilized to detect such effects.

Male and Female Reproductive Systems

The reproductive system in the embryo consists of paired gonadal ridges in the dorsal midline containing all components but the gonial cells; the gonial cells differentiate during embryogenesis, external to

the embryo in the yolk sac, and migrate into the embryo and into the gonadal ridges along prescribed routes. The gonial cells in transit number in the hundreds; once they arrive, they proliferate, and the gonad develops into sex-specific structures.

Male System

The mammalian male reproductive system consists of the testes and associated structures: epididymides, vas deferens, accessory sex glands (seminal vesicles, prostate, Cowper's (bulbourethral) glands, and preputial glands), and intromissive organ (the penis) (**Figure 1**). The testis consists of two major compartments: the seminiferous tubules and the interstital compartment. The seminiferous tubules contain the spermatogonial cells, which differentiate into the spermatozoa (sperm), and the Sertoli cells, which provide support and nutrition to the developing sperm, produce androgen-binding protein to bind the male sex hormone testosterone (and produce a glycoprotein in the conceptus and neonate which suppresses female development), and which maintain the blood–testis barrier. The interstitial compartment contains Leydig cells which produce testosterone and other androgens for transport within and outside the testis.

The androgens control spermatogenesis, the growth and activity of accessory sex glands, and external masculinization. Interestingly, 17-β estradiol (the endogenous potent estrogen), made locally in the male brain, determines male-specific behaviors. *In utero*, androgen production by the fetal testis early in development (e.g., weeks 4–6 in humans) is essential for sexual differentiation of the gonads triggering male development and repressing female development (along with a product of the Sertoli

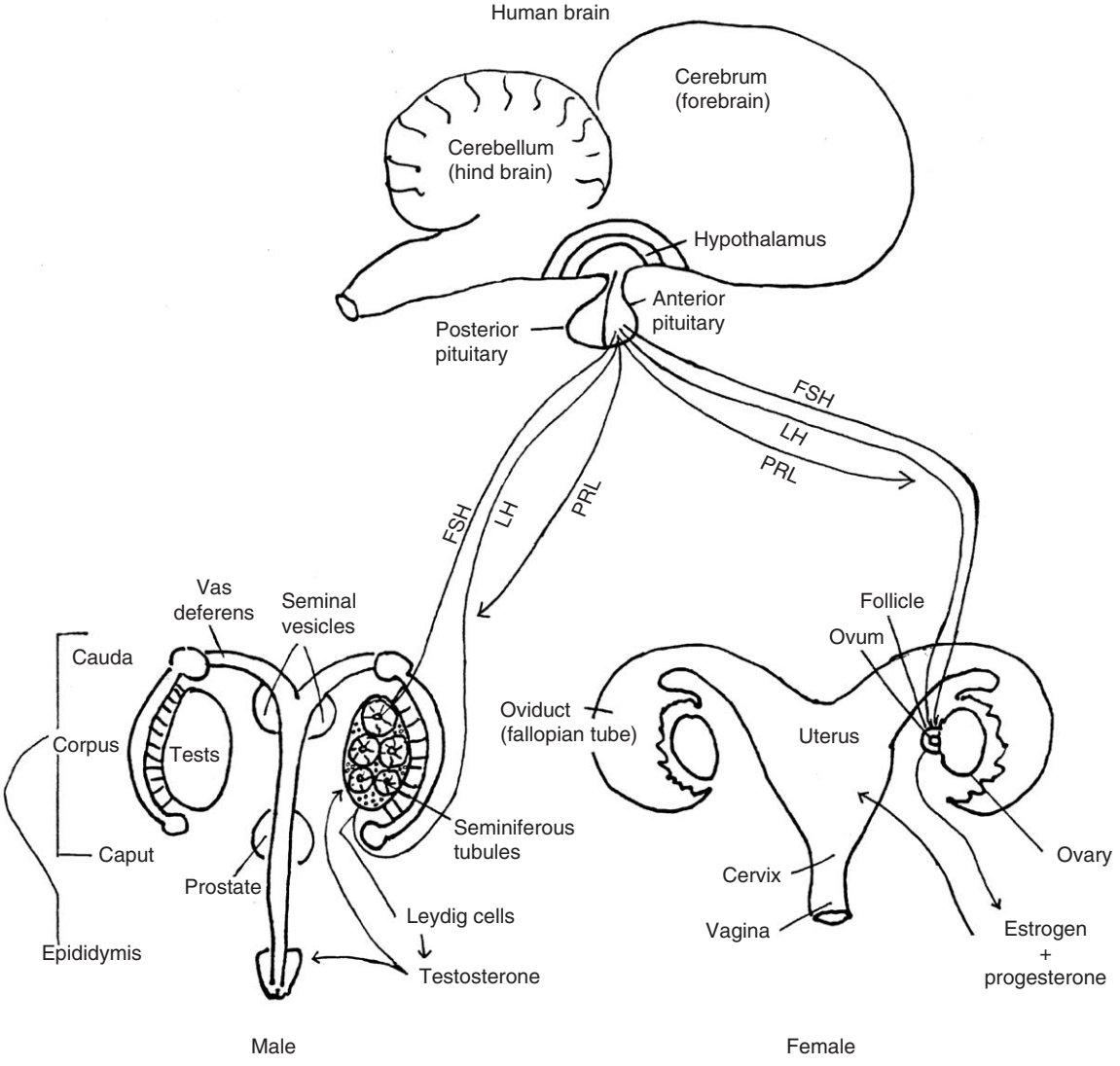


Figure 1 Male and female reproductive systems with hormonal controls from the brain (hypothalamus) and anterior pituitary gland.

cells). The developmental process in the indifferent gonad in the male is triggered by the activation of a gene on the Y chromosome that causes the interstitial cells in the gonad to differentiate into Leydig cells and produce testosterone. This in turn triggers the cascade of gene activations to direct development of male-specific organs. The predominant masculinizing hormone *in utero* is dihydrotestosterone, produced locally in the testis from testosterone by the enzyme 5-α-reductase. The increased presence of testosterone during puberty in males triggers the development of male secondary sex characteristics and the initiation of spermatogenesis.

The postnatal control of testicular function is via the hypothalamus–pituitary–testis axis (**Figure 1**). In the hypothalamus of the forebrain, neuroendocrine neurons secrete gonadotrophic hormone-releasing hormone (GnRH) into the anterior pituitary gland. Here GnRH stimulates release into the blood of the two gonadotrophic hormones, luteinizing hormone (LH) and follicle stimulating hormone (FSH), named for their first-discovered roles in the female reproductive system. Prolactin (PRL) is also released into the blood from the anterior pituitary under control of dopamine from the median eminence in the forebrain. FSH and LH act on the testis: FSH stimulates the Sertoli cells to enhance sperm production, and LH acts on the Leydig cells to stimulate synthesis of testosterone, which in turn is required in high concentrations in Sertoli cells for sperm production. PRL acts to enhance the effects of LH on the testes.

Spermatogenesis (the process which produces mature sperm) in the testis begins at puberty in mammalian males, when high blood levels of FSH and LH are attained (**Figure 2**). The spermatogonial cells (diploid) at the periphery of each seminiferous tubule undergo repeated mitoses; one 'daughter' cell in each cell division replaces the original spermatogonial cell, and the other daughter cell becomes a primary spermatocyte and begins the process toward production of sperm. Each primary spermatocyte undergoes meiosis I (the first reduction division) to form two secondary spermatocytes; each secondary spermatocyte undergoes meiosis II (the second reduction division) to form two spermatids. Each spermatid undergoes a differentiation process, termed spermiogenesis, to produce a mature sperm (haploid); this process involves compaction of the DNA into the headpiece of the sperm, covered by an acrosomal cap (used to penetrate the egg); formation of a midpiece with mitochondria (to fuel the swimming function of the flagellum); and formation of a flagellum (tail) to drive the sperm. The process produces, from one primary spermatocyte, four functional sperm. The sperm are passively moved from the center (lumen) of each seminiferous tubule into the epididymis where the sperm acquire the capacity for movement and fertilization – the capacitation process. The epididymides, seminal vesicles, and prostate secrete fluids to nourish and capacitate the sperm, to provide the hydrodynamic force for ejaculation, and to neutralize the acidic environment of the female's reproductive tract. Large numbers of sperm are produced in waves, with 10 000–10 million ejaculated per time in males. The process of spermatogenesis takes ~ 5 weeks in mice, 8 weeks in rats, and 10 weeks in humans and occurs continuously from puberty until death.

Female System

The female reproductive system consists of the ovaries, oviducts (fallopian tubes), uterus, cervix, and vagina (**Figure 1**). The ovary consists of oocytes, follicles (containing thecal and granulosa cells) where the oocytes develop, and support cells. *In utero* development of female internal and external structures is by 'default'. In the absence of testosterone, dihydrotestosterone, and Müllerian-inhibiting substance (all made in the testes), male reproductive anlagen regress and female anlagen differentiate into the appropriate female reproductive structures. The prenatal female (at least in rodents) does not make 17β-estradiol in either her ovaries or adrenal glands. The follicles do produce estrogen and progesterone during the reproductive period of the female (in humans from puberty to menopause).

The control of ovarian function, beginning at puberty, is via the hypothalamus–pituitary–ovary axis (**Figure 1**). As with the male, cells in the hypothalamus secrete GnRH in the female that acts on the anterior pituitary to release FSH and LH in a cyclical pattern (PRL is also released). FSH and LH act on the ovary; FSH stimulates the growth of follicles which in turn secrete estrogen and progesterone to prepare the uterus for implantation of the fertilized egg (zygote), and LH, in a mid-cycle surge, triggers the rupture of the follicle and release of the ovum (ovulation). PRL plays a role in the maintenance of the corpus luteum (the collapsed follicle after ovulation which produces estrogen and progesterone), the rupture of the follicle, and in lactation.

Oogenesis (the process that produces mature ova) begins at puberty in mammalian females (**Figure 2**). Note that the series of mitoses of oogonial cells occurs only *in utero*; all the primary oocytes a female will have ($\sim 500\,000$ in humans, most of which will die) are present in her ovaries prior to her birth. *In utero*, each primary oocyte proceeds through the second (of four) phases of meiosis I and waits until puberty and the onset of the cyclical release of FSH

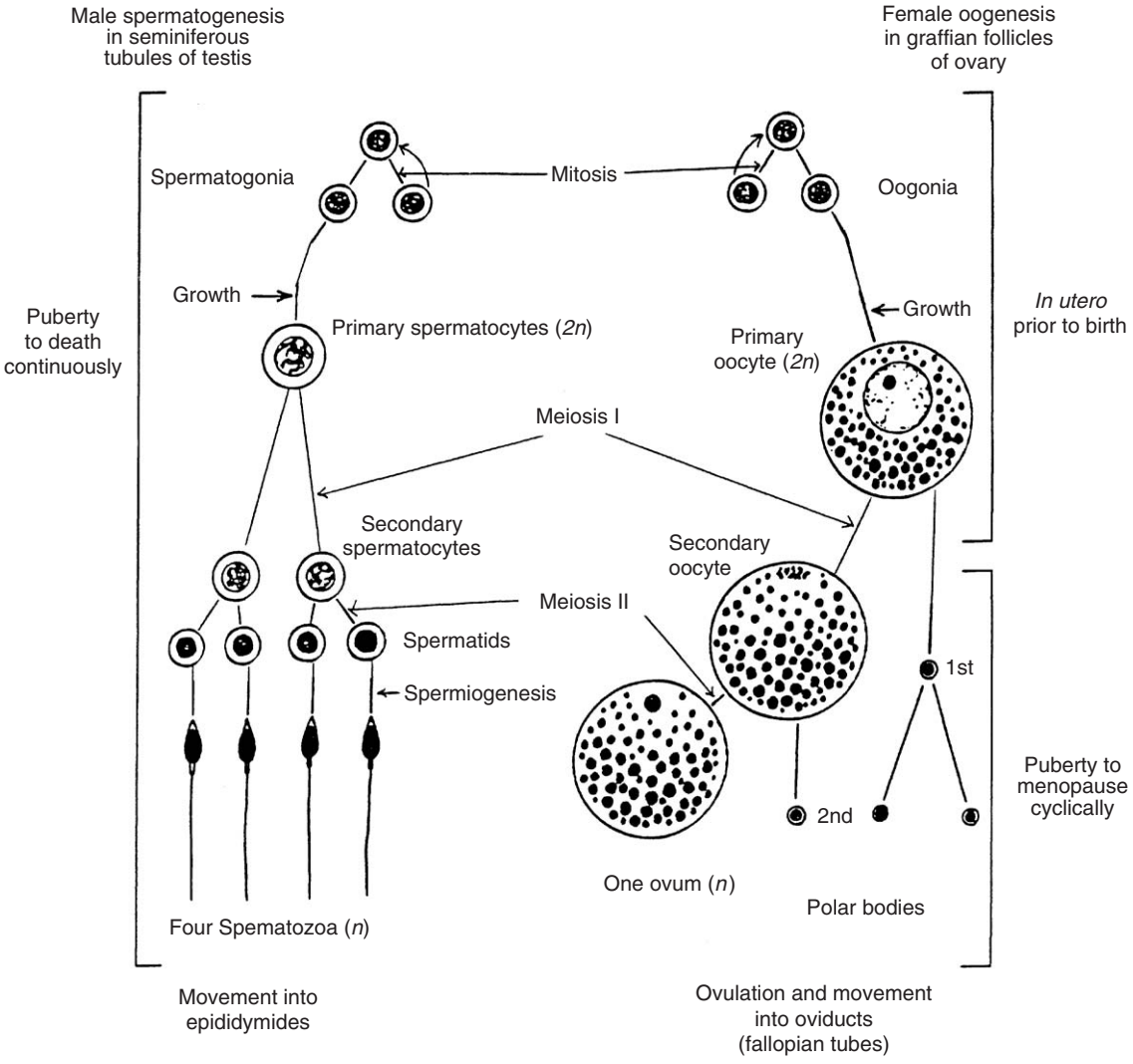


Figure 2 The process of male spermatogenesis and female oogenesis.

and LH. Beginning at puberty, a number of follicles begin the process of oogenesis during each cycle. In each follicle, the primary oocyte completes meiosis I to produce a large secondary oocyte and a very small first polar body. The secondary oocyte (and in some species, the first polar body) undergoes meiosis II to produce a very large ootid (haploid) and a second polar body (if the first polar body divides, it produces two secondary polar bodies). The objective of the 'lopsided' division, and the functions of the layers of accessory nurse cells which surround the developing oocyte in the follicle, is to produce a very large ovum, filled with nutrients and preformed genetic blueprints to sustain the early embryo. In response to the LH surge, the most advanced follicle(s) ruptures (the other ova die in a process termed atresia) and the mature ovum (or ova) is released into the oviduct; the collapsed follicle becomes a corpus luteum with endocrine functions (producing estrogen and progesterone to sustain the uterine lining). One or more eggs is ovulated during each cycle (up to 24 in rodents). The associated uterine cycles (termed estrous cycles in mammals with little or no postovulation luteal phase, and termed menstrual cycles in mammals with a long luteal phase; e.g., humans) build up and then shed the uterine lining if the egg or eggs are not fertilized and last a few days (rat and mouse) to several weeks (dogs, humans, and horses). Estrus, at the time of ovulation of the egg(s), corresponds to the period of female receptivity (high levels of circulating estrogens and LH surge) in species that undergo periodic 'heats'. In humans, there are ~ 500 ova ovulated from puberty to menopause.

Fertilization and Offspring Development

The mature ovum is released from the ruptured follicle and is drawn into the oviduct. The egg is

transported from the ovary to the uterus by movement of cilia at the opening of the oviduct, muscle contractions of the oviduct, and subsequent fluid movement; fertilization takes place in the oviduct. Meanwhile, the uterus, in response to preovulatory estrogen production from the follicle, increases blood flow and the uterine lining (endometrium) begins to thicken. After ovulation, the actions of progesterone (and estrogen) complete the growth of the endometrium including increased blood supply, formation of microvilli, increased synthesis of proteins, and other sources of nutrition.

Fertilization consists of penetration of the ovum by a sperm and union of the two haploid nuclei (one from each gamete); early cell divisions then begin (cleavage). The fertilized egg (zygote) continues to travel down the oviduct into the uterus and implants in the receptive uterine lining. The time from fertilization to implantation is relatively short and comparable among mammalian species: 5 (mice) to 8 (humans) days. Implantation is accomplished by the invasive destruction of the uterine lining by the outermost extraembryonic cells of the conceptus (trophoblast cells) and ultimate intimate association of these cells with the maternal uterine blood vessels; the uterine lining heals over the conceptus and the exchange of nutrients and wastes begins at the site of the future placenta. Once implantation is complete, the conceptus proper begins to differentiate into outermost (ectoderm), innermost (endoderm), and middle (mesoderm) cell layers and the major organ systems begin to form. This period of organogenesis lasts ~10 days in rodents and 58 days in humans. At the end of organogenesis (signaled by the closure of the secondary palate), the conceptus is termed a fetus and the fetal period of histogenesis (differentiation of cells and tissues within systems) begins. It lasts until parturition (birth), ~7 days later in rodents and 7 months later in humans. Delivery occurs after ~19–22 days of pregnancy in rats and 270 days in humans; the perinatal period involves adjustments of the offspring to air breathing, nursing, and rapid growth, development, and learning in a gravity-based environment, aided by required postnatal care by the mother (maternal behaviors such as pup retrieval, milk production and delivery, grooming, and teaching).

Considerations for Reproductive Toxicity Evaluations

The male reproductive system is at risk during fetal development *in utero*, postnatally during puberty, and during the male reproductive lifetime with targets including the processes and structures involved in primary sex differentiation (testes and accessory organs), secondary sex characteristics and sexual behaviors (e.g., libido), and performance (e.g., erection and ejaculation). The traditional endpoint of concern is the production of normal numbers of normal (genetic, chromosomal, and structural) sperm.

Identification of reproductive toxicants is made from clinical workups on men in infertility clinics or undergoing drug treatments, predominantly for cancer, from epidemiologic studies on general populations (environmental exposures), or on worker populations (industrial exposures in production plants or users of chemicals such as pesticides and commodity chemicals), and from animal studies (usually in rodents). Animal studies allow for more invasive examinations such as histopathology of the testes, close scrutiny of mating behavior, and mating to proven breeders. They can be used to confirm or extend initial observations in humans or to initially identify a potential reproductive toxicant. One epidemiologic study of male workers exposed to dibromochloropropane (DBCP) was apparently triggered by the men talking at work breaks about their wives' failure to conceive and a request from the workers to Occupational Safety and Health Administration for an investigation. DBCP proved to be a testicular toxicant that affected spermatogenesis in the male workers; however, data on rats exposed to DBCP with the same testicular findings were, in fact, available in the literature 15 years prior to the worker concerns. One additional unique characteristic of male reproductive toxicity is that if effects are limited to postspermatogonial cells, then the effect is transient (limited to the time when these cells become sperm and are ejaculated) and subsequent waves of spermatogenesis from the intact spermatogonial cells are not affected.

Since the definition of normal sperm includes normalcy of the haploid genetic complement as well as normal sperm structure, numbers, and functions (i.e., ability to swim in the female's reproductive tract, penetrate the ovum, and join its genetic material with the egg's haploid genetic material), analyses of sperm parameters are performed in all three categories of investigations: clinical, epidemiologic, and animal testing. Routinely, sperm numbers, motility (viability), and morphology are ascertained. The interpretation of the human worker reproductive data is usually confounded by, among many factors, job experience, exposures to multiple materials, lifestyle (e.g., smoking, drinking, 'recreational' use of drugs, and hobbies), age, health status, diet, status of spouse, number and ages of children, socioeconomic class,

educational level, and ethnic/religious factors. Exposure data for workers are usually very poor (e.g., workplace air concentrations are not precise for individuals or job descriptions/titles, and measurements are not frequent and not long term (over the job or employee working lifetime)). Exposure data for nonworking environments (i.e., contaminated foodstuffs, water, soil, or air) may be even worse.

The female reproductive system is also at risk during fetal development *in utero*, postnatally during puberty, and during her reproductive lifetime until menopause (cessation of ovulation). The traditional end points of concern are ovulation of a normal ovum, fertilization, uterine status, implantation and prenatal development, parturition, and lactation involving nursing (appropriate quality and quantity of milk) and other maternal behaviors.

Identification of reproductive toxicants in females is made from clinical workups (as with males), from epidemiologic studies in general populations, and to a lesser degree on worker populations (the industrial workforce in chemical production and end use has been traditionally male). Animal studies are also very important to initially identify an agent or to confirm and extend initial findings in women. The risk to women's reproductive status may be transient (limited to the pregnancy at risk) or permanent, if effects are to the primary oocytes, since no additional oocytes will be made during her reproductive lifetime. The confounders for male reproductive risk assessments are essentially the same for female reproductive risk assessments. The cyclical nature of the female's reproductive activity (e.g., hormone levels, ovulation, and uterine lining buildup and shedding) makes both human and animal research more difficult; synchronized test animal populations (by estrous cyclicity) are very useful in identifying some effects on reproductive structures and functions.

Categories of Reproductive Toxicants

Reproductive toxicants can be categorized by type of agents, for example, physical agents such as ionizing radiation; pharmaceuticals such as therapeutic drugs, especially those used in treatment of cancers (which target DNA and therefore act as mutagens and/or target cell proliferation and therefore affect spermatogenetic and oogenetic cell divisions); recreational drugs/drugs of abuse; pesticides (which are obviously biologically active); industrial chemicals (including solvents and commodity chemicals); environmental chemicals (contaminating air, water, soil, and foodstuffs); and naturally occurring toxicants such as phytoestrogens (in soy and clover), plant toxins (e.g., mushrooms and herbs) and animal toxins (from invertebrates such as certain shellfish, spiders, and insects and from vertebrates such as certain fish, frogs, toads, and snakes).

Categorization of reproductive toxicants by function or mechanism would include agents acting as mutagens (causing changes within and between genes), clastogens (causing changes in parts of chromosomes, including chromosome breakage), cytotoxins (killing cells in general or specific cell types such as gonial cells, Leydig cells, Sertoli cells, etc.), mitotic/meiotic poisons (interfering with cell division, e.g., by damage to the assembly/dissociation of spindle fibers which control the movement of chromosomes), agonists or antagonists of endogenous hormones (e.g., environmental estrogens/antiestrogens and androgens/antiandrogens), inhibitors of hormone synthesis, transport, or degradation, and neurotoxicants (affecting central nervous system control of reproduction). Toxic effects on other systems (e.g., the thyroid, liver, kidneys, adrenal glands, etc.) may also affect reproduction.

Governmental Regulations

The thalidomide disaster in the late 1950s and early 1960s resulted in over 8000 children in 28 countries with major drug-specific malformations. The US governmental agencies recognized that it was only extraordinary luck and Dr. Frances Kelsey of the FDA which averted huge numbers of children in the United States being affected (the manufacturers were not permitted to market the drug in the United States); there were no mandated testing procedures in place at the time which would have identified the risk. In response, in 1966, Dr. E. I. Goldenthal, Chief of the Drug Review Branch, FDA, sent a letter to all corporate medical directors establishing *Guidelines for Reproductive Studies for Safety Evaluation of Drugs for Human Use*. These guidelines were promulgated "as a routine screen for the appraisal of safety of new drugs for use during pregnancy and in women of childbearing potential." Three phases or segments were proposed which have since been modified by the International Conference on Harmonisation (ICH) and adopted by the regulatory bodies of the European Union, Japan, and the United States. Segment I, Study of Fertility and Early Embryonic Development to Implantation (**Figure 3a**), was designed to provide information on breeding, fertility, preimplantation survival, nidation (implantation), and early postimplantation survival and growth in rats. It involves exposure of weanling males for at least one full spermatogenic cycle (10 weeks) and/or of adult females for at least two ovulation cycles (2 weeks) and then a mating period,

with males necropsied after the mating period. Females continue exposure through gestation with necropsy of the parental females and their litters during gestation to identify pregnancy rate and pre- and early post-implantation loss. One rarely used alternative is to necropsy one-half of the dams and their litters on gestational day (gd) 13–15 (with exposure ceasing on gd 6), and to necropsy the other half of the females and their litters at weaning at the end of lactation (postnatal day (pnd) 21) to identify *in utero* losses, postnatal losses, and growth and development of the offspring. Acquisition of developmental landmarks such as surface righting, pinna (external ear) detachment, pilation, auditory startle, eye opening, incisor eruption, mid-air righting reflex, negative geotaxis, testis descent, etc., is noted. The offspring maintained beyond weaning allow ascertainment of acquisition of puberty (time of vaginal patency in females, preputial separation in males) and, if appropriate, motor activity, learning and memory, and mating competence. Segment III Study of Pre- and Postnatal Development (also see Toxicity Testing, Developmental), as initially designed, involved exposure of the maternal animal after major organogenesis is over (gd 15) through the 'last trimester' of rodent pregnancy, through parturition and lactation of their litters until weaning (pnd 21). One male and one female F_1 offspring/litter are selected to be

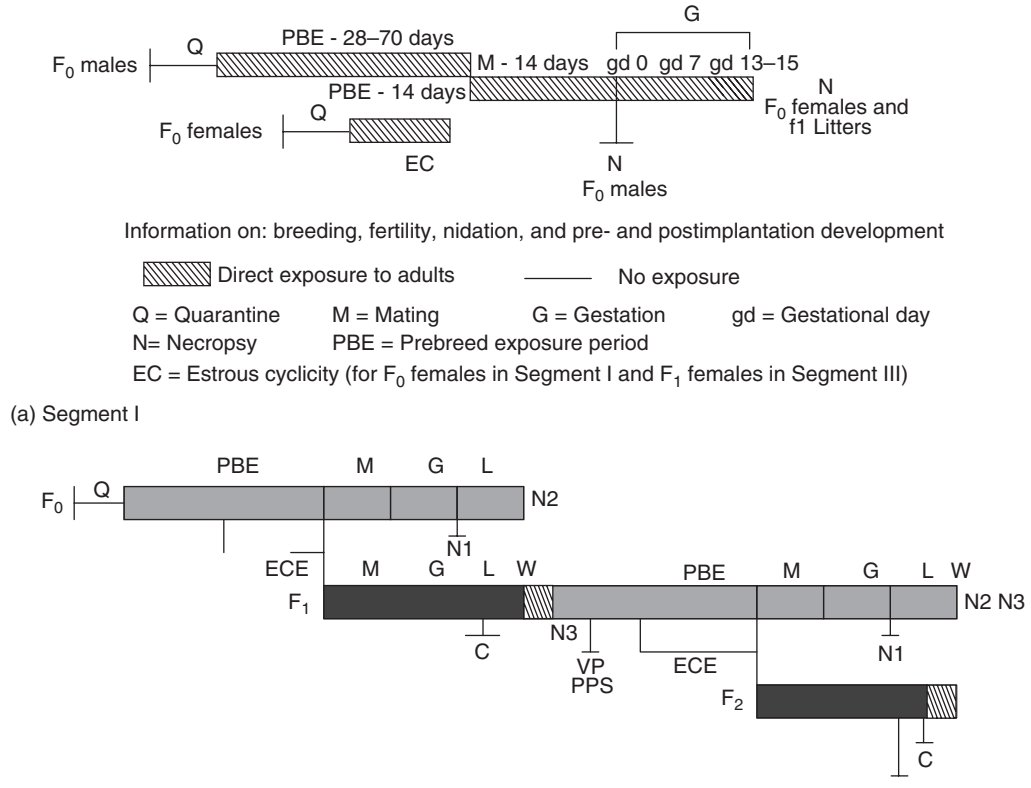
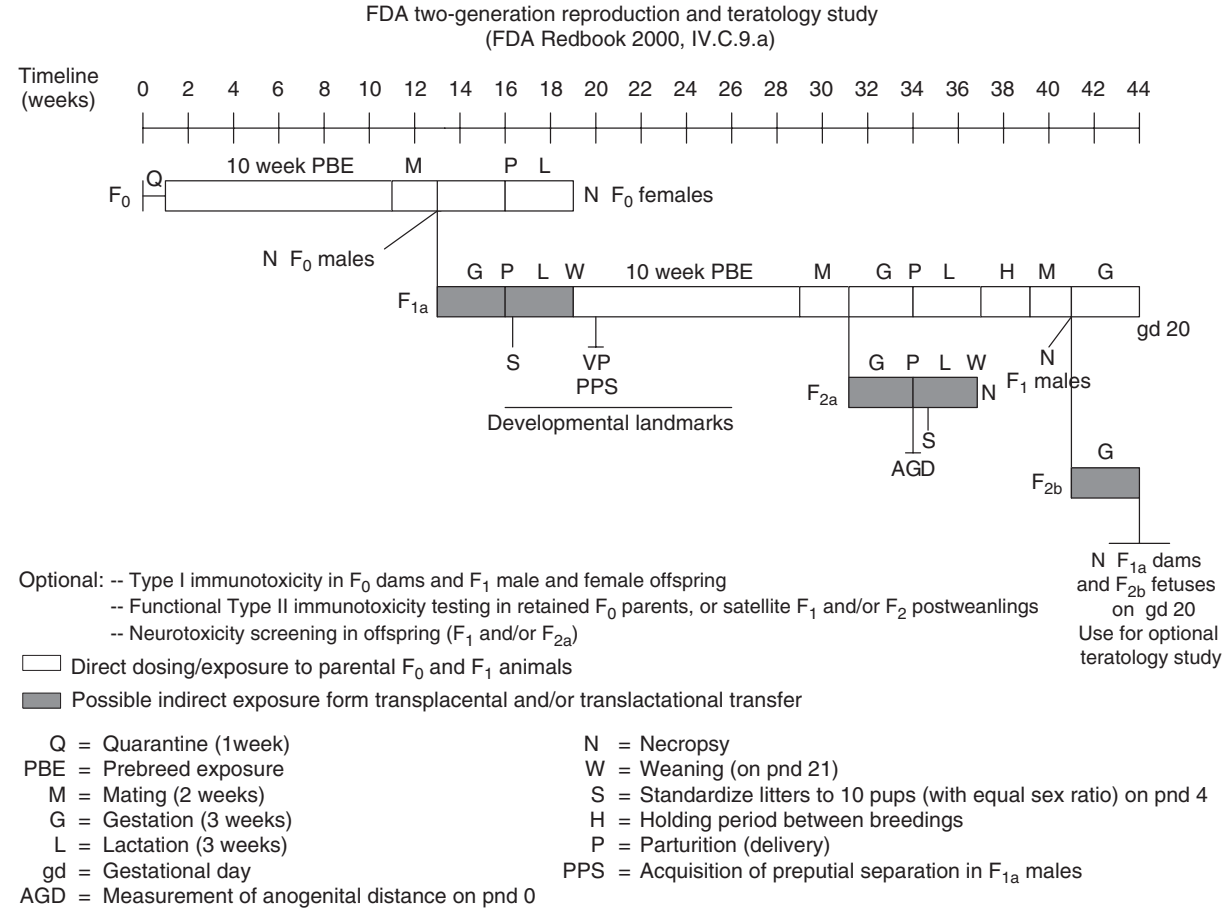


Figure 3 US government guidelines for study designs of reproductive toxicology. (a) Segment 1 (US FDA); (b) two generation (US EPA: TSCA, and FIFRA; OECD).



(c) Reproductive toxicity study design (two-generation, two litters/generation)

Figure 3 (Continued) (c) two generation (US FDA).

retained (with no direct dosing) to adulthood, with acquisition of developmental landmarks, puberty, behavioral, and other functional tests performed (see above for alternative Segment I postnatal evaluations). The retained F_1 offspring are then mated (1:1) within groups. The F_1 males are necropsied after mating, and the F_1 females and their F_2 litters are necropsied on pnd 4. Except for the Segment I alternative, this is the only FDA segment which evaluates postnatal consequences of *in utero* exposure, but it only addresses exposures that occur after major organogenesis is complete (see ICH Guideline 4.1.2). (Segment II studies are discussed under Toxicity Testing, Developmental.)

A two-generation reproductive toxicity study, used by the Environmental Protection Agency (EPA; OPPTS, TSCA, FIFRA) and OECD (**Figure 3b**), involves a long prebreed exposure (10 weeks in rats, 8 weeks in mice) of both sexes (designated F_0) begun after weaning, with continuing exposure during mating, gestation, and lactation (with F_0 males necropsied usually after mating), selection of offspring (designated F_1) and prebreed exposure, mating, gestation, and lactation of F_1 parents and F_2 offspring. The F_1 generation is the major focus of this protocol since it is the only generation that receives exposure from the time its members were gametes through their time of reproductive performance; treatment-related effects on structures and functions of the reproductive system would be discernible in the F_1 animals by this protocol. The new (1998) EPA test guidelines include prebreed estrous cyclicity and stage of estrus at demise for F_0 and F_1 females, andrology (epididymal sperm number, motility and morphology, testicular homogenization-resistant spermatid head counts to calculate daily sperm production (DSP) and efficiency of DSP) in F_0 and F_1 males, organ weights and histopathology of selected organs at adult necropsies, 'triggered' anogenital distance in F_2 newborns, and acquisition of puberty in F_1 postwean animals. A third reproductive toxicity protocol promulgated by FDA involves a three-generation, two litter per generation study design (**Figure 3c**). It is similar to the two-generation study, except that F_0 animals, after they produce the

first litters (designated F_{1a}) are rebred (within groups to different partners) to produce F_{1b} offspring. Usually the F_{1a} offspring are retained for prebreed exposure and generation of F_{2a} and F_{2b} offspring. (F_{1b} offspring are terminated at weaning, with representative animals, usually 10/sex/group, necropsied.) The F_{2a} animals are usually retained for prebreed exposure and generation of F_{3a} and F_{3b} offspring. The last breed of F_{2a} animals to produce the F_{3b} litters can be executed like previous breeds, with the offspring terminated at weaning, or the F_{2a} mothers can be necropsied on gd 20 (prior to expected parturition) and the F_{3b} fetuses evaluated for developmental toxicity (examination of external, visceral, and s keletal morphological development *in utero*).

The ICH has recently (1994) promulgated harmonized testing guidelines for reproductive toxicity in five study designs adopted by the European Union, Japan, and the United States in 1996 (**Figure 4**).

The first study design (**Figure 4a**), termed Study of Fertility and Early Embryonic Development (4.1.1), is similar to an FDA Segment I study except that male prebreed exposure is for 4 weeks and female exposure begins at mating and extends only to gd 6 (at the time of implantation). F_0 males are sacrificed after gd 6 and F_0 females are sacrificed at midpregnancy (gd 15) or just prior to term (gd 20). It is designed to assess the effects of exposure during prebreed (males), mating (both sexes), and the preimplantation period (females) on *in utero* reproductive indices.

The second study design (**Figure 4b**), termed Study for Effects on Prenatal and Postnatal Development (4.1.2), assesses exposure to the parental female from implantation (gd 6) through weaning of her litter (pnd 21) with selected offspring pups retained for mating (to produce F_2 litters). This design is similar to an FDA Segment III study, except that the treatment exposures begin at implantation and encompass the periods of major organogenesis and fetogenesis, with evaluation of postnatal consequences of *in utero* and lactational exposures.

The third study design (**Figure 4c**), titled Study for Effects on Embryo–Fetal Development, is essentially an FDA Segment II study with exposure of the mother during organogenesis of her offspring *in utero* (gd 6–15).

Additional study designs (**Figure 4d** and **e**) are essentially combinations of the first two or three designs.

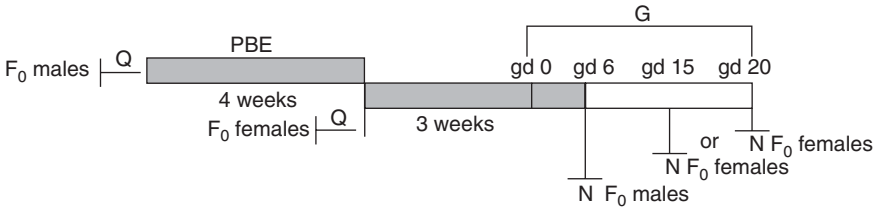
Obviously, specific studies are also designed to investigate a specific endpoint and/or agent.

Statistical Analyses

Statistical analyses of continuous data which distribute according to a bell-shaped curve (e.g., adult body weights, weight changes, feed consumption, pup body weights, and percentage male pups) employ parametric methods, including analysis of variance, tests for homogeneity of variances, tests for dose-related trends, and pairwise comparisons to the concurrent control group values. Continuous data which do not distribute as discussed previously are examined by nonparametric methods to identify trends and pairwise comparisons. Nominal (noncontinuous) data such as reproductive indices (e.g., mating, fertility, fecundity, and incidence of adult clinical signs) and survival indices are also analyzed for trends and pairwise comparisons. The unit of analysis for all tests is the male, the female, or the litter.

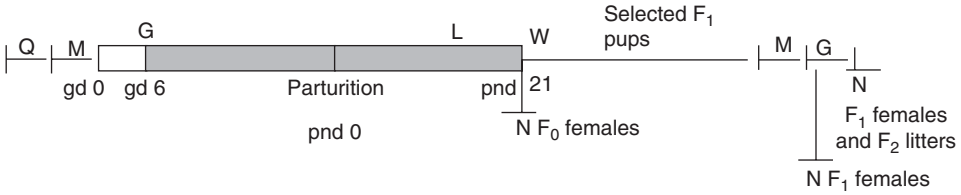
Risk Assessment

The US government's new approach (2000) to health assessment of agents involves the iterative interaction of four major components: basic scientific research (hazard identification), science-based toxicity/risk assessment (dose–response assessment), exposure assessment, and risk characterization (**Figure 5**). Animal studies usually provide hazard identification and dose–response assessment. Animal studies usually employ a route of administration which delivers a bolus dose for a worst-case scenario (i.e., gavage) or which duplicates the known or potential human route of exposure, usually dosed feed or dosed water for end-use consumer exposure, or inhalation or cutaneous routes (for industrial exposures). The highest dose in these studies which produces no effects is designated the no-observed-effect level (NOEL) or the no-observed-adverse-effect level (NOAEL). The NOEL/NOAEL is then divided by one or more uncertainty or safety factors to obtain the 'acceptable daily intake' value (ADI; EPA: FIFRA) or the reference dose (RfD; EPA: TSCA). These doses (ADI or RfD) are defined as an estimate of the daily human exposure that is likely to be without appreciable risk of adverse effect. The uncertainty factors typically include 10 for extrapolation from animal models to humans and 10 for the diversity of human populations (to protect the most sensitive human subpopulations) for a total of 100. (A third factor of up to 10 is proposed for pesticides to protect infants and children.) Another uncertainty factor (usually 10) is commonly used to cover possible lifetime exposures in humans versus the exposure period in the test animal species, especially for ADIs, for a total of 1000 (to protect against chronic, long-term exposure in the diet from food, water, etc. contaminated with pesticide residues). Exposure assessment is then performed for human exposure for all sources. A value termed margin of exposure (MOE) is defined as the



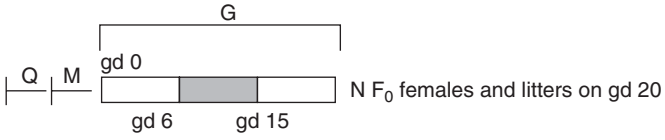
Assess: Maturation of gametes, mating behavior, fertility, preimplantion, implantation

(a) Study of fertility and early embryonic development, (4.1.1) rodent (see segment I)



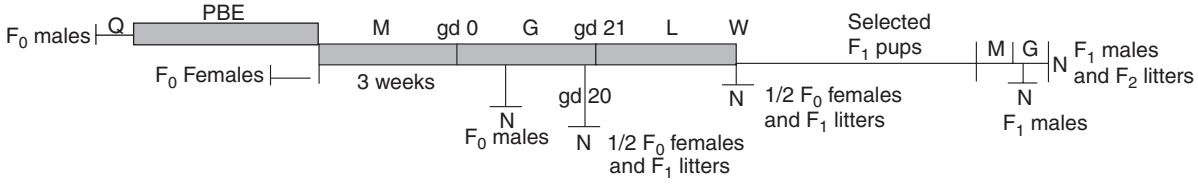
Assess: Toxicity relative to nonpregnant females, prenatal and postnatal development of offspring, growth and development of offspring, functional deficits (behavior, maturation, reproduction)

(b) Study for effects on prenatal and postnatal development, including maternal function (4.1.2) rodent (see segment III)



Assess: Toxicity relative to nonpregnant females, embryofetal death, altered growth of offspring *in utero*. Sructure changes of offspring *in utero*

(c) Study for effects on embryo–fetal development (4.1.3) rodent and non-rodent (see segment II)



(d) Single-study design (4.2) rodents (combine 4.1.1 and 4.1.2)

4.1.1 with 4.1.2 1/2 F_0 females and F_1 litters necropsied on gd 20
1/2 F_0 females and F_1 litters necropsied on pnd 21
(retained selected F_1 pups followed through mating gestation of F_2 liters)

(e) Two study design (4.3) rodents

Q = Quarantine
PBE = Prebreed exposure
M = Mating
G = Gestation
L = Lactation
W = Wean
N = Necropsy
gd = Gestational day
pnd = Postnatal day

Direct exposure to adults

Figure 4 International Conference on Harmonisation guidelines on detection of toxicity to reproduction for medicinal products. (a) Study of fertility and early embryonic development (4.1.1); (b) study for effects on prenatal and postnatal development, including maternal function (4.1.2); (c) study for effects on embryo–fetal development (4.1.3); (d) single-study design (4.2); (e) two-study design (4.3).

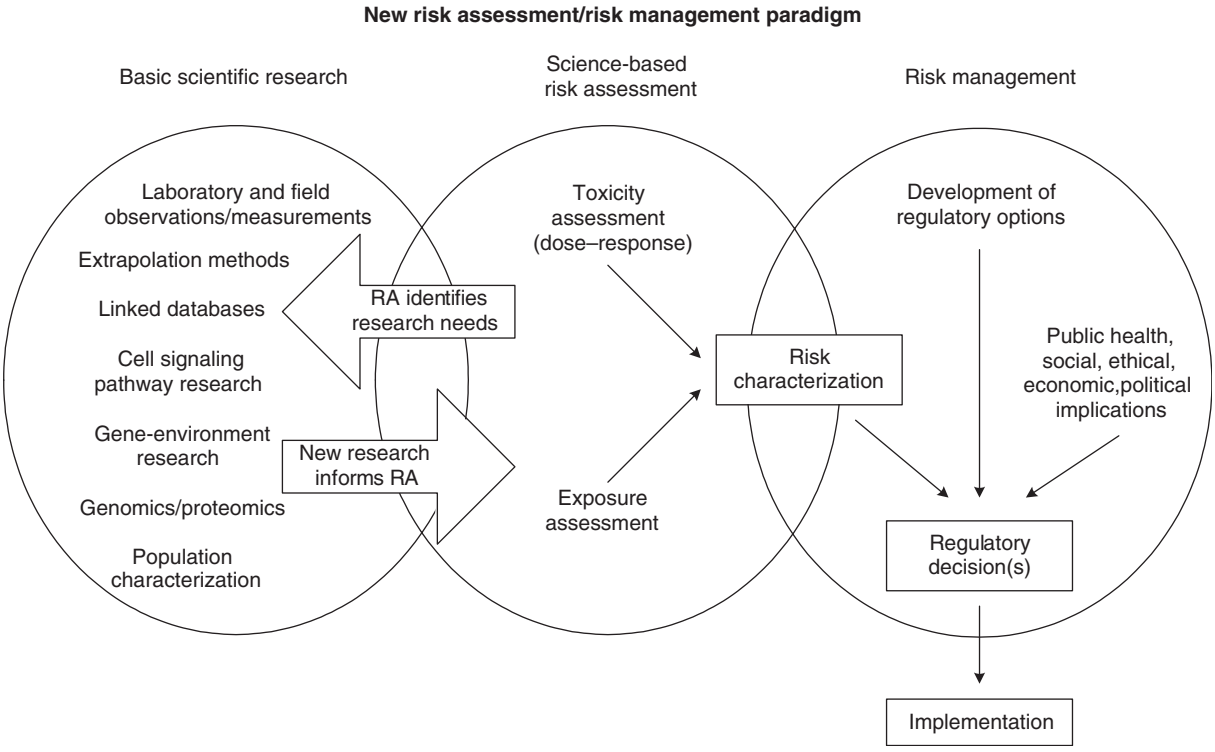


Figure 5 US National Research Council, new risk assessment/risk management paradigm. (Modified from National Research Council (2000) *Scientific Frontiers in Developmental Toxicity and Risk Assessment*, from National Academy Press.)

ratio of the NOAEL from the most sensitive test species to the human estimated exposure from all possible sources. If the MOE is very large, the risk to humans is perceived as low.

The EPA also employs a weight-of-evidence scheme to factor in the levels of confidence for the data from various animal and human studies/reports and to emphasize human data over animal data.

See also: Androgens; Developmental Toxicology; Dose–Response Relationship; Endocrine System; Levels of Effect in Toxicological Assessment; Neurotoxicity; Radiation Toxicology, Ionizing and Nonionizing; Reproductive System, Female; Reproductive System, Male; Risk Assessment, Human Health; Risk Characterization; Toxicity Testing, Developmental.

Further Reading

National Research Council (2000) *Scientific Frontiers in Developmental Toxicity and Risk Assessment*. Washington, DC: National Academy Press.

Tyl RW (2002) Section 16. Male reproductive toxicology. Chapter 16.1, *In vivo* models for male reproductive toxicology, and Chapter 16.2, Guidelines for mating rodents. In: Maines MD *et al.* (ed.) *Current Protocols in Toxicology*, vol. 2, pp. 16.1.1–16.1.15 and 16.2.1–16.2.11. New York: Wiley.

Toxicity Testing, Sensitization

Robin C Guy

Background Information

Skin sensitization (allergic contact dermatitis, allergic sensitization) can occur following exposure by the dermal or inhalation routes and presents problems to significant numbers of humans, both in the occupational field and in the general population. In the human, the responses may be characterized by itching, redness, swelling, skin lesions, or a combination of these. In other species the reactions may differ and only redness and swelling may be seen. Allergic reactions are of various types, but all involve at least

one exposure to initiate the process of sensitization, as they are immunologically mediated cutaneous reactions to a substance. Determination of the potential to cause or elicit skin sensitization reactions (allergic contact dermatitis) is a significant factor in evaluating a substance's toxicity. Early identification of any allergic potential is a prudent measure for identifying possible hazards to a population exposed repeatedly to a test substance and to ensure that appropriate methods of control can be applied.

The sensitization test selected should identify substances with significant allergenic potential and minimize false-negative results. Several animal models have been developed for identification of sensitizers, including

- Draize sensitization test;
- Freund complete adjuvant (FCA) technique;
- Buhler test;
- open epicutaneous test;
- Maurer optimization test;
- split adjuvant technique;
- guinea pig sensitization (Buehler);
- guinea pig maximization (Magnusson–Kligman);
- mouse ear swelling test;
- mouse local (auricular) lymph node assay; and
- photosensitization (photoallergy) models including:
  - photo-mouse ear swelling test (photo-MEST); and
  - photo-local lymph node assay (photo-LLNA)

In the traditional Draize test in guinea pigs, potent sensitizers are identified but moderate sensitizers in humans may give false-negative reactions in guinea pigs. Use of adjuvant in the guinea pig maximization test (GPMT) or the split adjuvant technique gives a lower probability of missing a sensitizer.

Sensitization testing is determined by examining the reaction to the challenge exposure and comparing this reaction with that of the initial induction exposure. In general, the test animals are initially exposed to the test material by intradermal or epidermal application. This step is the induction exposure, an experimental exposure of a subject to a test substance with the intention of inducing a hypersensitive state. The induction period follows. This is a rest period of at least 1 and possibly 2 weeks following the induction exposure. At this time, a hypersensitive state/immune response may develop. After the induction period, the animals are exposed to a challenge dose, which is an experimental exposure of the previously treated subject to the same test substance. At specific time points after the challenge dose, the extent and degree of the skin reaction to the challenge exposure are compared with those demonstrated by control animals that undergo vehicle treatment during induction and then receive the challenge exposure.

Experimental Design

The GPMT of Magnusson and Kligman (which uses adjuvant) and the nonadjuvant Buehler test are given preference over other methods and these procedures are described in detail.

Guinea Pig Maximization Test

Briefly, the GPMT uses intradermal injection with and without FCA for induction, followed on days 5–8 by topical irritation/induction, followed by topical challenge for 24 h on days 20–22. Readings are made ~24 h after removal of the challenge dose, and again after another 24 h. If the results are equivocal, the animals may be rechallenged 1 week later.

Animals A minimum of 10 animals are used in the treatment group and at least 5 animals in the control group. When fewer than 20 test and 10 control guinea pigs have been used, and it is not possible to conclude that the test substance is a sensitizer, testing in additional animals to give a total of at least 20 test and 10 control animals is strongly recommended.

Dose Levels The concentration of test substance used for each induction exposure should be well tolerated systemically and should be the highest to cause mild to moderate skin irritation. The concentration used for the challenge exposure should be the highest nonirritant dose. The appropriate concentrations can be determined from a pilot study using two or three animals.

Induction: Intradermal Injections *Day 0 treated group*: Three pairs of intradermal injections of 0.1 ml volume are given in the shoulder region, which is cleared of hair so that one of each pair lies on each side of the midline:

- *Injection 1*: A 1:1 mixture (v/v) of FCA/water or physiological saline.
- *Injection 2*: The test substance in an appropriate vehicle at the selected concentration.
- *Injection 3*: The test substance at the selected concentration formulated in a 1:1 mixture (v/v) of FCA/water or physiological saline.

In injection 3, water-soluble substances are dissolved in the aqueous phase prior to mixing with FCA. Liposoluble or insoluble substances are suspended in

FCA prior to combining with the aqueous phase. The concentration of test substance shall be equal to that used in injection 2. Injections 1 and 2 are given close to each other and nearest the head, while injection 3 is given toward the caudal part of the test area.

Day 0 control group: Three pairs of intradermal injections of 0.1 ml volume are given in the same sites as in the treated animals:

- *Injection 1*: A 1:1 mixture (v/v) of FCA/water or physiological saline.
- *Injection 2*: The undiluted vehicle.
- *Injection 3*: A 50% w/v formulation of the vehicle in a 1:1 mixture (v/v) of FCA/water or physiological saline.

Induction: Topical Application *Days 5–7 treated and control groups*: Approximately 24 h before the topical induction application, if the substance is not a skin irritant, the test area, after close-clipping or shaving is painted with 0.5 ml of 10% sodium lauryl sulfate in vaseline, in order to create a local irritation.

Days 6–8 treated group: The test area is again cleared of hair. A filter paper (2 cm × 4 cm) is fully loaded with the test substance in a suitable vehicle and applied to the test area and held in contact by an occlusive dressing for 48 h. The choice of the vehicle should be justified. Solids are finely pulverized and incorporated in a suitable vehicle. Liquids can be applied undiluted, if appropriate.

Days 6–8 control groups: The test area is again cleared of hair. The vehicle only is applied in a similar manner to the test area and held in contact by an occlusive dressing for 48 h.

Challenge: Topical Application *Days 20–22 treated and control groups*: The flanks of treated and control animals are cleared of hair. A patch or chamber loaded with the test substance is applied to one flank of the animals and, when relevant, a patch or chamber loaded with the vehicle only may also be applied to the other flank. The patches are held in contact by an occlusive dressing for 24 h.

Observations of Treated and Control Groups

- Approximately 21 h after removing the patch the challenge area is cleaned and closely-clipped and shaved or depilated if necessary.
- Approximately 3 h later (~48 h from the start of the challenge application) the skin reaction is observed and recorded according to the grades shown in **Table 1**.

Table 1 Magnusson and Kligman grading scale for the evaluation of challenge patch test reactions

Grade	*Reaction*
0	No visible change
1	Discrete or patchy erythema
2	Moderate and confluent erythema
3	Intense erythema and swelling

- Approximately 24 h after this observation a second observation (72 h) is made and once again recorded.

Rechallenge If it is necessary to clarify the results obtained in the first challenge, a second challenge (i.e., a rechallenge), where appropriate with a new control group, should be considered ~1 week after the first one. A rechallenge may also be performed on the original control group.

Clinical Observations All skin reactions and any unusual findings, including systemic reactions, resulting from induction and challenge procedures should be observed and recorded. Other procedures, for example, histopathological examination, the measurement of skin fold thickness, may be conducted to clarify doubtful reactions.

Buehler Test

Briefly, the standard Buehler test uses three 6 h dermal patches, one per week, to the same shaved site. After a 2 week rest period, the test animals and half of the control animals receive another 6 h patch at another site. Then, the test animals are tested again 7–15 days later. Reactions are graded according to a five-point scale. If the results are equivocal, the animals may be rechallenged 1 week later.

Animals A minimum of 20 animals is used in the treatment group and at least 10–20 animals in the control group.

Dose Levels

- The concentration of test substance used for each induction exposure should be the highest to cause mild irritation. The concentration used for the challenge exposure should be the highest non-irritating dose. The appropriate concentration can be determined from a pilot study using two or three animals.
- For water-soluble test materials, it is appropriate to use water or a dilute nonirritating solution of

surfactant as the vehicle. For other test materials 80% ethanol/water is preferred for induction and acetone for challenge.

Induction: Topical Application *Day 0 treated group*:

- One flank is cleared of hair (closely clipped). The test patch system should be fully loaded with test substance in a suitable vehicle (the choice of the vehicle should be justified; liquid test substances can be applied undiluted, if appropriate). The test patch system is applied to the test area and held in contact with the skin by an occlusive patch or chamber and a suitable dressing for 6 h.
- The test patch system must be occlusive. A cotton pad is appropriate and can be circular or square, but should be ~4–6 cm^2. Restraint using an appropriate restrainer is preferred to assure occlusion. If wrapping is used, additional exposures may be required.

Day 0 control groups: One flank is cleared of hair (closely clipped). The vehicle only is applied in a similar manner to that used for the treated group. The test patch system is held in contact with the skin by an occlusive patch or chamber and a suitable dressing for 6 h.

Days 6–8 and 13–15 treated and control groups: The same application as on day 0 is carried out on the same test area (cleared of hair if necessary) of the same flank on days 6–8, and again on days 13–15.

Challenge *Days 27–29 treated and control groups*:

- The untreated flank of treated and control animals is cleared of hair (closely clipped). An occlusive patch or chamber containing the appropriate amount of test substance is applied, at the maximum nonirritant concentration, to the posterior untreated flank of treated and control animals.
- When relevant, an occlusive patch or chamber with vehicle only is also applied to the anterior untreated flank of both treated and control animals. The patches or chambers are held in contact by a suitable dressing for 6 h.

Observations of Treated and Control Groups Approximately 21 h after removing the patch the challenge area is cleared of hair; ~3 h later (~30 h after application of the challenge patch) the skin reactions are observed and recorded according to the grades shown in the GPMT (**Table 1**); ~24 h after the 30 h observation (~54 h after application of the challenge patch) skin reactions are again observed and recorded.

Rechallenge If it is necessary to clarify the results obtained in the first challenge, a second challenge (i.e., a rechallenge), where appropriate with a new control group, should be considered ~1 week after the first one. The rechallenge may also be performed on the original control group.

Clinical Observations All skin reactions and any unusual findings, including systemic reactions, resulting from induction and challenge procedures should be observed and recorded. Other procedures, for example, histopathological examination, measurement of skin fold thickness, may be carried out to clarify doubtful reactions.

Additional Procedures for the GPMT and the Buehler Test

- The young adult guinea pig is the preferred species.
- Blind reading of both test and control animals is recommended.
- Removal of the test material should be accomplished with water or an appropriate solvent, without altering the existing response or the integrity of the epidermis.
- In a properly conducted test, a response of at least 30% in an adjuvant test and at least 15% in a nonadjuvant test should be expected for mild to moderate sensitizers. Preferred substances are hexylcinnamic aldehyde (CAS 101-86-0), mercaptobenzothiazole (CAS 149-30-4), benzocaine (CAS 94-09-7), dinitrochlorobenzene (CAS 97-00-7), or DER 331 epoxy resin. There may be circumstances where, given adequate justification, other control substances may be used.
- Depending on the test selected, animals may be used as their own controls, but usually there will be a separate group of vehicle-treated animals that are exposed to the test substance only after the induction period, whose reactions are compared to those of the animals that have received both induction and challenge exposures. Control groups which provide the best design should be used. Some cases may best be served by both naive and vehicle control groups.
- Periodic use of a positive control substance with an acceptable level of reliability for the test system selected is recommended.
- The dose level will depend on the test method selected. In the Buehler test, the concentration of the induction dose should be high enough to cause

mild irritation, and the challenge dose should use the highest nonirritating concentration. In the GPMT, the concentration of the induction dose should be well tolerated systemically, and should be high enough to cause mild to moderate skin irritation; the GPMT challenge dose should use the highest nonirritating concentration.
- If the formulation intended for the final product is used in testing, the concentration of a substance suspected of causing sensitization can be increased 10-fold in the formulation during induction as a safety factor in conducting sensitization studies; the original formulation should be used during challenge. The amount of the test substance applied to the guinea pig during challenge should not exceed the highest amount which is nonirritating in naive guinea pigs in a 24 h irritation study.

Alternatives to Guinea Pig Tests

The MEST or the mouse LLNA (auricular) may be used as screening tests to detect moderate to strong sensitizers. Historically, the naive guinea pig has been used as the model for evaluating drug and chemical hypersensitivity in sensitization tests since its response level is higher than that of other animals and similar to that of humans. The high cost associated with guinea pig studies and an increasing awareness of animal welfare issues have led to the development of more cost-effective and humane models.

Mouse Ear Swelling Test

Briefly, the MEST procedure involves weighting mice and anesthetizing, if necessary, so that the dorsal thorax of each mouse may be shaved. On the first day of dosing, the test article, a positive control, and the respective vehicles are applied to their respective sites on the shaved area. Following application, the animals are restrained long enough to allow the vehicle to start to volatilize. These procedures are repeated on days 2 and 3. Mice are then rested during days 4–7. On day 8, the pretreatment thickness of both ears on each mouse is measured.

Following this measurement, mice are challenged with the test article, vehicle, positive control, or the positive control vehicle on both sides of each ear. The same ears are then measured 24 and 48 h after challenge. Recorded raw data include pretreatment measurements, 24 and 48 h posttreatment measurements of the thickness of two sites on the right ear of all mice. The percent ear swelling is calculated as follows: ((mean thickness of both ears (24 or 48 h posttreatment)/mean thickness of both ears measured before treatment) $\times$ 100) $-$ 100. The percent ear swelling for the test article is compared to the percent ear swelling for the vehicle for significance and dose response.

Mouse LLNA

The LLNA may be used as an alternative to the GPMT or the Beuhler assay. The LLNA measures the response of the lymph nodes to a substance. Sensitization is mediated by lymphocytes, the pivotal cell type in the immune system. When susceptible individuals are exposed to a chemical allergen, those lymphocytes that are able to recognize it as a foreign substance divide and increase in number. It is this increase in the number of chemical allergen-responsive lymphocytes that renders the individual sensitized; the stimulation of lymphocyte division is, therefore, a central event in sensitization.

Advantages of the LLNA include:

- The ability to test colored materials that may otherwise be contraindicated, as it may interfere with any irritation scoring of the skin.
- An adjuvant is not needed.
- It is not necessary to clip or shave the fur from the mice, as must be done more than once during the guinea pig tests.
- Guinea pig sensitization tests require 6–8 weeks and, therefore, take a long time to complete.

Disadvantages of the LLNA include:

- Weaker sensitizers, as detected in the GPMT, were usually not detected.
- It is not recommended for metallic compounds (e.g., metals, metal salts, and organometallic materials) and high-molecular weight proteins.
- It is not very suitable for materials that do not sufficiently adhere to the ear for the treatment period. Particular care should be taken to ensure that hydrophilic materials are incorporated into a vehicle system that wets the skin and does not immediately run off. Therefore, very aqueous vehicles or test materials and runny liquids are to be avoided.

Briefly, the LLNA procedure involves the application of a test material to the backs of the ears of four or five young adult (6 to 16-week-old) female mice per concentration. Each mouse is treated for three consecutive days, and then rested for 2 days. On the sixth day after the start of dosing, the mice are euthanized and their lymph nodes are excised and examined. A test substance that causes a stimulation index (SI) of three or greater, meaning a threefold proliferation of lymph node cells in the test mice, at

one or more concentrations is considered to have skin-sensitizing activity. However, the magnitude of the SI should not be the sole factor determining the biological significance of a skin sensitization response. A quantitative assessment must be performed by statistical analysis of individual animal data in order to provide a more complete evaluation of the test substance. Factors to be considered in evaluating the biological significance of a response or outcome of the test include the results of the SI determinations, statistical analyses, the strength of the dose–response relationship, chemical toxicity, solubility, and the consistency of the vehicle and positive control responses.

Human Testing

More subjects are necessary for sensitization studies in humans than in animal studies because of the larger variation in immune responses and the need to use lower concentrations of the test material in human exposure. Usually, the sensitization of a material is assessed in a preliminary study with 20–25 subjects, and then expanded to a main study with up to 200 subjects. The sample size of test subjects in the main study must be large enough so that the results are valid for the population for which the preparation is intended. It may be of interest to use a particular component at a level 10 times the concentration in the finished product for induction. For the challenge dose, a nonirritating concentration should be used. In addition, the test conditions should reproduce the actual use of the final product, that is, the materials tested should contain all of the ingredients. In general, sensitization procedures are similar to the procedures described above:

- Multiple applications (24–48 h) of occlusive patches for the induction phase.
- A total of 9–15 applications are made over a 3 week period.
- Induction is followed by a 10–14 day rest phase to allow for development of latent sensitization.
- Subjects are then challenged by application of the test material at a different site for 48 h.
- Responses are evaluated 1, 24, 48, and 96 h after application of the patches.
- If results are positive, a second challenge 2 weeks after the original challenge may be conducted. At this time a particular component suspected as the sensitizer may be tested alone at different concentrations to confirm identification of the sensitizing component of the product.

Evaluation must distinguish between primary irritation responses, which may disappear within a couple of days, and sensitization responses which may develop more slowly, persist longer, and are characterized by pruritis, erythema, edema, papules, vesicles, bullae, or a combination of these. Further identification of sensitization reactions may involve microscopic examination of skin biopsy samples. Some issues which may be encountered in human studies include reactions early in the induction phase which may be indicative of preexisting sensitization to the test material, or a delayed response at 192 h instead of at 48 or 96 h. Follow-up of subjects not completing the study may yield valuable information on the adverse effects of a preparation.

See also: Environmental Protection Agency, US; Federal Insecticide, Fungicide, and Rodenticide Act, US; Food and Drug Administration, US; Immune System; National Institutes of Health; National Toxicology Program; Organisation for Economic Cooperation and Development; Toxicity Testing, Alternatives; Toxicity Testing, Dermal; Toxicity Testing, Irritation.

Further Reading

Kimber I, *et al.* (2001) Skin sensitization testing in potency and risk assessment. *Toxicological Sciences* 59(2): 198–208.

Kimber I, *et al.* (2002) The local lymph node assay: Past, present and future. *Contact Dermatitis* 47(6): 315–328.

Marzulli FA and Maibach HI (eds.) (1973) *Dermatotoxicology and Pharmocology, Advances in Modern Toxicology*, vol. 4. New York: Wiley.

Relevant Websites

http://www.epa.gov – OPPTS Harmonized Test Guidelines Test Guidelines, OPPTS 870.2600, Skin Sensitization. Developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency.

http://www.epa.gov – US Environmental Protection Agency, 40CFR798.4100, Subpart E, Specific Organ/Tissue Toxicity, Section 798.4100 Dermal sensitization.

http://www.fda.gov – US Food and Drug Administration, Protocol For Dermal Toxicity Testing for Medical Devices in Contact with Skin.

Toxicity Testing, Validation

Leon H Bruner, G J Carr, M Chamberlain, and R D Curren

Introduction

Toxicologists have developed an array of animal-based tests that are used to assess the toxicity of chemicals and mixtures of chemicals during the last half century. These methods have been adopted by regulatory agencies throughout the world to provide data that are ultimately used to protect public health and to warn chemical users of potential health dangers. For the most part, these methods have provided adequate information for the protection of plant workers and the general public.

The use of animals for routine toxicity testing is now questioned by a growing segment of society. If currently used animal tests are to be successfully replaced, it is important to demonstrate that the alternative methods provide chemical hazard data equivalent to that now available from animal-based tests. Additionally, in order for toxicologists to take the best advantage of new technologies that are constantly evolving, it is important that the validation process be conducted in a manner that efficiently and definitely characterizes the performance of new test methods.

The performance of numerous alternative methods has been assessed in validation programs, and these programs have provided a great deal of information about the validation process and the utility of the alternative methods. Additionally, there has been significant discussion on the theoretical and practical aspects of the validation process. This review summarizes some of the important lessons that have been acquired during this work, and to provide recommendations of design factors that should be considered in future validation programs. The role of validation studies in obtaining objective measures of alternative method performance is reviewed. Additionally, a multistep process is presented that may guide the design, execution, and evaluation of validation studies. Finally, factors that must be considered when the relevance of an alternative method is assessed are reviewed. The validation of alternative methods for eye irritation testing is used as a specific example to illustrate important points associated with the validation process.

The Definition of Validation

Validation has been defined as 'the process by which the reliability and relevance of an alternative method is established for a particular purpose'. The discussion below begins with what is meant by reliability and relevance from a test user's point of view, and provides a perspective on how these elements may be assessed in the validation process.

Reliability

Toxicologists must rely on results obtained from an alternative method if it is to serve as a replacement for an *in vivo* toxicity test. Two measures of alternative method performance must be known in order to define reliability from a test user's point of view. First, a toxicologist must know it is possible to consistently reproduce the data obtained from the alternative method over long periods of time. A test that does not provide the same results on the same test substance repeatedly would not be useful in the safety assessment process. Second, it must be possible to consistently predict *in vivo* toxicity endpoints at a known level of accuracy and precision. These measures of reliability are objective endpoints that can be measured experimentally. The part of the validation process that provides the data needed to confirm the reliability of an alternative method as proposed by its developers is the validation study.

Relevance

In practical terms, the assessment of relevance addresses the following question: Given the information known about the alternative method, are the data provided by the assay good enough to allow its acceptance as a replacement for a given *in vivo* test? In order to answer this question, all of the available information related to performance, operation, and mechanistic basis of an alternative method and the *in vivo* toxicity test it is intended to replace must be thoroughly reviewed. The benefits and risks associated with the adoption of the new method must also be defined. Once this information is available, it must be synthesized in a manner that allows those involved in a validation process to render a judgment that the performance of the alternative method is acceptable or not as a replacement for the *in vivo* toxicity test.

Based on the preceding discussion, it is clear that the processes used to establish the reliability and relevance of an alternative method are distinct. The confirmation of alternative method reliability is an objective process, since it provides data measured in the laboratory during a validation study. The assessment of relevance is a subjective process, since it is based on the evaluation and integration of

information and requires judgment. Since these processes are distinct, they will be reviewed separately.

Confirming Alternative Method Reliability in a Validation Study

As noted already, the tool used to obtain the data that provides objective measures of an alternative method's reliability is the validation study. Experience shows that conducting validation studies is complex. In order to provide a clear review of the steps that need to be considered, the discussion is organized around the flow chart depicted in **Figure 1**, beginning with a consideration of the information that must be available about the performance of an alternative method before it is included in a validation study.

Step 1. Define the Performance Measures to be Confirmed in the Validation Study (Figure 1)

In order to more easily design and ultimately interpret the results of a validation study, it is important to define two factors that define the reliability of an alternative method before the study starts. These factors are reproducibility and predictive capability of the alternative method. It is of critical importance that these performance factors are clearly stated before a validation study starts. When these performance characteristics are defined beforehand, they provide critical information needed to design the study so that it includes the appropriate number of laboratories, an acceptable set of test substances, and the appropriate range of toxicity. They also provide benchmarks that can be used to set the criteria that an alternative method must meet in order to be considered reliable. If the data obtained from the study meet or exceed these predefined performance criteria, then it confirms that the alternative method performs as described by its developers. If the method fails to perform at a level equivalent to the criteria set at the start of the study, then its performance cannot be confirmed.

Preliminary evidence of an alternative method's reproducibility is usually generated during its initial development. This information may be further supported by data obtained from formal method development programs that involve the collaboration of several laboratories.

Evidence demonstrating the predictive capability of an alternative method is usually also generated early in its development. This evidence is obtained by evaluating a subset of test substances of known toxicity in the alternative method. The results from the alternative method are directly compared with the *in vivo* toxicity data from each test substance. If this comparison

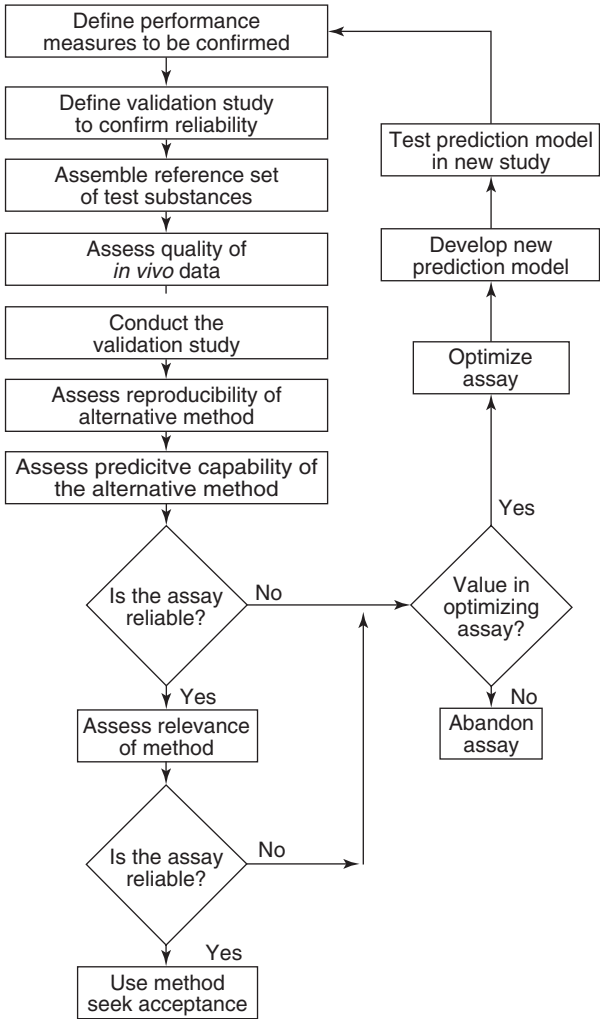


Figure 1 The validation process. The flow chart depicts a eries of steps that may be used as a guide to design and conduct a validation program. The steps proceeding down the left side of the chart represent the actual validation process. The steps proceeding up the right side of the chart depict the steps associated with improving the performance of the alternative method and defining another prediction model prior to inclusion of the method in a new validation study. Reproduced from *Toxicology In Vitro* 10: 479–501, 1996, Bruner, L. © Proctor & Gamble.

reveals the existence of a definable relationship between the two data sets, it indicates that the alternative method may be useful for predicting *in vivo* toxicity.

An example of a definable relationship between alternative method and *in vivo* test data is illustrated in **Figure 2**. This plot shows that the results from a hypothetical alternative method are directly related to the level of toxicity measured *in vivo*. In this case, the relationship may be described in terms of the standard equation for a line, $y = mx + b$, where m is the slope of the regression line, and b represents the value of the y intercept of the regression line. If this algorithm is true for all test materials, then any result

x, from this alternative method could be incorporated into the algorithm, $y = mx + b$, to obtain an output, y, that represents the prediction of toxicity *in vivo*. Other nonmathematical approaches, like binary classification schemes, can be used to define the relationship between two tests.

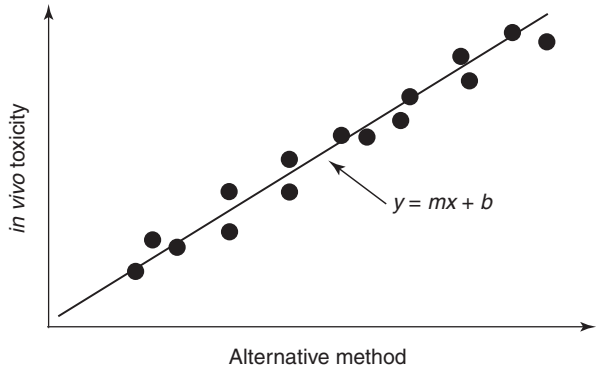


Figure 2 Plot showing a hypothetical relationship between a specific toxic endpoint measured *in vivo* and corresponding results from an alternative method. In order for an alternative method to be useful, there must be a consistent and definable relationship between toxicity measured *in vivo* and corresponding results in the alternative method. In this case, the relationship may be described in terms of a mathematical algorithm, $y = mx + b$. If this algorithm is true for all test materials, then any result, x, from this alternative method could be input into the algorithm $y = mx + b$, to obtain an output, y, which represents the prediction of toxicity *in vivo*. Such algorithms can be incorporated into prediction models that translate the results from an alternative method into a prediction of toxicity *in vivo*. Reproduced from *Toxicology In Vitro* 10: 479–501, 1996, Bruner, L. © Proctor & Gamble.

Since such algorithms constitute models that convert the results from an alternative method into a prediction of toxicity observed *in vivo*, they have been called 'prediction models'. A prediction model is essential because it defines exactly how an alternative method is used to predict *in vivo* toxicity. Therefore, if an assay does not have an adequate prediction model, there is no way to confirm the assay's reliability. Described next in detail are the key elements that make up an adequate prediction model.

A prediction model is adequate when it defines three elements (**Figure 3**). These elements include a definition of all the possible results that may be obtained from an alternative method (inputs), an algorithm that allows a conversion of each result into a prediction of the *in vivo* toxicity endpoints (outputs), and a description of the types of test materials for which the prediction model may be used.

A prediction model must define all of the possible results that may be obtained from the alternative method. This is important since there are many different types of data available from typical alternative methods. Examples of data types include quantitative data, censored data, qualitative data, descriptive data, default values, and nonqualified

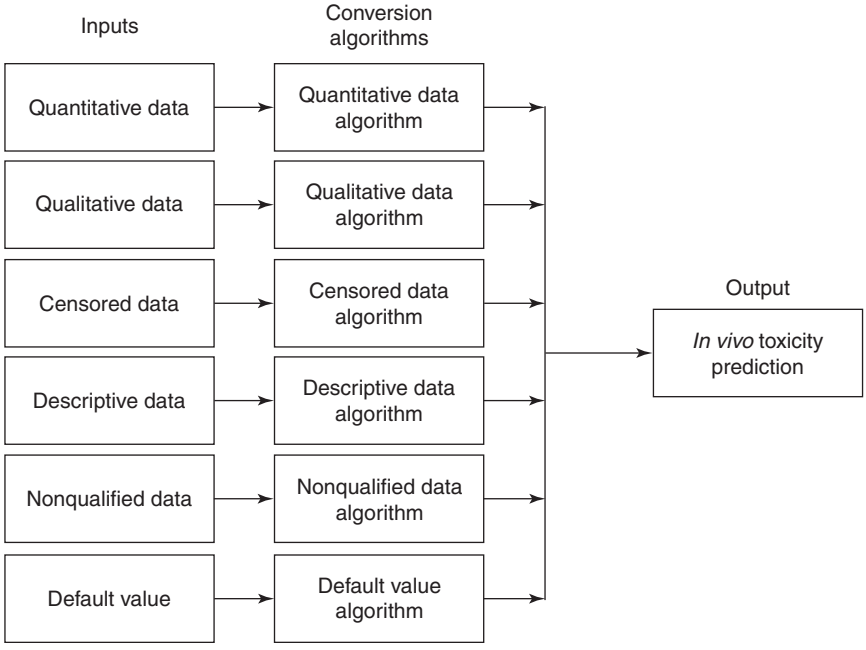


Figure 3 Elements of the prediction model. In order for an alternative method to predict toxicity *in vivo*, there must be a way to convert the results of the alternative method into a correct prediction of toxicity *in vivo*. The description of how to perform this conversion is called the prediction model. The conversion process followed must be input into the conversion algorithm that will lead to a prediction of toxicity as an output. A prediction model must define each of the data types available from the alternative method, an algorithm useful for converting the results of the alternative method into a prediction of toxicity, and the chemical classes, product categories, and physical forms for which the prediction model is valid. Reproduced from *Toxicology In Vitro* 10: 479–501, 1996, Bruner, L. © Proctor & Gamble.

data. 'Quantitative data' are specific numerical values obtained as endpoints from the assays. These data are most commonly ED_{50} values, but may be any other numerical values specifically measured in the assay. 'Censored data' occur in assays that have maximum or minimum obtainable quantitative data. The result '$>10\,000\,\mu g\,ml^{-1}$' is a specific example of a censored datum. Censored data usually occur due to technical restraints that limit the dynamic range of a particular procedure. 'Qualitative data' represent a classification of the effects caused by the test substance on the target of the assay. A result described as 'irritant', 'moderate irritant', or 'nonirritant' is an example of qualitative data. 'Descriptive data' are written phrases that characterize an observed effect of the test substance in the test system. The result 'test substance causes coagulation of the chorioallantoic membrane' is an example of descriptive data. 'Default values' result when two or more outputs obtained from an alternative method at the same time are combined to make a prediction. For example, two results from an alternative method at the same time are combined to make a prediction. For example, two results from an alternative method such as 'the ED_{50} is greater than $10\,000\,\mu g\,ml^{-1}$' and 'no denaturation of proteins' may be combined to give the default value of 'not irritating'. 'Nonqualified data' represent values obtained from an assay that cannot be used due to some kind of technical incompatibility of the test substance with the assay. For example, the buffering capacity of the tissue culture medium might neutralize an acid test substance. If the toxic effect of the test substance depends on its acidity, the result obtained from testing the material should not be considered an accurate indicator of the test substance toxicity. Other types of data in addition to these examples may be available. If so, each type must be defined in the prediction model.

Second, a prediction model must adequately define the conversion algorithms that translate each alternative method result into a prediction of the toxicity *in vivo* (**Figure 3**). The example illustrated in **Figure 2** depicts an alternative method where the quantitative data algorithm, $y = mx + b$, is used to predict an *in vivo* toxicity, y, given any alternative method result, x. The conversion algorithms do not necessarily need to be mathematical equations. For example, algorithms may describe how to convert the alternative method data into classifications that fit a particular *in vivo* toxicity test classification scheme. No matter what approach is used, each algorithm must provide an unambiguous description of how to arrive at a prediction of *in vivo* toxicity given any possible result obtained from an alternative method. Any reasonably trained individual should be able to perform this translation. In addition to providing a prediction, it is important that the prediction model provide an indication of the variability associated with any prediction.

Third, the prediction model should define the chemical classes, product categories, and physical forms of test substance for which it is valid. For example, a particular alternative method may be useful (or validated) *only* for predicting the toxicity of surfactant-containing liquids. If so, these limitations must be defined.

Step 2. Design a Validation Study to Test the Validity of the Prediction Model (Figure 1)

Once the performance measures of an alternative method have been defined in terms of reproducibility and predictive capability, the next step in the process is to design a validation study that will test whether or not the alternative method actually performs as described. The design of a validation study is crucial to its success, not only in terms of testing reliability but also in retaining credibility and gaining acceptance by regulatory agencies. The factors that need to be considered include how the validation study will be managed, the nature and competence of the participating laboratories, the protocols and standard operating procedures (SOPs) to be used, how test substances will be coded and distributed, how data will be collected and analyzed, how well laboratories comply with the principles of good laboratory practice (GLP), and what data will be needed in order to confirm the reliability of the alternative method.

Management Structure In order to be successful, large and complex validation studies must have a well-defined management structure. This structure is required in order to assure the study principles and overall design is followed as agreed by the sponsors of the study. Responsibilities of managers and participating laboratories should be defined to assure the program accomplishes the mandates outlined in the goals of the study.

Participating Laboratories Ideally, all participating laboratories should be independent in order to ensure the integrity of separate data sets. If more than one laboratory is in the same large organization, the laboratories should be able to demonstrate local management structure and operational as well as financial independence. In the case of commercial enterprises, the design of the study should ensure that their participation is as unbiased as any other. If it is necessary for technical staff to undergo a period of

training to ensure use of common methods, such training should be undertaken and documented.

Establishment of Common Protocols and Standard Operating Procedures It is essential that all factors relevant to the conduct of the alternative method that may affect the results, the collection of data, and interpretation of the alternative method results be clearly defined before the study begins. These are best documented in the study protocol and SOPs that define the alternative methods. In order to assess the adequacy of the SOPs, they should be examined to determine if they contain three key elements. First, each SOP must have a detailed step-by-step description of how to conduct the assay. Enough details need to be provided such that any appropriately trained and competent laboratory technician need use only this document as the guide to run the assay. Second, the SOP must indicate the steps used to calculate the endpoint of the assay and the number of replicates necessary. Any data transformation or algorithms applied to the data should be clearly documented and consistently applied across all laboratories conducting a particular assay. Third, the protocol must specifically describe the prediction model being tested in the validation study.

Test Material Selection, Coding, and Distribution The reference set of test substances (RSTS) included in the study should be commensurate with the prediction model being tested in the validation study (discussed in detail in step 3). The substances should be obtained with a specification stating source, purity, and whether there are any contaminants. These specifications should be identical to those of the material actually used to generate the *in vivo* data. If identical substances are not available, the potential effect that such discrepancies may have on test substance toxicity must be assessed. In the case of formulated products, the ingredients and their levels in the product should be identified so that the formulation can be made again if necessary. Commercial sources of all single substances should be stated so that substances of the same or similar specification may be purchased in the future. Since testing usually occurs under conditions where participants do not know the identity of the reference substances, procedures need to be established to distribute substances under a randomly generated code. The system established to code and distribute the test samples should be evaluated to assure that the coding is done correctly and that participants do not have access to the codes. Each laboratory should receive substances under different codes in order to assure that results generated in each laboratory are independent.

Data Collection and Analysis The methods for the submission of results from the participants should be established to assure that all the necessary information has been provided to the study statistician. The mechanisms used to assure there are no errors in transcription should also be established in order to ensure that all data are accurately entered into the analysis.

Good Laboratory Practice Acceptance of results and conclusions from a validation study may be compromised if the principles of GLP are not applied during the study. While this is unlikely to be an issue for industrial laboratories, it may be a more important concern in academic laboratories where adherence to GLP traditionally has been less of a concern. All efforts should be made to ensure that the principles of GLP were adhered to in all participating laboratories. This in large part can be achieved by determining whether the participants followed common protocols, SOPs, and data reporting procedures.

Step 3. Assemble an RSTS Appropriate for Confirming the Reliability of the Alternative Method (Figure 1)

The next step in the process is to assemble an RSTS appropriate for assessing the reliability of the alternative method. The factors that need to be considered are the chemical classes, physical form, distribution of toxicity, and the number of materials that need to be included.

Chemical Classes Included in the RSTS The chemical and physical forms of the substances included must be consistent with the stated prediction model. For example, if the prediction model indicates that the alternative method is valid for assessing the eye irritation potential of mild, moderate, and severely irritating liquid, surfactant-based formulations, then the RSTS should contain liquid surfactant-based substances of the relevant class that cover a range of toxicity from mild to severe. Quantitative structure-activity relationships may be useful in helping selection of relevant test chemicals.

Distribution of Toxicity in the RSTS The toxicity of the substances in the RSTS should be distributed as uniformly as possible across the range of interest. This is important because a nonuniform distribution of test substance toxicity in an RSTS may not allow an effective assessment of alternative method performance. Potential effects of nonuniform test substance distribution are illustrated in **Figure 4**. The ideal situation is shown in **Figure 4b**. In this example, the test substances are uniformly distributed across the range of possible toxicity. If such results were

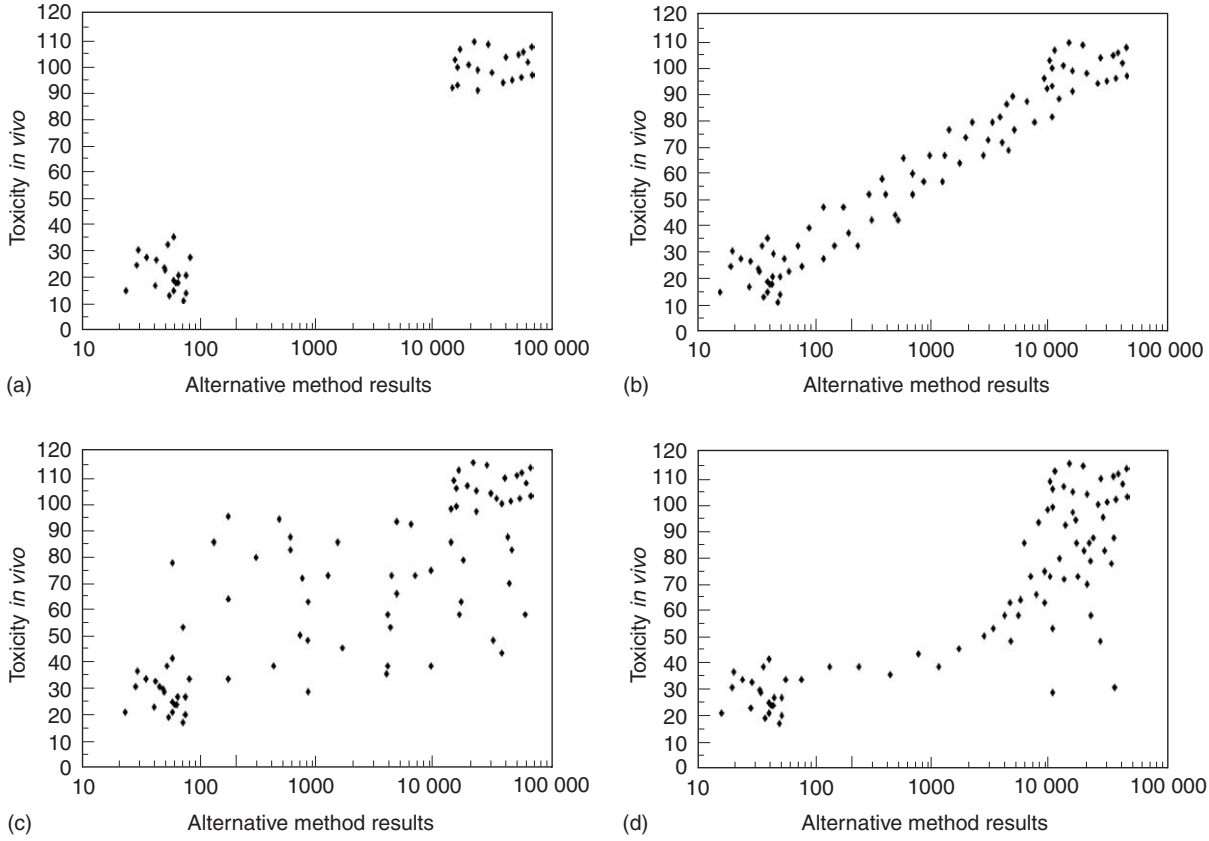


Figure 4 The effects of nonuniform data distributions in the reference set of test substances (RSTS). This series of figures illustrates why the irritancy of the materials in the RSTS must be uniformly distributed across the range of toxicity of interest. (a) includes test substances that are not uniformly distributed across the range of toxicity observed in the *in vivo* method. In this case, it is impossible to determine whether the method is useful for predicting the toxicity of moderately toxic materials. If moderately toxic materials were to be evaluated in the assay shown in part (a), it may be found that the performance of the alternative method is similar to that shown in part (b), (c), or (d). The ideal situation is shown in part (b) where there is a useful relationship between the *in vivo* and alternative method results across the entire range of toxicity assessed. The less satisfactory outcomes shown in parts (c) and (d) are also possible. The only way to determine whether the relationship between the alternative method results and *in vivo* toxicity is useful is to assess an RSTS with a uniform distribution of toxicity across the entire range of interest. Reproduced from *Toxicology In Vitro* 10: 479–501, 1996, Bruner, L. © Proctor & Gamble.

obtained from an alternative method, it would strongly suggest that it could be used to predict toxicity across the full range of possible responses. In **Figure 4a**, the substances in the RSTS are not uniformly distributed, but rather are either mildly or strongly toxic. The problem with such an RSTS is that it is impossible to determine whether the method is useful for predicting the toxicity of the moderately toxic materials. In fact, recent work has shown that the distribution of toxicity included in an RSTS can have profound effects on the performance statistics obtained from a validation study. If the distribution of toxicity used in a validation study were indeed similar to that shown in **Figure 4a**, it is good test performance. However, when materials of mild and moderate toxicity are tested, the measures of performance may be considerably poorer. It may be found that the performance is more similar to those shown in **Figure 4c** or **d**. Alternative methods performing similar to the former two examples are less useful than one similar to **Figure 4b**. The best way to distinguish between the possible outcomes illustrated in **Figure 4b–d** is to evaluate an RSTS having a uniform distribution of toxicity across the entire range of interest.

Number of Test Substances in the RSTS The number of test substances included in the RSTS must also be evaluated. Although it has been suggested that an RSTS should contain up to 250 substances, requiring such a large number is impractical for several reasons. First, it has proven extremely difficult to identify such a large set of test substances that have been evaluated in a common toxicity test procedure. Hence, it is unlikely that a set containing 250 substances can be assembled without conducting additional *in vivo* testing. Second, experience has shown that the cost of conducting a validation study

using such a large RSTS is prohibitive. Third, there is a diminishing returns phenomenon after the RSTS reaches a certain size.

In order to gain a better understanding of how many test substances would be acceptable, a computer simulation based on the Draize eye irritation test was used to investigate the effects of changing sample size on the precision of future predictions of an *in vivo* test result from an alternative method test score. For this simulation, it was assumed that the relationship between a hypothetical alternative method response, X, and a corresponding eye irritation response, Y, has the linear form:

$$Y = (1.1)X \qquad (1)$$

where we restricted the alternative response X to be in the range (0–100), so that the *in vivo* response Y is on the usual maximum average scores (MAS) scale (0–110).

Values for X were chosen with a uniform distribution across the range of interest. Corresponding Y values were then calculated using eqn (1). Since there is inherent variability in both alternative method and *in vivo* data, random error was added to the X and Y values, respectively. This was achieved through the use of independent beta distributions scaled to give a specified coefficient of variation. The coefficient of variation applied to the alternative method response, X, was maintained constant on the full range 0–100. The coefficient of variation applied to the *in vivo* response, Y, was based on the distance of a particular Y value from the closest end of the 110 point Draize scale. This was done because the variability in eye irritation scores decreases as the score approaches either extreme of the Draize irritation scale. Data were available to provide a basis for assigning a level of variability to the Y values. MAS were computed using data given in the original publication. The coefficient of variation for the MAS was also calculated for each test substance. The degree of variation among the laboratories conducting the Draize eye irritation test on the same substances was strikingly large, ranging between 40% and 60% for a six-animal rabbit eye irritation test. The variability in alternative method data is typically less than the *in vivo* test, with coefficients of variation (CV) ranging between 10% and 25% (**Table 1**).

Data sets were generated containing hypothetical RSTS sample sizes of 10, 20, 50, 100, or 250,

Table 1 Intralaboratory reproducibility

Eye irritation test, alternative method	*Positive control*	*n*	*Endpoint units*	*Mean*	*SD*	*CV* (%)
Bovine corneal opacity[a]	Acetone	119	Units	156.50	18.800	12.0
Bovine corneal opacity	Ethanol	44	Units	54.30	9.000	16.0
Bovine corneal opacity	Imidazol	20	Units	113.60	17.400	15.3
MICROTOX[b]	Phenol	123	$\mu g\,ml^{-1}$	20.10	3.900	19.4
Silicon microphysiometer[b]	SLS	163	$\mu g\,ml^{-1}$	78.60	12.200	15.5
Neutral red uptake[b]	SLS	191	$\mu g\,ml^{-1}$	4.24	0.920	21.7
SIRC plaque forming assay[c]	SLS	205	$\mu g\,ml^{-1}$	24.50	4.170	17.0
Neutral red release[d]	Triton X-100	26	$mg\,ml^{-1}$	0.20	0.038	19.0
CORROSITEX[e]	NaOH	44	Minutes	11.74	1.120	9.5
ZK1200 topical application[f]	SLS	44	% viability	45.40	11.800	26.0

Note: The coefficient of variation following multiple runs using the indicated positive control test substances in one laboratory is shown. The overall average of the intralaboratory CVs listed is ~17%. *n* is the number of times the assay has been conducted with the indicated positive control material; units indicates the measurement units obtained from the alternative method; mean indicates the average value obtained for all the indicated runs; SD is the standard deviations calculated associated with the mean of the alternative method sources; CV is the coefficient of variation (mean/SD); SLS is sodium lauryl sulfate.

[a] As described by Gautheron P, Dukic M, Alix D, and Sina JF (1992) Bovine corneal opacity and permeability test: An *in vitro* assay of ocular irritancy. *Fundamentals of Applied Toxicology* 18(3): 442–449.

[b] As described by Bruner LH, Kain DJ, Roberts DA, and Parker RD (1991) Evaluation of seven *in vitro* alternatives for ocular safety testing. *Fundamentals of Applied Toxicology* 17(1): 136–149.

[c] As described by North-Root H, Yackovich F, Demetrulias J, Gacula M Jr., and Heinze JE (1982) Evaluation of an *in vitro* cell toxicity test using rabbit corneal cells to predict the eye irritation potential of surfactants. *Toxicology Letters* 14(3–4): 207–212; and North-Root H, Yackovich F, Demetrulias J, Gacula M Jr., and Heinze JE (1985) Prediction of the eye irritation potential of shampoos using the *in vitro* SIRC cell toxicity test. *Food and Chemical Toxicology* 23(2): 271–273.

[d] As described by Reader S, Blackwell V, O'Hara R, *et al.* (1989) A vital dye release method for assessing the short term cytotoxic effects of chemicals and formulations. *Alternatives to Laboratory Animals* 17: 28–37.

[e] As described by Gordon VC, Harvell JD, and Maibach HI (1993) Dermal Corrosion: The CORROSITEX system: A DOT accepted method to predict corrosivity potential of test materials. *Alternative Methods in Toxicology* 10: 37–42.

[f] As described by Osborne RM, Perkins MA, and Roberts DA (1995) Development and intralaboratory evaluation of an *in vitro* human cell-based test to aid ocular irritancy assessments. *Fundamental and Applied Toxicology* 28: 139–153.

each having defined levels of error added to the *X* and *Y* terms. The 95% confidence interval for the prediction of a single future observation of a MAS of 55 (95% CI_{pred}) and the standard deviation of the 95% CI_{pred} values were then calculated for each data set.

The effects of the RSTS size on the precision of a prediction derived from an alternative method are summarized in **Table 2**. Each of the tabled 95% CI_{pred} values and standard deviations are based on 1000 simulations conducted for each sample size. In the first case (ideal conditions, **Table 2**) the imposed variation is relatively low. As expected, the 95% CI_{pred} values are relatively narrow, ranging from ± 7 to ± 16. As the number of test substances included in the RSTS increases from 10 to 250, the 95% CI_{pred} decreases slightly and the standard deviations of the 95% CI_{pred} decrease by about fourfold.

In the second case (typical conditions, **Table 2**), the CVs applied to the data are more consistent with those observed in the Draize test and currently available alternative methods. Under these circumstances, the 95% CI_{pred} is significantly wider, ranging from approximately 54–28 depending on the sample size and imposed variation. Again, the prediction intervals tend to be narrower as the sample size increases, but this improvement is not substantial when $n > 20$. Also, the standard deviation of the 95% CI_{pred} decreases approximately four- to eightfold.

These simulations therefore indicate that the width of the 95% CI_{pred} does not improve with larger RSTS sizes. Rather, the real benefit of increasing sample

Table 2 Ninety-five percent confidence intervals for the prediction of an *in vivo* eye irritation score of 55 from an alternative method (95% CI_{pred})

Coefficient of variation		*Sample size n = 10*		*Sample size n = 20*		*Sample size n = 50*		*Sample size n = 100*		*Sample size n = 250*	
Alternative method	*In vivo*	*95% CI_{pred}*	*SD* of 95% CI_{pred}*	*95% CI_{pred}*	*SD of 95% CI_{pred}*	*95% CI_{pred}*	*SD of 95% CI_{pred}*	*95% CI_{pred}*	*SD of 95% CI_{pred}*	*95% CI_{pred}*	*SD of 95% CI_{pred}*
Ideal conditions											
0.05	0.05	8.4	2.4	7.5	1.4	7.1	0.8	7.0	0.5	7.0	0.3
0.10	0.10	16.3	4.5	14.9	2.9	14.2	1.5	13.9	1.1	13.8	0.7
Typical conditions											
0.10	0.40	34.0	9.2	30.5	5.0	28.9	2.9	28.3	2.1	27.9	1.3
0.10	0.50	41.8	11.6	37.1	6.4	34.7	3.9	34.0	2.6	33.6	1.6
0.10	0.60	48.5	12.9	43.3	7.7	40.7	4.5	39.9	3.0	39.6	2.0
0.20	0.40	41.8	10.6	37.5	6.1	34.9	3.4	34.5	2.4	34.3	1.5
0.20	0.50	46.8	12.4	43.0	7.3	40.0	4.1	39.4	2.9	39.1	1.8
0.20	0.60	53.1	14.2	47.9	8.1	45.6	4.6	44.7	3.2	44.3	2.0
0.40	0.40	56.4	14.2	50.5	8.0	48.0	4.5	46.9	3.2	46.5	2.0
0.40	0.50	60.4	15.4	54.7	8.9	51.7	5.0	50.6	3.3	50.1	2.1
0.40	0.60	66.0	16.0	59.3	9.2	56.0	5.2	54.8	3.6	54.3	2.3

Note: The mean 95% CI_{pred} is shown for different numbers of materials in the reference set of test substances (RSTS). The 95% CI_{pred} for a predicted *in vivo* score of 55 were obtained from computer simulations designed to assess the effect of changing the size of the RSTS on the uncertainty in predictions obtained from an alternative method. The variability in the 95% CI_{pred} is indicated as the standard deviation of the 95% CI_{pred}. Each of the values shown is based on 1000 runs of the simulation. This simulation shows that the 95% CI_{pred} is relatively wide given the variability associated with the *in vivo* eye irritation test and current alternative methods. For example, if an alternative method having a CV = 0.2 predicts a maximum average score of 55, the 95% CI_{pred} is ± 40 if the CV = 0.5 for the *in vivo* data and the RSTS $n = 50$.

References: Gautheron P, Dukic M, Alix D, and Sina JF (1992) Bovine corneal opacity and permeability test: An *in vitro* assay of ocular irritancy. *Fundamentals of Applied Toxicology* 18(3): 442–449; Bruner LH, Kain DJ, Roberts DA, and Parker RD (1991) Evaluation of seven *in vitro* alternatives for ocular safety testing. *Fundamentals of Applied Toxicology* 17(1): 136–149; North-Root H, Yackovich F, Demetrulias J, Gacula M Jr., and Heinze JE (1982) Evaluation of an *in vitro* cell toxicity test using rabbit corneal cells to predict the eye irritation potential of surfactants. *Toxicology Letters* 14(3–4): 207–212; and North-Root H, Yackovich F, Demetrulias J, Gacula M Jr., and Heinze JE (1985) Prediction of the eye irritation potential of shampoos using the *in vitro* SIRC cell toxicity test. *Food and Chemical Toxicology* 23(2): 271–273; Reader S, Blackwell V, O'Hara R, *et al.* (1989) A vital dye release method for assessing the short term cytotoxic effects of chemicals and formulations. *Alternatives to Laboratory Animals* 17: 28–37; Gordon VC (1992) Utilization of biomacromolecular *in vitro* assay systems in the prediction of *in vivo* toxic responses. *Lens and Eye Toxicity Research* 9(3–4): 211–227; Gordon VC and Bergman HC (1987) Eytex: An *in vitro* method for evaluation of ocular irritancy. *Alternative Methods in Toxicology* 5: 87–90; and Osborne RM, Perkins MA, and Roberts DA (1995) Development and intralaboratory evaluation of an *in vitro* human cell-based test to aid ocular irritancy assessments. *Fundamental and Applied Toxicology* 28: 139–153.

size is that the 95% CI_{pred} estimation is more precisely defined. This is because increasing the sample size improves estimation of the confidence interval endpoints. This is because statistical theory assures that in arbitrarily large samples the estimated endpoints converge to the true endpoint values.

The simulations also indicate that the overall width of the 95% CI_{pred} is limited by the variability in the *in vivo* response. Low levels of variability in the populations are needed in order to predict individual responses both accurately and precisely. High levels of variability in individual predictions cannot be overcome by simply increasing the size of the RSTS. The quality of the *in vivo* data, therefore, is more important than the quantity of substances included in a validation study.

Step 4. Evaluate the Quality of the *In Vivo* Toxicity Data (Figure 1)

The quality of the *in vivo* data available for the substances in the RSTS must be reviewed. This is important. If the quality of the *in vivo* data is poor, then the results of comparison between the *in vivo* data and the alternative method results will be difficult to assess definitely.

It is difficult to obtain a set of test substances that has consistent, high-quality toxicity data. The difficulty arises because many different schemes are used for measuring and classifying toxicity endpoints. The situation is made worse by the fact that there is no consistent source for the information that currently exists. This has meant that toxicity data used in validation studies have often come from many laboratories that have used different protocols and produced different kinds of data.

Some of these problems have been addressed through the efforts of organizations such as the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC). This organization established a committee that developed a reference data bank containing 55 chemicals with *in vivo* eye irritation data that were generated using tests conforming to OECD (Organisation for Economic Co-operation and Development) Guideline 405. The ECETOC criteria provide a useful example of an approach that can be taken to assemble sets of test substances for which there is a uniform quality of *in vivo* data. The criteria the test substances had to meet in order to be included in the eye irritation data bank are shown in **Table 3**. If it is not possible to identify a set of test substances where the data meet such a set of standards, then it may be necessary to generate new *in vivo* data. If that is not possible, then all the shortcomings associated with the *in vivo* data in the RSTS must be documented and factored into the overall assessment of the performance of the alternative method at the end of the study. ECETOC have also prepared reference chemicals data banks for skin and respiratory sensitizes, and for skin irritation and corrosion.

Step 5. Conduct the Validation Study (Figure 1)

Once an adequate RSTS has been assembled and characterized, the next step is to test each of the materials in the alternative method. Many logistical issues need to be carefully monitored during this phase of typical large validation studies, and a few are particularly important. First, careful communication between the participants is essential. Those involved in validation studies must never underestimate the possibility for misunderstanding. Second, preliminary runs of the alternative methods using a small subset of test substances are particularly useful in helping to identify and solve start-up problems

Table 3 Acceptance criteria for *in vivo* data

All test materials should be defined entities available at a known high level of purity or specification
Each material should be chemically stable
In vivo data generated recently (since 1981 when GLP were introduced)
Good laboratory practices followed in generation of *in vivo* data
Studies carried out according to OECD Guideline 405:
At least three rabbits evaluated per test material
A volume of 0.1 ml or the equivalent weight of test substance was instilled into the conjunctival sac
Topical anesthesia was not used (Durham *et al.* 535–541)
Observations made at least at 24, 48, and 72 h
Enable reversibility/irreversibility to be assessed
Scoring done using the scheme of (Draize *et al.* 377–390) so that corneal opacity and area affected, iris inflammation, conjunctival redness, swelling, and discharge data are available for each test substance at each time point evaluated
Chemicals tested undiluted, except where testing materials undiluted would likely lead to severe effects

Note: The *in vivo* data available for materials in the reference set of test substances should meet a minimum quality standard before they are used in a validation program. An example of a set of criteria established for the selection of test substances is provided in the ECETOC Eye Irritation Chemicals Data Bank (ECETOC, 1992). The factors considered important by the Technical Committee who prepared the ECETOC Technical Report are listed here. Reproduced from *Toxicology In Vitro* 10: 479–501, 1996, Bruner, L. © Proctor & Gamble.

that invariably occur. Third, managers of validation studies must carefully monitor the progress being made throughout the study to assure the program proceeds as planned.

Step 6. Assess the Reproducibility of the Alternative Method (Figure 1)

Once the testing of the RSTS materials in the alternative method is completed, the next step in the validation process is to assess the reproducibility of the data generated by the participating laboratories. This analysis is important because it provides the half of the data needed to confirm the reliability of the alternative method.

Many validation studies use a nested or hierarchical design (**Figure 5**). These studies usually involve several laboratories that independently conduct the same alternative method on all the substances in an RSTS. There are four sources of variability in such studies. These include variation in the test substances, variation within experiments within a laboratory (intraexperiment variability), variation between experiments within a laboratory (intralaboratory variability), and variation between laboratories (interlaboratory variability). Reviewed next is the nature and importance of each. The differences between chemicals are ignored in this discussion since they can generally be minimized with well-controlled test article distribution and storage. Attention is concentrated on the variability in results obtained by testing a single chemical in a number of different laboratories.

Definitions (Figure 5) During a validation study, each participating laboratory generally carries out a number of separate executions of an alternative method on each material in the RSTS. Each of these independent executions is a 'repeat experiment'. Often, within each repeat experiment, duplicate, triplicate, or quadruplicate measures are obtained. These are 'replicate measurements'. The mean of results from several repeat experiments gives the 'laboratory mean', and the mean of several laboratory means gives the 'overall laboratories mean'. The importance of variation in the replicate measures, repeat experiments, and overall laboratories mean and how these measurements should be assessed is described in the following sections.

Intraexperiment Variability (Figure 5) The intraexperiment variability is evaluated by examining the variation in the replicate measures obtained within a given repeat experiment. This value is most useful for workers within a laboratory, since it is an indicator of the performance of a particular assay on a specific day. While useful for internal monitoring, this value does not provide a particularly good indication of how an assay performs over time. This is because replicate measures are obtained under conditions where sources of variability such as different technical staff, preparation of the test substances, preparation of cell cultures, and preparation of media are tightly controlled. Because of this control, results obtained from a group of replicate measures best represent a 'precise' estimate of test variability at the particular time under the particular conditions when the test was run. It is not necessarily an 'accurate' reflection of a test's performance over multiple runs. Thus, the performance of an assay over time within one laboratory is best measured at the level of the repeat experiment.

Intralaboratory Variability (Figure 5) Intralaboratory variability is assessed by evaluating the results obtained from repeat experiments conducted on the same substance in the same laboratory over a reasonable

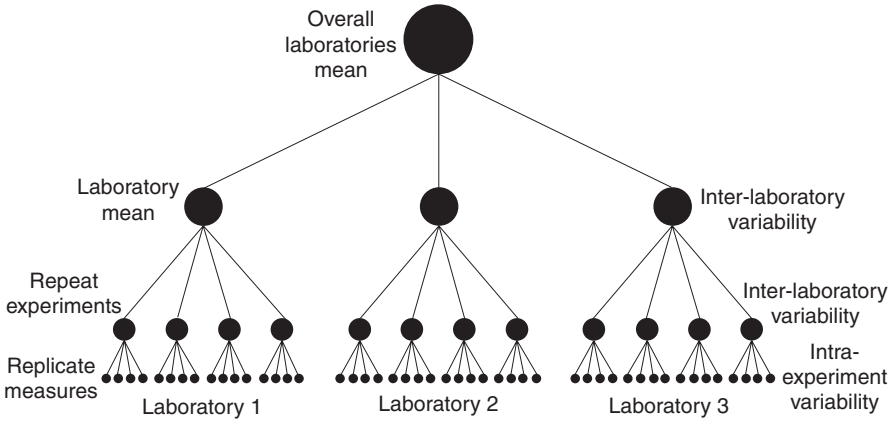


Figure 5 Hierarchical design of a validation study. The different levels of repeated measures obtainable from assays tested in a validation study are shown. The descriptions on the left side indicate the names of the endpoints at each level, and the descriptions of the right side indicate the variability term associated with each level. Reproduced from *Toxicology In Vitro* 10: 479–501, 1996, Bruner, L. © Proctor & Gamble.

period of time. This assessment will be most representative if it is performed using data from several repeat experiments conducted completely independent of each other in terms of substances, batches of chemicals, and possibly even the technical staff who performed the work.

What are the limits of interlaboratory variation that should be considered acceptable for a given alternative method? The consideration of this question must be done on a case-by-case basis. The example illustrated in **Figure 6** shows the results obtained from a test substance that has a laboratory mean score of 100 (bold horizontal line) in an alternative method that produces scores ranging between 0 and 200. Two diagonal lines indicate the upper and lower 95% confidence limits for the laboratory mean when the alternative method CV ranges from 0% to 50%. As the CV increases, the width of the 95% confidence interval for the laboratory mean score increases. Eventually this interval becomes so wide that the results obtained from the test become meaningless relative to the entire response range of the alternative method. As the CV reaches 20% in this example, the width of the 95% confidence interval is ± 40 (dotted horizontal lines, **Figure 6**). Because the ± 40 covers 40% of the total range of possible responses from the alternative method, if the CV is consistently greater than 20%, a good case could be made that this alternative method is not acceptably reproducible. On the other hand, if the range of responses from the alternative method covers several orders of magnitude, a CV of 20% might not cause concern. This is because an alternative method response in the range of 100 ± 40 may correspond to only a small percentage of the total possible range of responses *in vivo*. Ultimately, the toxicologist who uses the alternative method for making decisions in a safety assessment must define the level of uncertainty in a prediction that is acceptable for the purpose at hand.

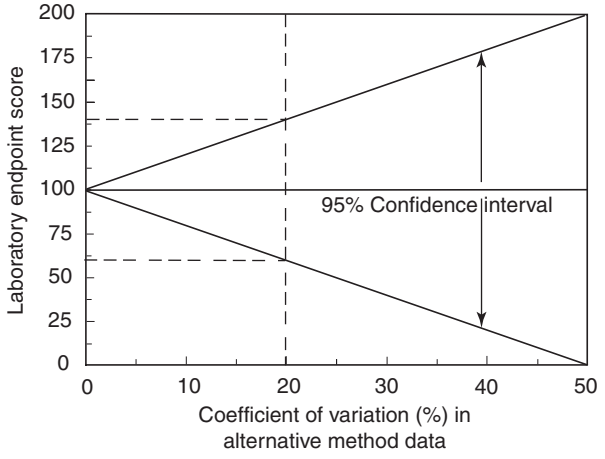


Figure 6 The effect of varying the coefficient of variation in the width of the 95% confidence interval is shown. The alternative method illustrated in this example has a laboratory mean score of 100. As the CV increases from 0% to 50%, the width of the confidence interval increases. In this specific example, when the CV = 20%, the 95% confidence interval is ± 40. An acceptable level of variability for an alternative method depends on the range of responses obtained from the alternative method and the effect of this variability on the precision of *in vivo* toxicity predictions. Reproduced from *Toxicology In Vitro* 10: 479–501, 1996, Bruner, L. © Proctor & Gamble.

Interlaboratory Variability (Figure 5) Interlaboratory variability gives an assessment of how well results can be reproduced across several independent laboratories. This level of reproducibility measurement is of greatest importance when assessing the reliability of a toxicity test to be used for regulatory purposes since regulators will receive results generated from any number of laboratories across the world. This value can be determined by evaluating results derived from the same alternative method protocol on the same test substances across several laboratories. An approach often used to assess interlaboratory reproducibility is to calculate the correlation coefficient that relates the results from different laboratories. Although this statistic provides useful information, it has some limitations. For example, the results of an alternative method in one laboratory may differ across the entire range of results by an order of magnitude compared to a second laboratory. Thus, even though the laboratories did not duplicate each other's results (i.e., poor interlaboratory reproducibility), the data will be highly correlated. Correlation, therefore, should be used with caution when comparisons between laboratories are required. Satisfactory evidence of interlaboratory variability could also be obtained by considering the CV obtained from the results of all the participating laboratories (i.e., the CV associated with the overall laboratories mean). As noted in the discussion of intralaboratory variation, the acceptability of a particular CV must be assessed on a case-by-case basis. The effect of the variability on the uncertainty in predictions must also be considered. This issue is discussed later relative to the assessment of alternative method relevance.

Number of Participating Laboratories The number of laboratories that need to be included in a validation study in order to obtain a precise assessment of reproducibility is dependent on the level of variability associated with the alternative method being evaluated. This is illustrated in **Figure 7**. The figure shows the relationship between the width of the 95% confidence interval for the overall laboratories mean and the number of laboratories included in a determination of the overall laboratories mean. Each curve shows the results obtained when the CV of the

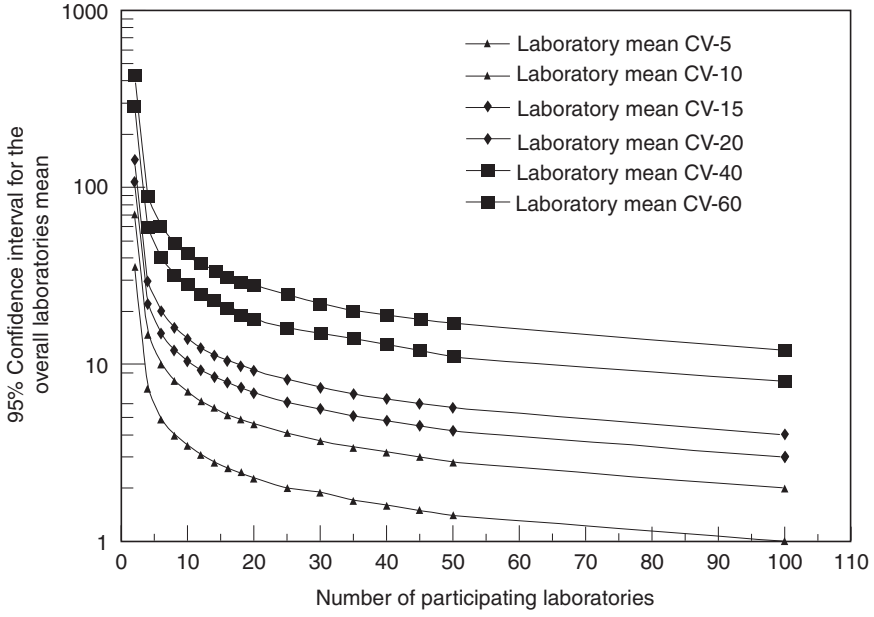


Figure 7 Number of laboratories in an interlaboratory variability assessment. This figure shows the relationship between the width of the 95% confidence interval for the overall laboratories mean and the number of laboratories participating in a validation study. Each curve shows the results obtained when the CV of the laboratory means range from 5% to 60%. The curves were calculated using a model that assumes that the true laboratory mean score is 100. As the laboratory mean CV decreases, and as the number of laboratories included in the evaluation increases, the width of the 95% confidence interval of the overall laboratories mean becomes narrower. Reproduced from *Toxicology In Vitro* 10: 479–501, 1996, Bruner, L. © Proctor & Gamble.

laboratory means range from 5% to 60%. These curves were calculated using a model that assumed the true laboratory mean score is 100 and that, on average, each laboratory is able to obtain this value. These calculations demonstrate that as the variability in the laboratory means decreases, and as the number of laboratories included in the study increases, the 95% confidence interval for the overall laboratories mean becomes narrower. The 95% confidence interval is especially wide when tests are highly variable (i.e., laboratory means CVs > 20%). This is particularly true when the number of participating laboratories is low (note the ordinate in **Figure 7** is a log scale). For example, the width of the 95% confidence interval for the overall laboratories mean of 100 is ± 59 if the four laboratories have a laboratory mean CV = 40%. If only two laboratories participate, the 95% confidence interval is so wide (± 287) that such results would have to be viewed with considerable caution. The choice of how many laboratories to include in a validation study ultimately becomes a trade-off between reducing the variability in the estimate of interlaboratory variation versus the cost of including more laboratories. Certainly there is a diminishing returns aspect to number of laboratories used. The largest benefits occur with smaller sizes: there is a large benefit to using three instead of two, and five instead of three.

Step 7. Assess the Predictive Capability of the Alternative Method (Figure 1)

The next step in the process is to assess the predictive capability of the alternative method. This may be done by confirming that alternative method data input into the predefined prediction models provides outputs that predict *in vivo* toxicity at the level of both accuracy and precision defined at the beginning of the study. Once the data are available from the validation study, each result from the alternative method should be converted by the algorithm(s) in the prediction model into a prediction of *in vivo* toxicity. Then the predicted toxicity should be directly compared with the actual toxicity of each test substance. If the method predicts toxicity within the limits defined by the prediction model, then it would provide strong evidence supporting the predictive capability of the assay. If the results from the alternative method poorly predict *in vivo* toxicity, then there would be little evidence supporting the utility of the method.

If the conclusion reached is that the method is reliable in terms of reproducibility (see step 6) and predictive capability, then the results of the validation study may be used in the next part of the validation process that is the determination of relevance (discussed next). If the methods are shown not to be reliable, then two courses of action may be followed (**Figure 1**). If it appears there is merit

in further developmental work (test method optimization), then additional research should be undertaken. When the assay is adequately modified and/or a new prediction model is developed, it may be evaluated in a subsequent validation study. Alternatively, the method may be abandoned if it is apparent that additional effort is unlikely to be fruitful.

Assessing Alternative Method Relevance

Once the reliability of an alternative method has been confirmed in a validation study, its relevance as a replacement for an *in vivo* toxicity test must be assessed. As noted earlier, the assessment of relevance addresses the question: Is the performance of the method good enough to allow its acceptance as a replacement for a given *in vivo* test? Answering this question requires assembly and review of as much information as possible about the performance of the alternative method and the *in vivo* test it is intended to replace. This review process must ultimately allow the formulation of a judgment of whether the alternative method is acceptable or not for its intended use. Reviewed next is the information that must be considered in order to judge the relevance of an alternative method, and provide recommendations on how to establish objective benchmarks that can be used to help make this judgment.

The factors that must be considered in defining the relevance of an alternative method include an assessment of: (1) the best performance that can be expected from an alternative method given the performance characteristics of both the alternative method and the *in vivo* test it is intended to replace, (2) the performance of the *in vivo* test being replaced, and (3) the supplemental data available for use in conjunction with the alternative method during a safety assessment. Each of these factors is reviewed next.

Assessing the Best Performance that can be Expected from an Alternative Method

Ideally, alternative method results should provide nearly perfect predictions of the toxic endpoints measured in the *in vivo* method. However, there are important technical factors that prevent this ideal from being reached. One of the most important is the variability in the *in vivo* and alternative method data. If perfect prediction is unrealistic, then what is the best performance that can be expected from an alternative method? One approach that can be taken to answer this question is to use computer simulations based on the known performance characteristics of the *in vivo* test and the alternative method to create a picture that describes how the results from the validation study will appear if the prediction model is true. The results of these simulations can be used as benchmarks for objectively judging the acceptability of the alternative method that was measured in the validation study. In order to provide a practical example of this process, one can again return to the assessment of eye irritation alternatives. In order to assess the performance that may be expected from an alternative method evaluated in a validation program, a computer simulation based on the simple linear relationship of eqn (1) was used:

$$Y = (1.1)X$$

The effect of variability on the overall performance of the method was assessed by adding an error term to X and Y in each run of the simulation as described earlier (see step 3). After a large number of data points were simulated for each set of alternative method and *in vivo* test CVs, the Pearson's correlation coefficient was calculated in order to determine the correlation between the X and Y values. A second set of X values ranging from 0 to 40 were also run to simulate results for eye irritation scores that might be observed with a more restricted set of test substances such as cosmetics products.

Results from the simulations are summarized in **Table 4** and **Figure 8**. Each of the correlations shown in **Table 4** is based on 10 000 simulated responses. The effects on the size of the Pearson's correlation coefficient due to error imposed on the *in vivo* alternative method responses are shown under several conditions. In general, it is known that a tight linear relationship with nearly perfect correlation will exist when there are negligible levels of error in either X or Y. The expected level of correlation will be reduced as error is introduced into either X or Y. In the first case (ideal conditions, **Table 4**, **Figure 8**a), the imposed variation is set relatively low. As expected, Pearson's correlation coefficients are large, ranging between 0.97 and 0.99. Furthermore, restricting the alternative method results to the least irritating portion of the Draize scoring scheme ($X = 0$–40) has little effect on the correlation coefficients. In the second case (typical conditions, **Table 4**, **Figure 8b**), the CVs applied to the data (*in vivo* $CV = 50\%$, alternative method $CV = 20\%$) are consistent with those observed in the Draize test. Under these circumstances, the correlation is still high (> 0.8). Importantly, restricting the range of alternative method responses to the least irritating materials ($X = 0$–40) results in a decrease in the correlation coefficient to an approximate range of 0.6–0.7. Setting the imposed CV for the alternative method at 0.4 further decreases the correlation

Table 4 Expected Pearson's correlation coefficients when the error *in vivo* and alternative method data are considered

Imposed coefficient of variation		*Expected Pearson's correlation coefficient*	
Alternative method	*In vivo*	*Full range* ($x = 1$–100)	*Restricted range* ($x = 1$–40)
Ideal conditions			
0.05	0.05	0.994	0.990
0.10	0.10	0.975	0.960
Typical conditions			
0.20	0.40	0.860	0.719
0.20	0.50	0.828	0.652
0.20	0.60	0.803	0.608
0.40	0.40	0.719	0.604
0.40	0.50	0.690	0.542
0.40	0.60	0.672	0.504
Theoretical best conditions			
0.00	0.40	0.930	0.787
0.00	0.50	0.891	0.706
0.00	0.60	0.862	0.647
Alternative method equivalent to *in vivo* method			
0.40	0.40	0.719	0.604
0.50	0.50	0.635	0.490
0.60	0.60	0.543	0.403

Note: Computer simulations were used to assess the effects of variability in eye irritation test and alternative method data on the correlation coefficients expected between the data sets. The model used in the simulation assumed that the algorithm, $y = (1.1)x$, describes the relationship between the *in vivo* and alternative method data. Values for $x = 0$–100 were used to simulate responses across the entire Draize eye irritation scale. The simulations were conducted with test substances having the full range of response ($x = 1$–100) and for a restricted range representing the least irritating part of the eye irritation scale ($x = 1$–40). Each result is based on 10 000 runs of the simulation. Results are shown for the simulations where the variability is set relatively low (ideal conditions), and where the variability was set at a level consistent with performance of currently available alternative methods and the *in vivo* test (practical conditions). Additionally, simulations were conducted where the variability was set at zero for the alternative method (theoretical best conditions) and where the variability of the alternative method was set equivalent to the eye irritation test (alternative method equivalent to *in vivo*). The results of these simulations demonstrate that variability in the data sets can have a significant effect on the performance of the alternative method in predicting the *in vivo* response. Thus, the effect of variability must be taken into account when the performance of an alternative method is assessed. Reproduced from *Toxicology In Vitro* 10: 479–501, 1996, Bruner, L. © Proctor & Gamble.

coefficients (**Table 4**). If an alternative method could be perfected technically, its CV would approach 0. Third case shows the effect of setting the CV = 0 (theoretical best conditions, **Table 4**, **Figure 8c**). Under these conditions, the expected Pearson correlation coefficients range between 0.85 and 0.95 when $X = 0$–100. When the alternative method results are restricted to the least irritating substances ($X = 0$–40) the correlation coefficients are lower, ranging from $\sim$0.65–0.80. Finally, in the fourth simulation, the alternative method CV was set to a level equivalent to that observed in the Draize eye irritation test. Under these conditions, the simulations estimate that the correlation coefficients will range from $\sim$0.5–0.7 when all levels of alternative method response ($X = 0$–100) are included (alternative method equivalent to the *in vivo* method, **Table 4**, **Figure 8d**). When the alternative method results are restricted to the least irritating range ($X = 0$–40), the coefficients are lower, ranging from 0.4 to 0.6.

It is important to note that these simulation studies were conducted under idealized conditions. The underlying assumption is that the relationship between the *in vivo* and alternative method data is linear. Also, the number of substances included in the simulations was large (10 000). Hence, the results of the simulations shown in **Table 4** and **Figure 8** represent a long-run average of the most optimistic correlations. In practical terms, even if all of the assumptions were true, the observed correlation might be higher or lower than the long-run average. Other deviations from the conditions assumed in these simulations, such as nonlinearity or nonuniform distribution of responses, can also be expected to reduce the level of correlation. Since it is unlikely the results from an alternative method are so simply related to a particular *in vivo* toxicity, it can be expected that validation studies will result in lower correlation coefficients, even for those alternative methods that may actually be reasonably predictive of the *in vivo* response.

Once completed, the results of the simulations may be used as benchmarks to objectively compare against the actual results obtained in the validation study. If the data from the validation study meet or exceed the simulated benchmarks, a strong case could be made that the method performs at an acceptable level. For example, if an eye irritation alternative method has a prediction model algorithm of $Y = (1.1)X$ and a CV = 20%, and if the *in vivo* test has a CV = 50%, then the best performance that may be expected would appear as follows: the relationship between the *in vivo* and alternative method data would look similar to that shown in **Figure 8b**, the correlation coefficients would be within the range of $\sim$0.6–0.8 (**Table 4**), and 95% CI_{pred} for a MAS prediction of 55 would be in the range of ± 40 ($n = 50$, **Table 2**). A method performing at these levels should be considered a reasonable performer. If the alternative method performance was less than such a

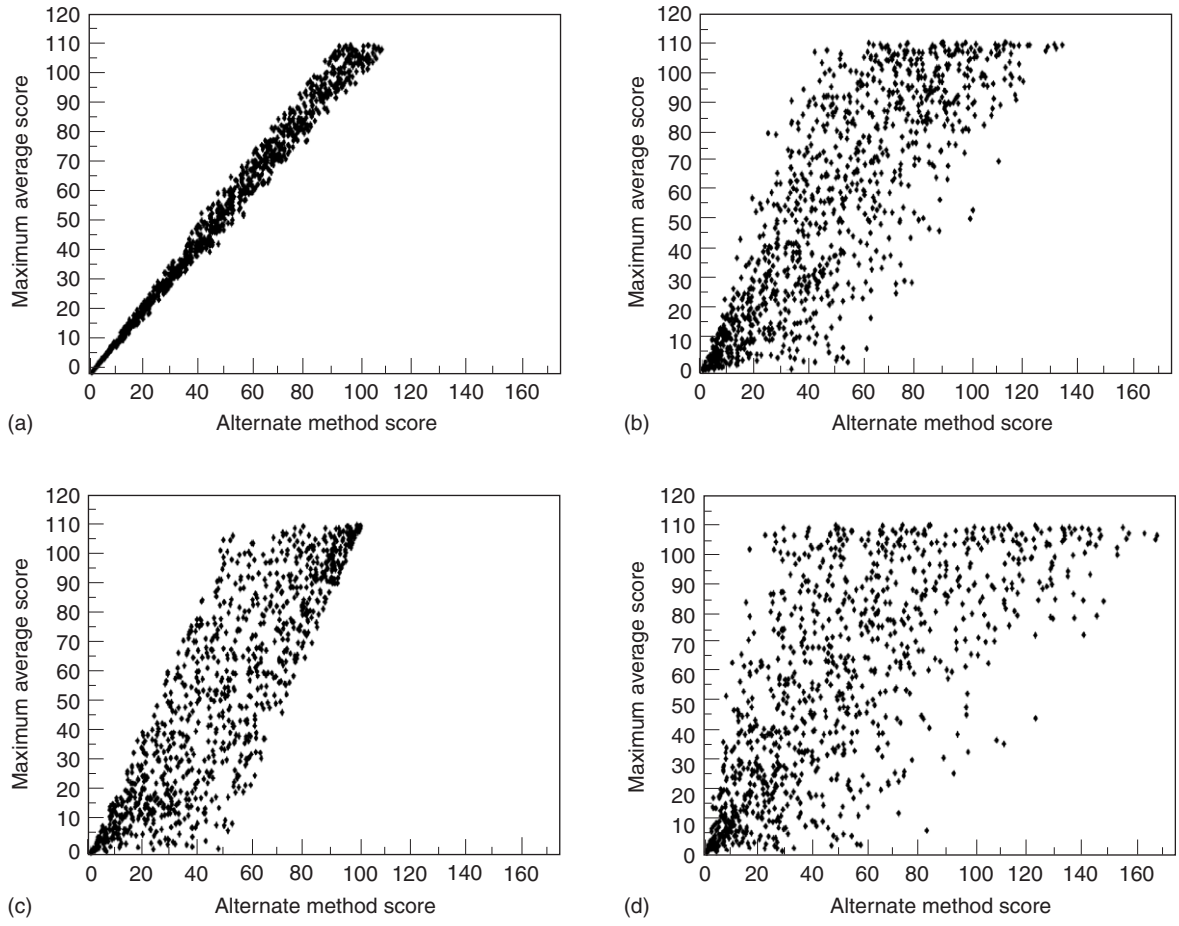


Figure 8 Effects of variability in the Draize test and alternative methods on correlation. Computer simulations were used to assess the effects of variability in the eye irritation test and alternative method data on the relationship between the two data sets. (a) The CVs applied to both the *in vivo* and alternative method data were 5%. (b) The CVs applied to the *in vivo* and alternative method data were 50% and 20%, respectively. (c) The CVs applied to the *in vivo* and alternative method data were 50% and 0%, respectively. (d) The CVs applied to the *in vivo* and alternative method data were 50% and 40%, respectively. Reproduced from *Toxicology In Vitro* 10: 479–501, 1996, Bruner, L. © Proctor & Gamble.

simulated benchmark, then there would be little evidence supporting its relevance.

Assess the Performance of the *In Vivo* Test that will be Replaced

Once estimates of the best possible performance of the methods are defined, another useful benchmark that can be used to assess the relevance of an alternative method is to compare its performance against that of the *in vivo* toxicity test it will replace. If the capability of the alternative method to predict an *in vivo* toxicity endpoint was at least equivalent to the capability of the *in vivo* test to predict its own result, then it would provide strong evidence supporting the relevance of the alternative method.

The capability of an *in vivo* method to predict results across multiple laboratories can be determined if its performance characteristics are known. If these data do not exist, then the interlaboratory variability terms needed to define the performance characteristics can be obtained by testing a common set of substances in several independent laboratories. If an alternative method is capable of predicting toxicity at a level equivalent to or better than the test it is intended to replace, then it would provide strong evidence supporting the contention that the alternative method can substitute for the *in vivo* toxicity test. Returning to the specific example of assessing the validity of eye irritation alternatives, the results of simulations that have conducted to assess the capability of the Draize test to predict results across laboratories are similar to those obtained from the simulations presented in **Tables 2** and **4** and **Figure 8**b. Thus, if the capability of the Draize test to predict eye irritation responses is used as the criterion for judging the relevance of eye irritation alternatives, then methods that perform similar to or better than those illustrated under typical conditions in **Tables 2** and **4** and **Figure 8**b should be considered adequate performers.

Assessing Supplemental Data Available for use in Conjunction with the Alternative Method During a Safety Assessment

Once the performance of the alternative method is compared against the appropriate benchmarks (such as the computer simulations described earlier), there are other factors that need to be considered when assessing the relevance of an alternative method. One factor considered particularly important is the mechanistic basis supporting the alternative method. Having an understanding of the mechanistic basis is important because it increases the probability that predictions of *in vivo* toxicity by the alternative methods are correct. Unfortunately, the science of toxicology has not progressed to the point that all toxic mechanisms are well understood. Thus, if alternative methods are to be accepted in the foreseeable future, they will be used under conditions where a full understanding of mechanisms is not available. Therefore, it is important to consider the approaches that the developers of an alternative method recommend following in order to compensate for this lack of knowledge.

Toxicologists who use alternative methods in the safety assessment process generally utilize three approaches to help decrease the uncertainty of the predictions when mechanistic understanding is weak. The first is to restrict the use of an alternative method to the same chemical classes that were used to develop the prediction model. This is important because similar materials are more likely to act by the same mechanisms of action. If the materials tested diverge significantly from those used to develop the prediction model, then the reliability of the predictions will decrease. This will occur because the divergent materials may exert effects through different toxic mechanisms that should perhaps be tested in different alternative methods that are sensitive to different chemical parameters and that use different prediction models.

The second approach commonly used to decrease the uncertainty of predictions is to compare the results obtained from an unknown test substance with one or two similar benchmark substances tested in the alternative method at the same time. If a material is intermediate in toxicity between two well-known benchmark standards in the alternative method, then it provides evidence that the material will be intermediate in toxicity *in vivo*. This approach provides greater confidence in a prediction than can be derived from testing isolated test substances on an absolute scale without any reference to other materials.

The third approach used to decrease the uncertainty associated with the predictions is to define specific limitations on the use of the assay. For example, developers may recommend restricting the use of a particular method to specific physical forms of test materials (liquids only or solids only) or for predicting limited ranges of irritancy (mild irritancy or severe irritancy only). These limitations may depend on many factors, especially specific technical incompatibilities associated with testing certain kinds of substances.

Next, it is important to consider whether the width of the 95% CI_{pred} from the alternative method is small enough to provide an acceptably precise prediction of *in vivo* toxicity. The analysis presented earlier demonstrated that the 95% CI_{pred} from an alternative method might be large. A benchmark that can be used to assess the acceptability of a large 95% CI_{pred} could be derived from an examination of the precision of toxicity measurements obtained from the *in vivo* toxicity test. If an alternative method provides predictions as precise as those obtained from the *in vivo* method it is intended to replace, then it would provide evidence that the 95% CI_{pred} alternative method is acceptable (for more information on the utility of the 95% CI_{pred}).

The margin of safety provided by a prediction from an alternative method compared to that obtained from the *in vivo* method must be considered. *In vivo* tests, such as the Draize eye irritation test, significantly overpredict the human response. This has been considered important because use of an overpredictive method decreases the probability of false-negative results that may be associated with a highly variable test. Establishing an acceptable margin of safety for an alternative method will depend on finding an appropriate balance between the risks to humans associated with possible underprediction due to variability versus the losses associated with a higher incidence of false-positive results that invariably results from setting more stringent cut-offs.

Finally, it is important to consider the experience that has been gained in use of the alternative method outside formal validation programs. Although the quality of data from other sources may be variable and not collected under blind conditions, it may provide additional useful insights into an alternative method's performance. If these additional data are consistent with the results obtained from a validation study, it would provide further evidence supporting the relevance of the alternative method.

Once all of this information is assembled and assessed, the participants in the validation study must render a final judgment on whether or not the method is relevant for the stated purpose. If (1) the measurement of method reliability from a validation study, (2) the actions taken to compensate for lack of mechanistic understanding, the performance of the method relative to calculated benchmarks, (3) the

width of the 95% CI_{pred}, the margin of safety, and (4) the breadth of experience toxicologists have with the method are judged to be adequate, this would provide strong evidence supporting the relevance of the alternative method.

If the alternative method is judged both reliable and relevant at the end of this process, then the new assay should be considered validated. Once validated, the alternative method may be used routinely in the safety assessment process and should be considered for acceptance by regulatory authorities (**Figure 1**). If the alternative method is judged not relevant, then it should not be used or considered for acceptance by regulatory authorities. The reasons for the rejection should be clearly stated so that the deficiencies can be identified and resolved in follow-up research if such work is likely to be fruitful.

Discussion

The development of an alternative method begins with the creation of a test followed by generation of a database that supports its utility. This work provides the preliminary evidence that a method is reproducible and has predictive capability. Once available, this information can be used to construct a prediction model that describes how to convert the results from an alternative method into predictions of toxicity *in vivo*. When a method has been technically advanced to this point, it may be assessed in the validation process.

Relative to the development of valid toxicity tests, the assessment of a toxicity test's validity is a relatively simple matter. Validation is relatively simple when the studies are designed to test the performance of an alternative method relative to performance criteria established prior to the start of the study. Defining a prediction model prior to the commencement of the study allows those evaluating an alternative method to construct a clear picture of what the results from a valid assay will look like before the study begins. When the results from the validation study become available, objective comparisons can be made between the predetermined picture and the actual study results. If the results are consistent with this picture, it provides strong evidence that the alternative method is reliable. If the results do not fit the picture, it provides evidence that either further developmental work on the alternative method is needed, or that the method should be abandoned. Such an approach has the advantage that it allows an objective evaluation of the data, while avoiding post hoc data fitting that does not provide definitive answers on alternative method validity.

Once the reliability of an alternative method has been confirmed in a validation study, the next step in the process is to review the relevance of the alternative method. This requires thorough consideration of all the performance data related to both the alternative method and the *in vivo* test it will replace. Ultimately, those conducting a validation program must take this information and render a judgment on whether the performance is good enough to allow replacement of the alternative method.

See also: Toxicity Testing, Alternatives; Toxicity Testing, Irritation; Toxicity Testing, Modeling.

Further Reading

Bruner LH, Carr GJ, and Curren RD (2000) Is it good enough? Objective assessment of toxicity test method performance in validation studies. In: Balls M, Zeller A-M, and Halder M (eds.) *Progress in the Reduction, Refinement and Replacement of Animal Experimentation*, pp. 375–384. Amsterdam: Elsevier.

Bruner LH, Carr GJ, Harbell JW, and Curren RD (2002) An investigation of new toxicity test method performance in validation studies: 1. Toxicity test methods which have predictive capacity no greater than chance. *Human & Experimental Toxicology* 21(305): 312.

Bruner LH, Carr GJ, Harbell JW, and Curren RD (2002) An investigation of new toxicity test method performance in validation studies: 2. Comparative assessment of three measures of toxicity test performance. *Human & Experimental Toxicology* 21: 313–323.

Bruner LH, Carr GJ, Harbell JW, and Curren RD (2002) An investigation of new toxicity test method performance in validation studies: 3. Sensitivity and specificity are not independent of prevalence or distiribution of toxicity. *Human & Experimental Toxicology* 21: 325–334.

Bruner LH, Kain DJ, Roberts DA, and Parker RD (1991) Evaluation of seven *in vitro* alternatives for ocular safety testing. *Fundamentals of Applied Toxicology* 17(1): 136–149.

Draize JH, Woodard G, and Calvery JO (1944) Methods for the study of irritation toxicity of substances applied topically to the skin and mucous membranes. *Journal of Pharmacology and Experimental Pharmacology* 82: 377–390.

Durham RA, Sawyer DC, Keller WF, and Wheeler CA (1992) Topical ocular anesthetics in ocular irritancy testing: A review. *Laboratory Animal Science* 42: 535–541.

European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) (1992) Eye Irritation, Technical Report No.48. Reference Chemicals Data Bank, Brussels.

Fentem JH, Archer G, Balls M, *et al.* (1998) The ECVAM International Validation Study on *in vitro* tests for skin corrosivity. 2. Results and evaluation by the management team. *Toxicology In vitro* 12(4): 483–524.

Gautheron P, Dukic M, Alix D, and Sina JF (1992) Bovine corneal opacity and permeability test: An *in vitro* assay of ocular irritancy. *Fundamentals of Applied Toxicology* 18(3): 442–449.

Gordon VC (1992) Utilization of biomacromolecular *in vitro* assay systems in the prediction of *in vivo* toxic

responses. *Lens and Eye Toxicity Research* 9(3–4): 211–227.
Gordon VC and Bergman HC (1987) Eytex: An *in vitro* method for evaluation of ocular irritancy. *Alternative Methods in Toxicology* 5: 87–90.
North-Root H, Yackovich F, Demetrulias J, Gacula M Jr., and Heinze JE (1982) Evaluation of an *in vitro* cell toxicity test using rabbit corneal cells to predict the eye irritation potential of surfactants. *Toxicology Letters* 14(3–4): 207–212.
North-Root H, Yackovich F, Demetrulias J, Gacula M Jr., and Heinze JE (1985) Prediction of the eye irritation potential of shampoos using the *in vitro* SIRC cell toxicity test. *Food and Chemical Toxicology* 23(2): 271–273.
Reader S, Blackwell V, O'Hara R, *et al.* (1989) A vital dye release method for assessing the short term cytotoxic effects of chemicals and formulations. *Alternatives to Laboratory Animals* 17: 28–37.
Spielmann H, Liebsch M, Kalweit S, *et al.* (2000) Results of a validation study in Germany on two *in vitro* alternatives to the Draize eye irritation test, the HET-CAM test and the 3T3-NRU cytotoxicity test. *Alternatives to Laboratory Animals* 24: 741–858.
Worth AP and Balls M (2001) The importance of the prediction model in the validation of alternative tests. *Alternatives to Laboratory Animals* 29(2): 135–144.
Worth AP and Balls M (2001) The role of ECVAM in promoting the regulatory acceptance of alternative methods in the European Union. European Centre for the Validation of Alternative Methods. *Alternatives to Laboratory Animals* 29(5): 525–535.

Toxicity, Acute

Donald J Ecobichon

By definition, acute toxicity studies are conducted to determine the total, adverse, biological effects caused during a finite period of time following the administration of single, frequently large doses of a chemical, physical (dusts, fibers), or biological (proteins, vaccines, genetically modified foods) agents, or some form of energy (radiation, ultraviolet light). The objectives of such studies are to discover any adverse health effect that could be attributed to the agent under investigation, including any immediate biochemical, physiological, and/or morphological changes, any delayed changes suggesting some secondary injury to body organs/tissues, as well as the death of the animal. In general, the effects observed in the experimental subjects, usually animals, are directly related to the amount of the poisonous substance administered. It is a common misconception that acute toxicity studies are designed only to express the potency of an agent in terms of the median lethal dose (LD_{50}), a value representing the estimated dose causing death of 50% of the population of test subjects exposed under the defined conditions of the test. Nothing could be further from the truth; acute toxicity studies encompass a number of experiments. The usual battery of acute toxicity tests is shown in **Table 1**.

Since information concerning the toxicity of the agent must be obtained before humans are exposed to it, this necessitates the use of animal models as surrogates or substitutes for the human. Several strains of rodents (mice, rats) are used routinely to determine the acute toxicity of new agents. Experiments are

Table 1 A battery of tests for the evaluation of acute toxicity

Test	*Description*	*Study period*
Lethality	LD_{50} (LC_{50}) or an estimated value	24 h
	Surviving animals – close observation permits determination of duration of toxicity; recovery; development of secondary toxicity; changes in hematology, blood chemistry, urinalysis; changes in organ and tissue function	14 days
Primary irritation	Skin	
	Exposure	4–24 h
	Evaluation	24, 48, 72 h
	Eye	
	Exposure	1.0 s
	Evaluation	1.0, 24, 48 and 72 h
Sensitization	Repeated (5 days week^{-1}) dermal application	14 days
	Rest period of 10–14 days	
	Challenge dose at days 28–30	
	Evaluation	24, 48, 72 h
Photoallergic and phototoxic reactions	Repeated treatment (oral, iv, dermal) for 10–14 days	
	Rest period of 14–21 days	
	Retreatment with UV light on shaved skin patch	
	Evaluation	24, 48, 72 h

conducted using both males and females of the species because of known sex differences in response(s) to various agents. Indeed, an extensive body of data has been built up over several decades using rodent species, thereby permitting chemical-to-chemical, inter- and intraspecies comparisons. It is desirable to have acute toxicity data from nonrodent (rabbit, guinea pig, dog, monkey) species, particularly if the results from the mouse and rat differ greatly, suggesting distinct species differences in response(s). In contrast, should similar toxicity be seen in a number of experimental animal species, the same toxicity might be produced in the human at some, as yet unknown, dosage to the agent.

The agent should be administered via the route by which the species might be expected to obtain the toxicant. In general, the usual routes of exposure for the human include ingestion, inhalation, or by contact with the skin. Accidental exposure to industrial or home products might also include having them splashed into the eyes or onto the skin. However, if an agent being tested is a drug, exposure might require intravenous, intramuscular, or subcutaneous routes of administration.

Why are acute toxicity studies necessary? With any new agent, there will be workers exposed to relatively high concentrations during its manufacture, handling, packaging, and use. Accidents may occur not only in the workplace but during the transportation (ship, rail, truck) of the agent, with exposure of bystanders in the immediate vicinity of the accident and risk to personnel involved with the accident or cleanup of the spillage (e.g., police, firefighters, emergency response teams, sanitation crews). There is also the potential of accidental and/or intentional exposure of the product user, members of his/her family, neighbors, children, and pets. It is essential to know just how toxic or nontoxic the agent may be under the most bizarre circumstances of exposure (e.g., ingesting or inhaling the agent or getting it on the skin). How much is safe? How little is too much? The information obtained from acute toxicity studies can be found printed in a material safety data sheet (MSDS) required by law to be prepared for each and every product manufactured and to be available to the public, to industries using the products, and to health and safety professionals.

Determination of the Lethal Dose

The LD_{50} is a statistical estimate of the acute lethality of an agent administered to a specified sex, age, and strain of a species of animal. The value provides a measure of the relative toxicity of an unknown agent compared to other agents administered by the same route to the same species, strain, age, and sex of the animal. As listed in **Table 1**, the LD_{50} value, an indicator of lethal potency, is frequently the first biological safety test determined for a new chemical, the agent being administered via the route by which the human might acquire a high concentration, and animal mortality being assessed in the 24 h period after treatment. Traditional LD_{50} tests have been replaced by abbreviated test protocols that minimize animal use. Given that people might acquire the chemical by different routes, it might be necessary to carry out two experiments, choosing two of the three possible routes (ingestion, inhalation, dermal) of administration in anticipation of quite different values. Although accurate determinations of the lethal potency are no longer required (designing experiments in which 60 to 100 animals might be used in the classical determination of the LD_{50}), some insight into the potency, even a rough estimate of the range of acute toxicity, is essential. Regulatory agencies are still concerned about massive spills and the impact of these on the health of local populations. The Bhopal incident revealed just how little information was available on the toxicity associated with inhalation, dermal, and ocular exposure to methyl isocyanate. While criticism has been leveled that the LD_{50} values are just numbers, they are valid predictors of acute toxicity, albeit only of mortality and not of morbidity or long-term adverse health effects.

Animal rights activists have repeatedly challenged the need for the LD_{50} determination, in terms of the inflicting of injury to the test animals and the needless waste of large numbers of animals to obtain a number that is only a rough estimate. A properly designed study will yield much more information than just 'a number'. While, by definition, 50% of the animals will die, close observation of these animals during the first 12 h period after treatment may reveal several biological clues to possible mechanisms by which the toxicant may be causing an effect, clues that are valuable to the clinical toxicologist in attempts to alleviate human suffering. However, 50% of the animals will survive the treatment and these survivors are a repository of biological effects elicited by the test agent. These effects are studied over the next 14 day period to assess the short or long duration of toxicity; the rapid or slow recovery; the appearance of any additional, delayed, or secondary toxic effects; changes in hematology, serum biochemistry, and urinalysis; and changes in organ/tissue function (liver, kidney, and nervous system) measured by relatively noninvasive techniques, all without having to destroy the animals. When the animals are euthanized at 14 days

after treatment, organs/tissues will be available for detailed microscopic examination to correlate observed biological effects and/or injury with possible morphological changes. Thus, the animals surviving the toxic insult are a veritable treasure trove of information concerning the mechanism(s) of the chemical-induced toxicity.

Given the spectrum of observed and measured biological effects, one important aim of the acute toxicity study is to develop a quantitative relationship between the intensity of a measured response or adverse health effect and the concentration(s) of agent administered. Assuming that the dosage of agent can be 'delivered' to the test animals accurately with minimal variability, this leaves interanimal variability in response, one major reason why the classical LD_{50} determination uses 8–10 animals per treatment group. The biological responses and variability are usually presented in graphic form, the *x*-axis representing the range of dosage while the *y*-axis reflects the biological response in some quantitative manner (**Figure 1**). From such graphs, a dosage-related appearance of target organ toxicity may be determined, some organs/tissues responding to low levels of the agent (**Figure 1a**), others responding only at elevated concentrations (**Figure 1b**). In addition, the slope of the dose–effect relationship for each organ/tissue can be determined, indicating whether or not small changes in dosage produce marked biological changes (a steep slope, potent agent; **Figure 1a**), or the reverse, where large increases in dosage are accompanied only by weak to modest changes in responses (a shallow slope, weakly toxic agent, **Figure 1c**).

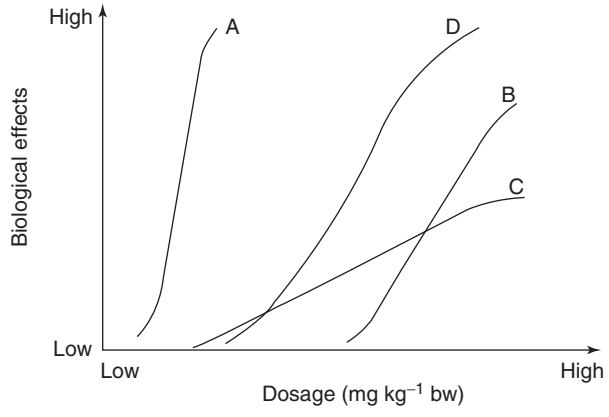


Figure 1 Theoretical dose–effect relationships illustrating possible, different target organ (A, B, C, D) responses over a wide range of agent concentrations, indicating the importance of the slope of the relationship to predict whether or not a small or large change in dosage is required to induce marked, moderate, or weak biological effects.

Range-Finding for the Lethal Dose

With a view toward reducing, refining, and replacing animal testing procedures, the 'three Rs' initiative, a simplified range-finding (or up-and-down) procedure using, at most, six animals has been developed. An arbitrarily selected, initial dosage (mg kg^{-1} body weight) is administered to a pair of suitable animals, with subsequent close observation for effects over a predetermined time period. If little or no toxicity is observed, the second pair of animals receives a dosage 50% (1.5/l.0) higher than the initial dosage. If no toxicity is observed, the second pair of animals receive a dosage double (2.0/1.0) the original dosage. If, however, the second level (1.5/1.0) is lethal to one or both animals, some concentration between the original and second dosage is given to the third pair of animals. If the originally chosen dosage causes severe intoxication and mortality, the dosage administered to the second pair will be downscaled to the order of 50% or 66% of the initial dosage.

How close do the above 'estimates' relate to the actual LD_{50}? Studies have shown that the approximate lethal dose for 86% of those chemicals tested in this manner were within 30% of the known LD_{50} values determined by the classical approach. The method is not infallible: for example, some 14% of chemicals were outside this range; and no dose–response or slope information can be obtained. International agreement has been reached that the up-and-down procedure could replace the conventional acute toxicity test for the purposes of hazard classification and label (including color-coding) production.

Primary Irritation Studies

Regulatory agencies demand the preparation of MSDSs for each chemical manufactured and sold. These must include testing for ocular and dermal irritancy as well as dermal hypersensitivity, sensitization, and phototoxicity. Such information is also required to meet regulations for packaging and labeling, hazard classification, and transportation. Products freely available for purchase by the public, including cosmetics, pesticides, cleaners, detergents, polishes, waxes, health care products (soaps, bath gels, shampoos, creams, mouthwashes) must receive the same assessment.

The Skin Test

Testing procedures for dermal irritation evolved from the product safety tests of the early 1940s using a variety of animal species, rat, rabbit, guinea pig, and dog, as surrogates for the human, with the addition of miniature swine and rhesus monkeys at later

Table 2 Factors governing the selection of animal species for dermal irritation testing

Physical properties of the test agent (liquid, powder, emulsion)
Solubility of the test agent in aqueous or organic-based solutions
Inherent toxicity of the test agent (e.g., low toxicity requiring application of large volumes)
Known species sensitivities of test animal dermis compared to that of the human skin from earlier experience
Application of different concentrations of test agent and the need for comparable control sites where the dermis is similar
Application of the test agent to intact and abraded dermis, the latter having slight damage to the stratum corneum (dead cell layer) by mechanical means to mimic the loss of the protective barrier through abrasions and cuts

Table 3 *In vitro* methods for testing dermal toxicity

Test system	*Biological endpoint*
Corrositex™	Corrosion
Skintex™	Corrosion
Testskin™	Irritation
Epiderm™	Phototoxicity
Episkin™	Percutaneous absorption
Melanoderm™	DNA damage
Immunoderm™	Immunological parameters
Epiderm-FT™	
Skin™ 2K1350	
Transcutaneous electrical resistance assay – mouse skin disk	Corrosion

Source: Data from various chapters in Salem H and Katz SA (eds.). (2003) *Alternative Toxicological Methods*. Boca Raton: CRC Press.

dates. As has been observed for humans, absorption of test agents through the skin varied considerably from region to region on the body surface. The choice of test species may be dictated by a number of factors such as those shown in **Table 2**.

The test substance (liquid, solid, paste, emulsion) is usually applied to a shaved test area (6.0 cm^2 or 1.0 $in.^2$) of skin on the back in a uniform layer, the site being covered by a gauze patch taped in place to prevent the animal from licking the material off the site during normal grooming. The trunk may be wrapped with an impervious plastic sheet, this practice being particularly useful when multiple sites are treated. At the end of the test period, usually 24 h, the coverings are removed, the residual test agent being wiped off with gauze wetted with warm soapy water, and the test areas are evaluated for (1) edema (puffiness, swelling) and (2) erythema (redness, inflammation). This evaluation is repeated at 48 and 72 h after treatment. The Organization for Economic Cooperation and Development have introduced a 4 h rabbit covered patch test in place of the 24 h study.

While highly subjective, a scoring system has been developed to measure visually the degree of puffiness (edema) and redness (erythema). The scoring system assigns a number (0–4) for each toxicological endpoint, ranging from none through very slight, slight, moderate, to severe effects. Average numerical scores for the primary irritation index are obtained from the results on five or six animals per treatment level over the test period (24, 48, or 72 h) to provide a dose–response relationship concerning the potency of the test agent to cause irritation (edema, erythema) as well as the rate of recovery from effects. This information provides guidance to regulatory agencies for cautions/warnings to be printed on product labels.

In Vitro Irritation – Skin

Concerns relating to pain and suffering as well as the numbers of animals used in the classical dermal irritation test have resulted in much effort to develop rapid, *in vitro*, test systems suitable for screening out highly irritant or corrosive chemicals, assessing hypersensitivity, sensitization, and phototoxicity. Unfortunately, the validation criteria of reliability, reproducibility, and relevance (predictability, biological basis for stated purpose) have not been attained and, consequently, no tests have been validated by national or international regulatory agencies. A list of such test systems and their biological endpoints is presented in **Table 3**. A few of these tests are considered as 'stand alone' indicators of corrosivity, thereby eliminating the need for further confirmatory animal studies.

Most commercial *in vitro* test systems are human tissue equivalents – three-dimensional tissue culture models of human skin at an air–liquid interface forming a reconstructed, differentiated dermis and a functional stratum corneum, usually composed of keratinocytes grown on a collagen matrix with fibroblasts, and/or melanocytes or Langerhans cells. The skin-like layer is suspended in a two-chamber cell, the test agent being added to the upper chamber either in solution or applied directly to the moist surface (stratum corneum). Samples of medium from the lower chamber can be removed for analysis or the 'skin' can be stained specifically for microscopic study of cell damage. The chemical or biological endpoints examine cell viability and membrane integrity (Neutral Red dye penetration, tetrazolium dye metabolism, protein leakage), cell proliferation and protein synthesis (Coomassie Blue or Kanacid Blue dyes), release of inflammatory mediators (cytokines, prostaglandins), and mediators of apoptosis (p53, p21, caspases) (**Table 4**). Isolated dermis from human cadavers or from swine can be used in double-chamber systems in a similar manner to that described for skin equivalents. Eventually, a battery of *in vitro* tests will be approved by

Table 4 Biological endpoints for testing skin toxicity

Skintex system: cell-free complex mixture of vaious macromolecules, with physicochemical changes occurring on interaction with irritants, resulting in changes in light transmission
Cell cultures: microorganisms and mammalian cell lines, examining Neutral red dye penetration Protein leakage, colorimetric assay Coomassi Blue or Kenacid Blue for cell proliferation protein synthesis
Testskin: human tissue equivalents (human kerationcytes on collagen or collagen – glycosaminoglycan matrix with human fibroblasts) forming an epidermis for examining Dye penetration (Neutral Red) Dye exclusion Cellular damage by agents Chemical penetration Cell growth and development Cellular metabolism
Isolated tissues: prepared for a double chamber; agent applied to the top and sampled from the bottom fluid to test penetration through the skin; can use candaver skin or isolated animal skin (mouse, rat, swine)

regulatory agencies, the guidelines requiring the submission of a spectrum of results from tests having well defined biological endpoints.

The Eye Test

Damage to the eye is an all-too-common consequence of an accidental splashing of industrial chemicals, home and health care products, pesticides, solvents, etc., resulting in painful and frequently permanent injury. The Draize eye test, first described in 1944, has become a target of animal welfare groups, antivivisectionists, and concerned scientists who claim that it is not required, is inhumane, causes unnecessary pain to the test animal, generates a subjective result even with the available detailed scoring systems used, and is prone to interlaboratory variability in results such that the test is meaningless. The thought of knowingly placing some highly irritating agent in an animal's eye and causing pain is abhorrent. In fact, if the dermal irritancy test is positive, there is little scientific basis for carrying out the eye test, since the agent will almost certainly be positive in the eye. Hence, dermal irritancy tests will screen out the highly toxic agents. However, between the highly damaging, strong acids or bases, and completely innocuous agents, lie a wide variety of seemingly neutral, slightly acidic or basic soaps, detergents, shampoos, cosmetic creams, and lotions, all of which may show minimal effects on the skin but still be irritating if accidentally introduced into the eye. Literally thousands of products must be tested annually. For the sake of occupational, bystander, and consumer safety, these products must still be subjected to an eye irritation test.

The basic ocular irritation test in the rabbit will be described in detail in another section, but it is important to point out that the number of test animals can be reduced from the usual six at each exposure level to two or, at most, three animals per dose without sacrificing much accuracy. Many test series have shown 88–91% accuracy with two animals per treatment group. The agent, instilled in the pouch formed by the lower eyelid, is held in place for 1 s and then released. The treated eye is not washed, allowing the animal's own tear secretions to flush out the material. The untreated eye serves as a control. Both eyes are examined at 1, 24, 48, and 72 h after treatment, the irritation (or damage) to the cornea, the conjunctiva, and the iris being scored numerically in a subjective manner. The test is open in that the experiment can be terminated at 72 h if there is no evidence of irritation, but observed effects can be assessed for a longer time period.

In Vitro Irritation – Eye

Considerable effort has been made by industry, national and international regulatory bodies to replace the eye irritancy test with suitable *in vitro* assays for such toxicity endpoints as cytotoxicity, corneal opacity, and inflammation, replacing the subjective nature of the assessment with objective and quantitative measurements (**Table 5**). What may evolve from the myriad of test tube and cell culture assay systems and *in vitro*, isolated eye or corneal test systems undergoing development and validation at the present time is one or more battery of test, none of them giving a complete answer to the question, but each contributing some quantifiable information for a selected endpoint. These test batteries will be used as screening devices to identify the strong-to-moderate irritants and the nonirritants, leaving those agents showing suspicious or equivocal results to be tested in animals. These *in vitro* test systems will aid significantly in reducing the number of animals subjected to the eye test, but they will never totally replace it.

None of the *in vitro* alternative 'eye' tests has proven applicable as a valid replacement for the Draize eye irritation test or has been acceptable for regulatory purposes (**Table 5**), though some are considered either reliable or reproducible. The most frequently used test has been the *ex vivo* bovine cornea opacity and permeability assay. The newer human corneal equivalents system, an *in vitro* culture of immortalized human corneal cells that develops into

Table 5 *In vitro* methods for testing eye toxicity

Cytotoxicity
Immortalized mammalian cell lines such as HeLa, V79, human keratinocytes and mouse fibroblasts and canine kidney cells to study
Dye uptake/exclusion – viable cells
Dye penetration – cell integrity
Dye penetration – membrane damage
Opacity
Eyetex assay: formation of high-molecular-weight protein aggregates causing reduced light transmission
Isolated bovine cornea and permeability (BOCP): prepared in a two-compartment chamber; chemical-induced damage causing changes in light transmission through the cornea; e.g.,
– an increase signifying corneal cell loss
– a decrease indicating opacity
Fluorescein dye uptake assessing cell damage
Inflammation
Bovine corneal cup method: inflammatory response releasing chemotactic factors and then reacted with neutrophils
Bovine corneal cup assay: inflammatory response releasing specific mediators (histamine, serotonin, prostaglandins, leukotrienes, thromboxanes) that can be collected in the bath medium and quantitated by chemical assay
Rat vaginal tissue assay: similar to bovine corneal cup assay with release of specific mediators
Fertile chicken egg chorioallantoic membrane (CAM) assay: scoring for vascular changes in the membrane blood vessels with fluorescein dye as well necrotic damage

all three elements of the cornea (stratified epithelium, stroma with keratinocytes, endothelial cell layer), may prove exceedingly useful as it mimics key physical, morphological, and physiological properties of the cornea.

Skin Sensitization Studies

Dermal reactions are seen whereby exposure to a certain chemical causes little effect following initial contact with it but, with repeated (daily, weekly, or even once a month) exposure of the skin, an effect, usually an erythema or red spot, is seen that occurs earlier in time, is more severe, and persists for a longer duration. Subsequent exposures, even though weeks or years apart, result in what appears to be an allergy-like, delayed reaction at the site of exposure or even on parts of the body where no exposure has occurred. The pattern of development of this skin condition, frequently found in the workplace, is suggestive of an allergy.

It is known that certain chemicals, upon penetrating the skin, act as antigens, reacting with immature, dermal, dendritic cells called 'Langerhans cells', which process the antigen while migrating to the drainage lymph nodes where they interact with T-cells to stimulate lymphocyte proliferation. The new lymphocytes are 'primed' effector cells capable of recognizing this new antigen (or allergen). With time, the entire immune system becomes 'alerted' to this antigen, the antibodies responding to its presence even at a much later date to cause a contact hypersensitive reaction or allergic contact dermatitis.

While there are a number of animal models for dermal sensitization studies, all based on the Draize test, the two most frequently accepted and used by regulatory agencies are the guinea pig maximization test (GPMT) and the Buehler assay (BA). In both, repeated, daily low doses of the test agent are injected intradermally or applied topically on closely shaved skin over a 14 day period (induction phase). This treatment period encourages the development of an immunological response as described above. Following a suitable 10–14 day resting period, a challenge dose, usually a lower concentration than was used as a sensitizing dose, is applied to a fresh, untreated site (elicitation phase). The severity of the responses of the animals (erythema, edema) is scored as described earlier over a period of 24, 48, and 72 h after the challenge dose. A greater irritation (edema, erythema) after the challenge dose is indicative of chemical sensitization. The GPMT and BA differ in that Freund's complete adjuvant is used in the former, and the latter test requires 21 days of sensitization exposure, the challenge dose being administered on day 28.

With time (28 days), the number of animals required (20 guinea pigs), costs, plus discomfort and/or pain to the animals, more rapid tests using fewer animals have been sought to screen chemical antigens. The mouse local lymph node assay requires that the test agent be applied for three consecutive days to the dorsal portion of the ears. On day 6, a radiolabeled compound (^{3}H-methylthymidine, ^{125}I-iododeoxyuridine) is injected in the tail vein and, 5 h later, the draining lymph node of each ear is excised, macerated, and incorporation of the radiolabel into proliferating lymphocytes is measured by β-scintillation or gamma counting for comparison with controls. A second test, the mouse ear swelling test, is conducted by application of the suspected antigen to the abdomen or back followed, in a few days, by a challenge dose applied to the ears. The endpoint analysis is the measurement of edema, the thickness of the swollen ears being assessed with calipers.

Photoallergic and Phototoxic Reactions

These skin conditions, found in the workplace and in some cases of therapeutic treatment, involve the interaction of certain wavelengths (275–325 nm) of ultraviolet (UV) light that can penetrate skin to the

depth of the subdermal blood capillaries and a host of drugs (salicylates, sulfonamides, tetracyclines, thiazides, phenothiazines, chlordiazepoxide, cyclamates, hexachlorophene, griseofulvin) and chemicals (coal tar derivatives, dyes, etc.), resulting in the formation of highly reactive intermediates. In the phototoxic situation, current theories suggest that, in the presence of light of suitable wavelength (UV < 320 nm), the chemical molecules are converted into reactive intermediates that can cause direct local cellular toxicity displayed as delayed erythema and hyperpigmentation (urticaria, rash), followed by a desquamation (shedding or scaling) of the skin (eczema). With photoallergic skin reactions, the light-induced activation of the chemical results in the strong binding of some reactive intermediate to cellular and blood plasma proteins to produce antigens that will stimulate antibody formation and recognition by the complete immune system.

The guinea pig or rabbit are species of choice for studying UV light induced chemical toxicity in the skin. In most cases, small amounts of the test agent will be administered orally or by intravenous injection for 10–14 days. Following a resting period of 14–21 days, the animals will receive the same dosage of test agent via the same route of administration, with exposure to light of an appropriate wavelength on an area of closely shaved skin, scoring the edema and erythema by the subjective numerical system described above. Such animal studies are complicated by the necessity of finding the correct wavelength of UV light to activate the particular chemical being tested, the narrower the band on either side of the specific wavelength, the more intense the biological effect that will be seen. More frequently, one sees screening tests for photoallergy and phototoxicity being included in toxicity data submissions since, as the test systems and diagnostic techniques improve, more of these toxicities are being detected in the workplace, the home, and in patients receiving certain medications.

A number of *in vitro* test systems have undergone assessment for detecting phototoxicity. None, other than the 3T3 mouse fibroblast – neutral red dye uptake (NRU) – have yielded reproducible results correlating well with the *in vivo* data. Having been validated, the 3T3NRU method is ready for regulatory acceptance. However, only the European Community has proposed that this test become the standard method for testing UV light absorbing cosmetic ingredients for phototoxic potential.

See also: Eye Irritancy Testing; *In Vitro* Test; *In Vivo* Test; LD_{50}/LC_{50} (Lethal Dosage 50/Lethal Concentration 50); Photoallergens; Skin; Toxicity, Chronic.

Further Reading

Bruner LH (1992) Ocular irritation. In: Frazier JM (ed.) *In Vitro Toxicity Testing*, pp. 149–190. New York, NY: Dekker.

Chan P-K and Hayes AW (1994) Acute toxicity and eye irritancy. In: Hayes AW (ed.) *Principles and Methods of Toxicology*, pp. 579–647. New York, NY: Raven Press.

DeLeo VA (1992) Cutaneous irritancy. In: Frazier JM (ed.) *In Vitro Toxicity Testing*, pp. 191–203. New York, NY: Dekker.

Ecobichon DJ (1997) *The Basis of Toxicity Testing*, 2nd edn., ch. 3, pp. 43–86. Boca Raton, FL: CRC Press.

Gordon VC, Harvell J, Bason M, and Maibach HI (1994) *In vitro* methods to predict dermal toxicity. In: Gad SC (ed.) *In vitro Toxicology*, ch. 4, pp. 47–55. New York, NY: Raven Press.

Patrick E and Maibach HI (1994) Dermatotoxicology. In: Hayes AW (ed.) *Principles and Methods of Toxicology*, pp. 767–803. New York, NY: Raven Press.

Salem H and Katz SA (eds.) (2003) *Alternative Toxicological Methods*. Boca Raton, FL: CRC Press.

Sina JF and Gautheron PD (1994) Ocular toxicity assessment *in vitro*. In: Gad SC (ed.) *In Vitro Toxicology*, pp. 21–46. New York, NY: Raven Press.

Toxicity, Chronic

Donald J Ecobichon

Chronic toxicity studies may be defined as those involving the characterization of adverse health effects following the long-term, repeated administration of a test substance over a significant portion of the lifespan of the test animal species. In general, the term usually denotes a study conducted for longer than 3 months. However, depending upon the test species being used, a 24–72 h exposure period could represent a chronic study for aquatic insects between hatching and flying, a span of some months for birds, etc., or a number of years if dogs or monkeys were to be used. Originally, in regulatory terms, a chronic mammalian study signified a duration of 2 years, ~70–80% of the lifespan of a laboratory rodent. However, the length of chronic studies has been shortened in the past decade and currently stands at 6 months duration. This reduction was based partly on results from 286 repeated dose toxicity studies in

which new findings were noted after 6 months for only seven compounds. All significant findings were detected within 6 months for 91% of studies in rats, 98% of those in dogs, and 87% of investigations using monkeys. To accommodate for animals with longer lifespans or the shortening of the study duration, the dosage regimen is usually adjusted upward so that the level obtained in a lifetime will be acquired in the shorter time interval. There are, of course, major problems inherent in overloading the animals' physiological capacity to distribute, biotransform, and excrete the excessive amounts administered. These difficulties lead to interference in function and to secondary toxic effects seen in other organs.

In chronic studies, it is essential to distinguish between a study defining the shape and nature of the dose–effect relationship for some or any toxicological end point, and one in which the primary objective is to evaluate the presence or absence of a particular toxicological effect, for example, neurotoxicity or carcinogenesis.

Experimental Design

With the longer duration of chronic studies and the labor-intensive nature of the investigations involving the employment of a number of individuals to care for the animals, to obtain samples of biological fluids (blood, urine), to carry out various analyses (hematology, blood biochemistry, urinalysis, quantitation of tissue residues of test agent, etc.) and to prepare and examine histological slides of various body tissues, careful attention must be paid to the design of the study. Such investigations can become very expensive particularly if they have to be repeated because of some oversight, a mistake made, or the appearance of unexpected toxicity. It is important to develop an experimental protocol based on the following questions:

1. How many animals will be acquired?
2. How many dosage levels should be used?
3. When does the 'lesion' or toxicity begin?
4. How rapidly does the toxicity progress toward signs and symptoms?
5. Does the toxicity disappear (rapidly, slowly or never) when exposure is stopped?
6. How long should the study be conducted?
7. How can the main 'theme' of the study be retained (or regained) when other, unexpected toxicity is observed, including excessive mortality within a single treatment group, etc.?

A piece of paper and a pencil are the most valuable tools, at this stage of the study, to develop responses to the question 'what if' this or that might happen during the investigation and attempting to anticipate what might happen with repeated administration of the agent. Study designs should be as open-ended as possible, retaining flexibility to react to the unforeseen, unpredicted events, as well as to those that are anticipated. An example of an experimental protocol is shown in **Figure 1**.

All too frequently, chronic studies are carried out according to the guidelines of national or international regulatory agencies rather than according to good scientific principles. The regulatory guidelines are only there to guarantee a minimum of requirements, information, results, etc., standardized or harmonized within and between national and international governmental bodies.

There may be scientific justification in carrying out a study in a particular manner not commonly ascribed to by such agencies. Few agencies would react

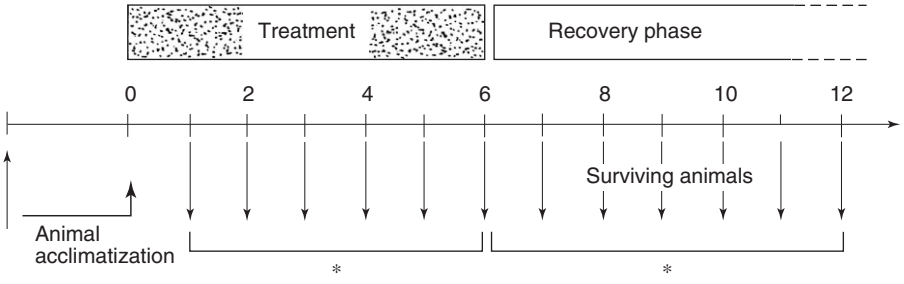


Figure 1 The design of a chronic (6 months, 180 days) study, the planning chart enabling the investigator to determine the total number of animals required based on the number of dosage levels and the number of treated animals required for euthanasia at each selected time interval (30 days). Periodic selection of representative subgroups of each population (controls, low, intermediate, and high levels of test agent) would permit both a dosage- and time-related study of the development of toxicant-related lesions as well as changes in physiological and/or biochemical tests of organ and tissue function/injury. Included in the design is a post-treatment recovery phase open to a further 6 months to assess the permanence or reversibility of the toxicant effects. *Animals (representative subgroups) killed at predetermined time intervals for physiological, biochemical, and morphological study.

unfavorably to a design defended by valid scientific principles.

The most difficult task in developing the protocol for a chronic toxicity study is the selection of an appropriate range of dosages to be used, based on limited knowledge about the effects of long-term exposure of an animal model to the test agent. Some prior knowledge of the shape of the dose–effect relationship is required, most frequently obtained from subchronic studies conducted beforehand. The objective is to use dosages of the test agent, administered by inhalation, in the diet, in drinking water, or by oral gavage, that will cause adverse health effects within the study period but will not cause excessive mortality. The maximum tolerated dose (MTD) is frequently used as the highest dose. This exposure level is being defined as the highest dose that causes no more than a 10% decrease in body weight, and does not produce mortality, clinical signs of toxicity or pathologic lesions that would be predicted to shorten the animal's natural lifespan. Usually, two lower levels, an intermediate (MTD/4) and a low (MTD/8) level will be selected in anticipation of observing a gradation in both appearance and severity of effect(s). Suitable numbers of control, untreated animals must be carried throughout the study, resulting in a four-dose design.

The MTD has caused considerable controversy when used in chronic toxicity studies. Many scientists believe that the highest dose should be above the MTD to be certain of eliciting some quantifiable deleterious effect(s), for example, to demonstrate that the model 'works'. Other scientists feel just as strongly that the highest dose should be lower than the MTD. In these scenarios, either a dose-related increase in adverse health effects or little or no toxicity may be detected independent of minimal body weight changes. The latter scenario poses a number of problems in interpreting the results for regulatory purposes. In other study designs, the lower dose levels may be fractions of the selected highest dose, either equally spaced as is shown in **Figure 2** (50% and 25%, respectively, of the highest dose chosen) or unequally spaced (20% and 1.0% of the highest dose chosen).

A basic principle of toxicology is that there is a correlation between biological effects and the level(s) of exposure, a dose–effect relationship. With a three-dose design (0, X/2, X), straight line relationships can always be determined. However, this may not reflect the true situation whereas, with a four-dose design, the usual curvilinear (concave or convex) relationship will be seen. Prior to embarking on the chronic study, an initial trial period of 2 or 3 weeks duration should be carried out at the selected dosage range, even to the point of conducting dose-dependent kinetic and

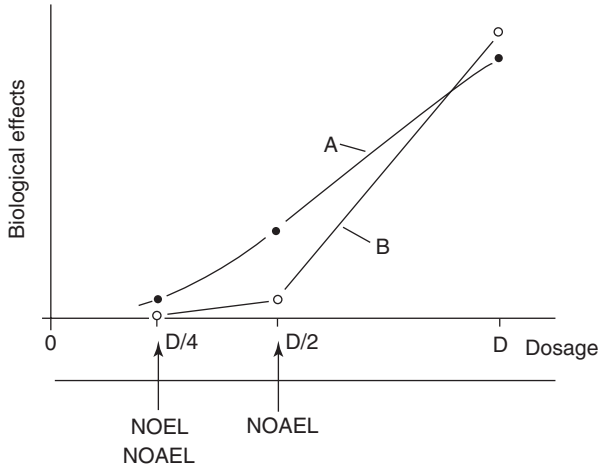


Figure 2 An experimental design showing unequally spaced dosages and theoretical results used for extrapolation to estimate the no-observed-adverse-effect level (NOAEL) or the no-observed-effect level (NOEL). In curve A, some slight degree of toxicity was observed at the lowest dose administered, permitting only the estimation of an NOAEL. In curve B, no toxicity was observed at the lowest dose, and slight toxicity at the intermediate dose, permitting the estimation of a NOEL and a NOAEL.

tissue distribution studies on a few animals at each treatment level in order to assess whether or not the test animals will tolerate the dosages selected. Adjustments to the preselected dosages can be made at this time without compromising the remainder of the study. Frequently, some downward adjustment of the highest dose may be necessary when excessive toxicity is observed.

The duration of the study may be dictated by guidelines from certain national or international regulatory bodies. As was indicated earlier, formerly, chronic studies were of 2 years duration. The exorbitant costs involved plus the concept that the same toxicity could be detected and quantitated in a shorter period of time by giving higher dosages resulted in a reduction in the duration to a 6 month time period. This is still a contentious issue among toxicologists, many maintaining that 6 months is only a fraction (20%) of the lifespan of a rodent and that toxicity may not appear until the animal is older than 12 months, when geriatric dysfunction begins to occur.

In earlier chronic studies, the animals were allowed to proceed until obvious toxicity was seen or the animals became moribund, these animals being killed in a humane manner for study. However, by that time, the toxicity was well advanced and, of course, the question of when the toxicity began or subtle changes in organ function occurred, the usual signals of impending toxicity could not be answered. Such questions can only be answered by the periodic (every 30 days) selection of representative subgroups from each treatment group and from control animals

for euthanasia and an in-depth study of biochemical, physiological, and morphological indices of toxicity (**Figure 1**).

Such an approach does not preclude the periodic sampling of blood from animals or the collection of urine and feces for analysis using techniques that are not life threatening. Such a protocol will permit the investigator to identify pretoxic changes in organ function and morphology as well as to determine when, in a dose-dependent manner, toxicity appears initially, both as obvious signs and symptoms and as morphological changes.

How does one know whether or not toxicity persists following termination of the exposure or that the signs and symptoms disappear slowly or quickly? Are there any long-lasting effects? Is the tissue damage reversible or irreversible? To answer these questions, additional animals should be incorporated into the study protocol so that, at the end of the treatment period, there is a reasonable population of animals remaining, sufficient to participate in a recovery phase study. Once again, small representative subgroups from each treatment group and controls will be killed and subjected to detailed study at predetermined time intervals (e.g., at 30, 60, and 90 days).

How many animals will be needed to provide biochemical, physiological, and morphological data for each of the previously mentioned, planned intervals of subgroup selection (during and after treatment), as well as for the unexpected toxicity and mortality that almost certainly will be encountered if the study is being conducted properly? What constitutes a representative subgroup? If one accepts the premise that five animals of each sex, selected at each time interval for euthanasia, are representative of the population being studied, the number of animals required can be calculated quickly. A larger number of controls, for example, 10 animals, would be required at each interval so that variability in the population as a whole can be monitored. Ten to 15 additional animals should be included in each treatment group in anticipation that some mortality may occur during treatment. Thus, for a 6 month chronic toxicity study having three dosage levels plus control animals, with subgroups being killed at 30 day intervals during treatment and at 30 day intervals over a 3 month recovery phase, an investigator might consider a minimum of 150 male and 150 female animals undergoing treatment with 90 control animals of each sex, a total of 480 animals. The number of animals could be reduced by spacing out the time intervals of subgroup selection but perhaps at the risk of missing some subtle change in one or more parameters being assessed, thereby not recognizing the appearance of the toxic effect(s).

A wide range of biochemical and physiological parameters should be monitored throughout the entire study, both during and after treatment (**Figure 3**). The moribund animals or those killed at preselected intervals will undergo an extensive morphological examination, both gross and microscopic, in order to identify possible organs or tissues where the test agent may exert an effect. Changes in body weight, food, and water consumption can provide information concerning the tolerance/aversion of the test animal to the agent in the food or water. A reduction in body weight, particularly if it is dose-related, over the study period is a simple but effective indicator of the animals' well-being; any sharp deviation will alert the investigator to a possible chemical-related event. If the animal does not feel well, it will not eat sufficient food to maintain normal growth and development, this being particularly critical in small rodents that have an elevated basic metabolic rate. Taste aversion to the test agent can be identified quickly by measuring the amount of food or water ingested in a 24 h period. A spectrum of biological markers–general tests of hematology, blood serum biochemistry and urinalysis–should be planned before the experiment is started, picking parameters that, if they are seen to change, will point in a meaningful way to some organ/tissue that may be affected by the test agent. All of these tests should be broad enough in scope to detect the unexpected as well as the anticipated toxicity. During this prolonged treatment period, various noninvasive tests of neurological competence (behavioral, sensory perception, motor function, learning skills) can be conducted in addition to organ (liver, cardiac, pulmonary) function tests that will pose minimal risk to the animals' health. At euthanasia, the animals will be dissected and a wide range of organs will be removed, fixed, sliced, and stained appropriately for light and electron microscopic examination. An attempt will be made to correlate morphological changes or damage with the biochemical and physiological changes observed and quantified.

Carcinogenicity Studies

Chronic studies include one additional end point of toxicity; any carcinogenicity related to exposure to the test agent. Traditionally, and mainly because tumor formation is seen in older animals, carcinogenicity studies in rodents are conducted for an 18 or 24 month period for mice and rats, respectively, separate from the shorter-term, 6 month chronic studies. The dosage range used for carcinogenic assessment is lower than that used for chronic toxicity

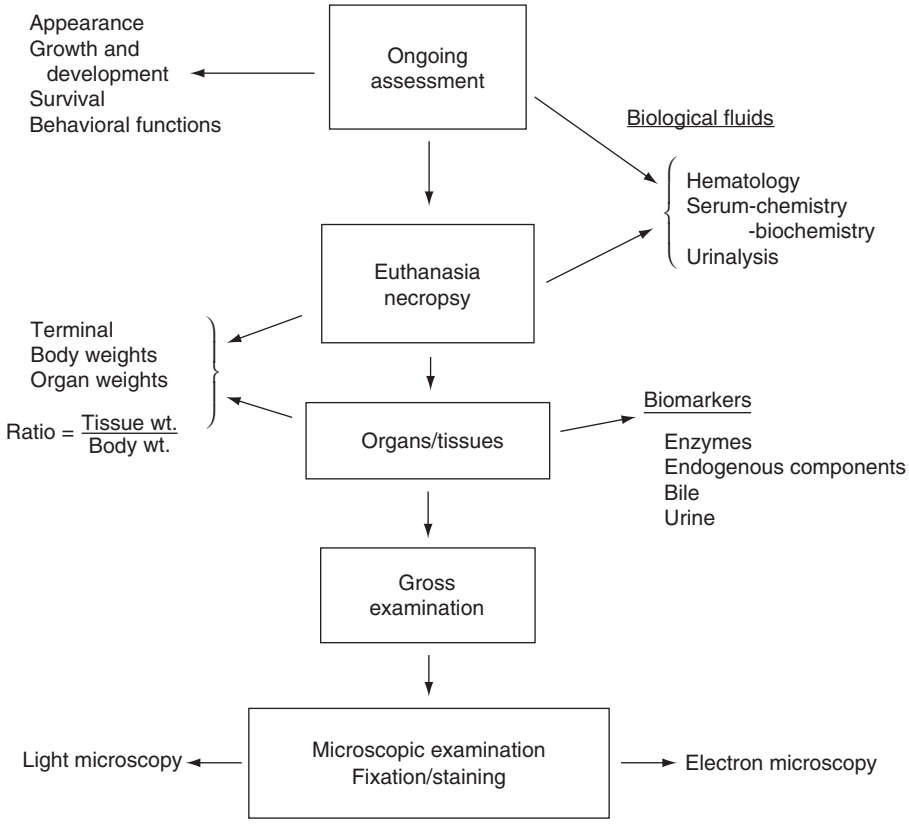


Figure 3 A flow chart depicting the various parameters or end points of toxicity to be monitored during the chronic study, both during and after treatment, as well as those studied following euthanasia and necropsy. The routine, periodic assessment of chosen parameters may detect the onset of impending toxicity, proving invaluable for the detection of developing lesions and as predictors of target-organ toxicity.

because the end point is tumor formation, independent of any other sort of toxicity.

Considerable controversy has arisen among scientists over the selection of the highest dosage to be used in carcinogenic studies. One faction suggests a value known as the MTD–the highest dose of the test agent during a chronic study that can be predicted not to alter the animals' normal longevity from effects other than carcinogenicity. This dosage level is also known as the 'minimally toxic dose'. With this dosage, selected from a chronic study as a reference point, two lower dosage levels, equally or unequally spaced, can be calculated so that, hopefully, the lowest level of exposure that will cause tumor formation can be determined. Another faction claims that doses near the MTD may cause significant cell mortality and/or compensatory changes (mitogenesis) that would make the damaged organ/tissue more susceptible to tumor formation, and that these levels are far higher than the human would encounter. Other investigators recommend that the top dose be some appropriate multiple of the expected human exposure. While this controversy has not been settled, retrospective review of a large number of chemical-induced carcinogenicity studies has revealed that two-thirds of the carcinogens would have been detected even if the estimated MTD had not been included but that, in many studies, some site-specific carcinogenic effects would not have been observed. Among the remaining one-third of the studies, $\sim 80\%$ had elevated rates of site-specific tumors at lower doses as well. Most carcinogenic effects observed at the highest dose were also present at reduced incidences at lower doses (MTD/4, MTD/2), although the results might or might not be statistically significant. The choice of dosages for long-term carcinogenicity studies will remain a contentious issue.

In the future, genetically engineered, transgenic animals may be used in carcinogenicity studies because these strains are more highly susceptible to early induction of cancer within 6–9 months. The 1997 International Conference on Harmonisation agreement gave study sponsors the option to replace one of the two species required for current carcinogenicity assessment with a short- or medium-term alternative model, usually a transgenic mouse. A number of mouse strains, for example, TgAC, TgrasH2, P53 $+/-$, and XPA $-/-$ P53 $+/-$, have

been examined, the data being reviewed recently. The replacement of an 18 month mouse carcinogenicity study with an alternative assay requires scientific justification for the selection of the model. To date, data obtained for TgrasH2 together with P53 +/− appear to provide the best combined coverage for detecting carcinogens. However, these various transgenic models are just beginning the arduous process of experimentation and validation.

Interpretation of Results

The main objective of any chronic study is to supply a database which will provide assurance to public concerns about the safety of chemicals found in the human environment, the sources usually being air, food, and water, and the results of such studies being used in safety evaluation, risk assessment, and risk management decisions. The objective of long-term toxicity testing, usually in rodents, is to assess the potential chronic toxicity of a chemical, including carcinogenicity, effects that would not be evident in subchronic studies. Pertinent to the studies is the development of a dose–effect relationship that ranges from no observed effect through minor changes to overt toxicity and the determination of dosages at which these observations occur. These values will be used by regulatory agencies to determine safe levels of exposure stated as a maximum allowable concentration, recommended maximum levels, reference dose, virtually safe dose, tolerance or acceptable daily intake, etc.

End points of toxicity and the severity of observed adverse health effects obtained from a four-dosage range study may be represented by arbitrarily determined dosage values such as the lowest-observed-adverse-effect level (LOAEL), the no-observed-adverse-effect level (NOAEL), or the no-observed-effect level (NOEL) (**Figure 2**). These values, of course, must be derived at the end of the chronic toxicity study based on the observations. It is unlikely that all three values would be obtained from a study. Usually, one of these indices might be determined with a degree of reliability in the estimated value. Either the NOAEL or NOEL can be used by regulatory bodies to establish reasonable, estimated values for the indices mentioned in the previous paragraph.

See also: Toxicity, Subchronic.

Further Reading

Arnold DL, Blumenthal H, Emmerson JL, and Krewski D (1984) The selection of doses in chronic toxicity/carcinogenicity studies. In: Grice HC (ed.) *Current Issues in Toxicology*, pp. 9–49. New York, NY: Springer.

Ecobichon DJ (1997) *The Basis of Toxicity Testing*, 2nd edn., ch. 4, pp. 87–116. Boca Raton, FL: CRC Press.

Haseman JK and Lockhart A (1994) The relationship between use of the maximum tolerated dose and study sensitivity for detecting rodent carcinogenicity. *Fundamental and Applied Toxicology* 22: 382–391.

Salem H and Katz SA (eds.) (2003) *Alternative Toxicological Methods*. Boca Raton, FL: CRC Press.

Stevens KR and Mylecraine L (1994) Issues in chronic toxicology. In: Hayes AW (ed.) *Principles and Methods of Toxicology*, ch. 18, pp. 673–695. New York, NY: Raven Press.

Stringer C (1992) Safety workshops-ICH. *Regulatory Affairs Journal* 3: 350–356.

Toxicity, Subchronic

Donald J Ecobichon

While acute exposure to high concentrations of chemicals can occur in any environment (the outdoors, the home, the workplace), individuals are more frequently exposed over much longer periods of time to agents at levels lower than those that might prove fatal. Acute exposure studies will not identify those adverse health effects, both immediate and/or delayed, that might arise as a consequence of long-term, lower level exposure. The simulation of such exposure requires the development of more carefully designed experiments in which larger numbers of animals are used, lower levels of the potential toxicant are administered by a suitable route of exposure, over a longer time period and a number of preselected biochemical, physiological, and morphological endpoints of toxicity are monitored and quantified throughout the study period. In general, a subacute or subchronic study is one conducted over a 21–90 day period, using surrogate animal species to mimic conditions anticipated to be found in human exposure.

These long-term studies are designed to examine the nature of the toxic effects from lower dosages at the organ, tissue, and cellular level in order to determine possible mechanisms of toxicant action. The repeated administration/exposure to the agent will permit examination of possible cumulative effects as body burdens of the agent and/or biotransformation products (metabolites) are acquired with time. Subchronic

studies will allow the investigator to ascertain the variation in response(s), by making close observations of a continuum of biological changes and/or unique events occurring over a wide range of dosage levels in both sexes and ages of different animal species. This will permit the identification of the appropriate dosage at which biochemical, physiological, and morphological changes, both macroscopic and microscopic, occur in relation to the level and/or duration of exposure. The last objective of such studies is to be able to predict the long-range adverse health effects in the test animal species, using the results to extrapolate whether or not toxicity might be expressed in the human at some, as yet unknown, level of exposure.

The old adage holds true that 'the more species of animals in which the same biological response(s) to an agent can be produced, the greater is the chance that, at some dosage, the same effect might occur in the human'. Invariably, subchronic studies are conducted in at least two species – one rodent species (with a choice of the mouse, rat, or possibly the hamster) and a nonrodent species (frequently the dog (purebred beagle), the rabbit, or occasionally, a strain of monkey (rhesus or macaque)). With such diverse species being studied, distinct variations in response(s) related to physiological (distribution, storage, excretion) or biochemical (e.g., biotransformation rate, type of metabolites formed) differences should be anticipated, thus, it is hoped, permitting a better appreciation of how the human might respond.

Experimental Design

The design of subchronic studies is extremely important not only because of the longer time period involved, but also because such studies are labor-intensive, involving a number of people in caring for the animals, obtaining blood samples, carrying out analyses on samples (hematology, blood chemistry, urinalysis) or examining morphological specimens (e.g., preparing and staining slides of tissue sections, light and electron microscopic evaluation). Since these studies become very expensive, it is important to set up an experimental design before the studies are begun, asking the following questions:

1. How many animals are required?
2. How many dosage levels should be used?
3. When does the 'lesion' or toxicity begin?
4. How rapidly does the toxicity progress toward signs and symptoms?
5. Does the toxicity disappear (rapidly, slowly, or never) when exposure is stopped?
6. How can the main 'theme' of the study be retained (or required) when other, unexpected toxicity is observed, including excessive mortality within a single treatment group, etc.?
7. How long should the study be conducted?

Any study design should be open-ended, allowing for unforeseen and unpredicted events that frequently appear in long-term studies as well as those events predicted. A piece of paper and a pencil are the most valuable tools at this stage of the design, asking the question 'what if' this or that might happen during the study. A simple experimental design is shown in **Figure 1**.

Having selected the agent for study, the first question is what dosage range will be used, and how many dosage levels will be necessary. Generally chosen from

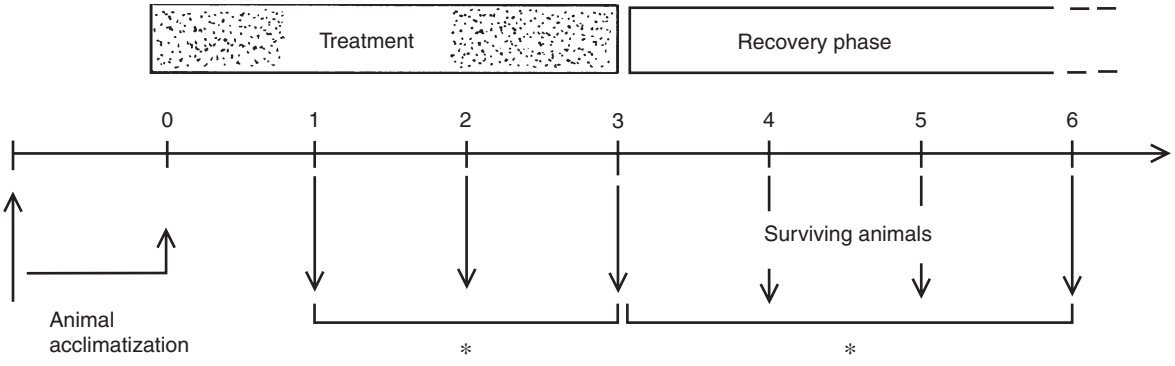


Figure 1 The design of a subchronic (3 month, 90 day) study: the planning chart enables the investigator to determine the total number of animals required based on the number of dosage levels and the number of treated animals required for euthanasia at each selected time interval (30 days). Periodic selection of representative subgroups of each population (controls, low, intermediate, and high levels of test agent) would permit both a dosage- and time-related study of the development of toxicant-related lesions as well as changes in physiological and/or biochemical tests of the organ and tissue function/injury. Included in the design is a post-treatment recovery phase open to a further 90 days to assess the permanence or reversibility of the toxicant effects. *Animals (representative subgroups) killed at predetermined time intervals for physiological, biochemical, and morphological study.

the dosages used in acute toxicity studies, one should always use three dosage levels, a high level guaranteed to elicit toxicity in the animal model and two lower (intermediate and low) dosages in the hope that a gradation in the appearance and severity of toxicity will be observed. Comparable control (untreated) animals must be carried through the study as well. A major objective of the study is to establish a relationship between biological effects and a level of exposure, for example, a dose–effect relationship which may be linear, or more likely, will be curvilinear.

The duration of the study may be dictated by guidelines from specific regulatory agencies. In general, subchronic feeding studies, particularly if the agent may be incorporated into the diet, drinking water, or given by oral gavage, are of 90 days duration. Inhalation or dermal exposure studies may be from 21 to 90 days in duration.

It will be unlikely that the investigator can predict when toxicity will begin to appear based on the acute toxicity results. One can allow the subchronic study to proceed until sick animals are observed before killing them in a humane manner but, by that time, toxicity will be in an advanced state. The question of when toxicity begins to appear can only be answered by the periodic (every 7 or 30 days in 21 or 90 day studies, respectively) selection of representative subgroups from each treatment group for euthanasia and in-depth study of biochemical, physiological, and morphological indices of toxicity. Such a design allows the investigator to identify pretoxic changes in organ function and morphology as well as to determine when, in a dose-dependent manner, toxicity appears.

If exposure is stopped, does toxicity persist or do the signs and symptoms slowly or quickly disappear? Is the tissue damage reversible or irreversible? By incorporating additional animals into the treatment groups at the beginning, there is a good chance that, at the end of the treatment period, there will be sufficient animals surviving to permit a recovery phase to be studied, again, selecting small, representative subgroups for euthanasia and detailed study at predetermined time intervals (30, 60, 90 days).

How many animals are needed to cover all the eventualities mentioned above – three dosage levels, periodic euthanasia of subgroups during treatment and after termination of exposure, possible expected and unexpected toxicity, some mortality among the animals, related or unrelated to treatment, etc.? What constitutes a representative group or subgroup? How many untreated control animals should be included? If one accepts the premise that five animals of each sex, selected at each time interval for euthanasia, are representative of the population under study, then the number of animals required can be calculated quickly with a few (10–15) additional animals being included for 'safety'. By the time the study is under way, the animal cost is the least expensive item in the investigation. Thus, one should not be reticent at including more animals. For a 90 day feeding study, an investigator would conservatively consider 150 male and 150 female rodents to adequately protect the study from the vagaries of Murphy's Law (if anything can happen, it will), including adequate numbers of control and 'spare' animals undergoing treatment.

A wide range of parameters can and should be measured during the entire study. Some, as simple as body weight, growth/development, food and water consumption are noninvasive and pose no risk to the animals. A change in body weight, particularly in small rodents having normally high basal metabolic rates, is a simple yet effective indicator of general well-being. A sharp decrease alerts the investigator to perhaps a chemical-related appetite depression, although the effect may be as simple as an aversion to the taste of the test agent in the diet rather than toxicity. Frequently, one can see a nice gradation in growth curves between the control animals and those in the three treatment groups. A spectrum of biological markers – general tests of hematology, blood serum chemistry, urinalysis – should be planned before the start of the study along with other specific physiological and biochemical markers. These parameters should be based on anticipated target organ toxicity but, of course, remaining broad enough in scope to detect the unexpected toxicity as well. Such a scheme is shown in **Figure 2**. During the treatment period, various noninvasive tests of neurological competence (behavior, sensory perception, motor function, learning skills) can be conducted along with liver, kidney, cardiac, and pulmonary function tests that pose minimal risk to the survival of the test animal.

Information Management

As you can appreciate, with over 300 animals in a subchronic study being monitored periodically for any adverse health effect, one can accumulate literally thousands of continuous and terminal data points on biochemical, physiological, and morphological parameters which become a significant burden to the investigator and the staff. Management of this data is crucial. Most of the data flow control has become automated on computer.

Good Laboratory Practices (GLP) regulations insist on the appropriate management of animal data so that quality assurance/quality control (QA/QC) personnel can, at any time, select an animal number

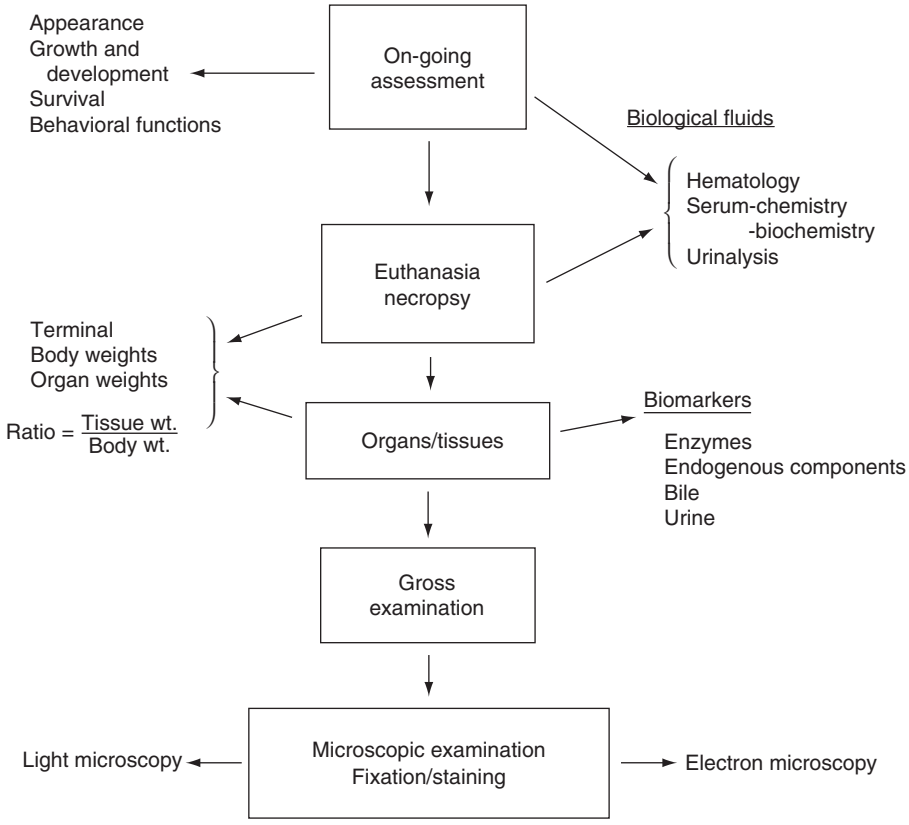


Figure 2 A flow chart depicting the various parameters or endpoints of toxicity to be monitored during the subchronic study, both during and after treatment, as well as those studied following euthanasia and necropsy. The routine, periodic assessment of chosen parameters may detect the onset of impending toxicity, proving invaluable for the detection of developing lesions and as predictors of target-organ toxicity.

and track it and its data throughout the study until it either dies or is killed. The laboratory carrying out the study will have a QA/QC staff while the company for whom the study is being done will have its QA/QC personnel check the study in progress and at the completion of the report before it is submitted to another QA/QC evaluation carried out by the regulatory agency.

Interpretation of Results

Depending upon the eventual use of the chemical, the results of a well designed and conducted subchronic study may provide all of the information required for the agent, for example, for a drug that will be used for only a limited time period of treatment. However, if exposure is anticipated to be longer or that the individual may be exposed to the agent (pesticides, food additives, industrial chemicals, etc.) for a lifetime, the results of the subchronic study may only justify making a decision on the need for additional or more extended and perhaps specific studies to determine more clearly the toxicological profile of the agent. In the time period of the study, some preliminary evidence may have been obtained to show that, if treatment had persisted for a longer time at the same or at lower dosages, some specific target organ toxicity might have become manifest. In such a situation, the subchronic study results have at least established a dosage range for administration over a significant proportion of the animal species lifespan (e.g., the characteristic chronic toxicity study).

Changes observed in body weight gain, organ weight, hematological and biochemical data, organ function, etc. should be subjected to trend analysis with those parameters measured in control animals. These findings should be correlated with the pathological and histopathological data. Since a basic tenet of toxicology is that there should be some correlation between observed biological effect(s) and the concentrations of test chemical, much effort is expended in establishing a trend in a dose–effect relationship for each parameter being measured.

Subchronic studies are of limited value for predicting the toxic effects of lifespan exposure. The nature and degree of toxicity vary, the sensitivity performance, and metabolic capability of organs/tissues change with aging and the spontaneous

occurrence of other diseases. No predictions can be made from subchronic exposure concerning the mutagenic, teratogenic, or carcinogenic potential of the test agent, and any effects on reproduction can only be related to primary effects on the testes and ovaries.

Given the known uncertainties which arise from qualitative and quantitative differences as well as similarities in toxicological effects observed in animals and man, interpretation of the dose-related effects must be done cautiously. If a no-observed-adverse-effect level of dosage can be determined from the experiment, this may be used to establish values for acceptable daily intake or a reference dose for setting tolerances of additives in food, for residue levels of unintentional contaminants, or for acceptable levels of exposure (threshold limit values, maximum acceptable concentrations) to chemicals in the workplace. However, beyond these indices, extrapolation to what might occur during a lifetime of exposure is extremely risky.

See also: Toxicity, Chronic.

Further Reading

Ecobichon DJ (1992) *The Basis of Toxicity Testing*, ch. 4, pp. 61–82. Boca Raton, FL: CRC Press, Inc.

Wilson NH and Hayes JR (1994) Short-term repeated dosing and subchronic toxicity studies. In: Hayes AW (ed.) *Principles and Methods of Toxicology*, ch. 17, pp. 649–672. New York, NY: Raven Press.

Toxicokinetics *See* Pharmacokinetics/Toxicokinetics.

Toxicology

Gabriel L Plaa

All chemical or physical agents can affect living organisms. Some of these effects can be beneficial while others can be adverse to the well-being of the organism. 'Toxicology', in its broadest sense, is the science that concerns itself with the adverse effects of chemical or physical agents on living organisms. There are two important elements in the definition of toxicology: the first is the aggressor substance (the chemical or physical agent), and the second is the target (the living organism affected). Since the target is a living organism, toxicology, as a consequence, is a biological science. The science of toxicology, however, draws heavily from the knowledge acquired in other sciences: chemistry, physics, physiology, biochemistry, pathology, pharmacology, immunology, genetics, molecular biology, mathematics, statistics, etc. Although the aggressor substance may be a physical agent (e.g., an electromagnetic field), the major concern of modern toxicology deals with chemicals (medicinal products, drugs of abuse, occupational chemicals, pesticides, industrial effluents, hazardous wastes, etc.). Biological targets include humans as well as other species. In our egocentric fashion, we humans place much emphasis on ourselves as potential biological targets, but we must not forget that chemicals can have an important impact on other biological targets. While humans are considered a target of particular interest, other terrestrial and aquatic species are of considerable importance as potential biological targets. Toxicological problems worthy of societal concern are not limited only to those that affect human beings.

Toxicology is a very broad science. Toxicity studies are essential to the safe use of chemical substances in various aspects of our lives. In medicine, one must know the adverse effects of therapeutic agents as well as their beneficial utility. In the workplace, chemicals used as solvents, components of a process, or as intermediates must be handled safely. In agriculture, the safe use of pesticides, feed additives, or growth regulators as well as the problem of food residues are major considerations. Industrial effluents and their impact on the environment are societal preoccupations. Identification of chemically induced diseases and their prevention is an important public health undertaking. Regulatory controls essential for the safe use of chemicals require a broad and detailed understanding of toxicology.

Subdivisions of Toxicology

There are a number of subdivisions to the science of toxicology, and these vary according to the particular interests of the toxicologist concerned. No single classification system of categorization is entirely satisfactory. About 35 years ago, however, T.A. Loomis divided the science of toxicology into three

major subdivisions: environmental, economic, and forensic. These subdivisions were in large part based on how humans would come in contact with potentially harmful chemicals. Generally, the scheme is still valid today.

Environmental toxicology, according to Loomis, is concerned primarily with the harmful effects of chemicals that are encountered by humans because of the presence of chemicals in the atmosphere, or in the occupational setting, or through recreational activities, or by ingestion as food residues. Environmental toxicology is the branch of toxicology that deals with the incidental exposure to chemicals that appear basically as contaminants of air, food, or water. This characterization of environmental toxicology is still appropriate today, although toxicologists are also interested in the impact of chemical substances on species native to various parts of the environment.

Economic toxicology, according to Loomis, deals with the potentially harmful effects of chemicals that are intentionally administered to living organisms for the purpose of achieving a specific beneficial effect. Here we find drugs developed for medicinal therapeutic purposes in human or veterinary medicine, chemicals developed for use as pesticides or insecticides, or substances designed as food additives. The term 'economic' used by Loomis stems from the work of Adrian Albert, who coined the phrase 'selective toxicity' to describe the use of chemicals by one species (humans) to eliminate an undesirable species, such as insects. In this context, humans were called the 'economic species' and the insect the 'uneconomic species'. Regardless of the terminology, economic as used by Loomis denotes that the potentially toxic chemical in question is being developed for some specific purpose, and we are interested in the undesirable effects that may accompany the beneficial effect.

Loomis categorizes forensic toxicology as the subdivision of toxicology that deals with the medical and legal aspects of the harmful effects of chemicals on humans. Therefore, here one finds those aspects of toxicology related to the diagnosis and treatment of chemical intoxications. The legal aspects of the subdivision pertain to cause-and-effect relationships between exposure to an aggressor agent and the adverse consequences observed in humans. We are very familiar with certain aspects of forensic toxicology, like the operation of a motor vehicle while under the influence of alcohol, or the use of performance enhancing drugs in sporting events. The detection and quantification of chemicals in biological fluids or tissues is a very important phase of forensic toxicology.

Scope and Activities of Toxicology

While Loomis' three-category scheme covers the broad use of applied toxicological information, it does little to denote the wide scope that represents the activities of toxicologists. In 1987, E. Hodgson and P.E. Levi formulated another set of characteristics in an attempt to cover the scope of the many activities encompassed by the discipline. They chose to organize toxicology into five broad categories, each with a number of subcategories:

A. Mechanisms of toxic action – all events leading to adverse effects at the level of the organ, cell, or molecular function

1. biochemical toxicology (enzymes, receptors, molecular events, etc.);
2. behavioral toxicology (peripheral and central nervous system, endocrine system etc.);
3. nutritional toxicology (influence of diet on the expression of toxicity);
4. carcinogenesis (chemical and biochemical events that lead to cancer);
5. teratogenesis (effects on embryonic and fetal developmental processes);
6. mutagenesis (effects on the genetic material and inheritance of these defects); and
7. organ toxicity (effects at the level of organ function).

B. Measurement of toxicants and toxicity – these include the use of analytical chemistry, bioassays, and applied mathematics

1. analytical toxicology (identification and assay of toxic chemicals in biological material);
2. toxicity testing (use of living systems to estimate toxic effects);
3. toxicological pathology (branch of pathology dealing with the effects of toxic substances);
4. structure–activity study (relationship between chemical structure and toxicity);
5. biomathematics and statistics (determination of significance, risk estimates); and
6. epidemiology (occurrence of toxicity).

C. Applied Toxicology – Applications as they occur in the field

1. clinical toxicology (diagnosis and treatment of human poisoning);
2. veterinary toxicology (diagnosis and treatment of poisoning of animals);
3. forensic toxicology (medicolegal aspects of clinical poisonings, including analytical detection);

4. environmental toxicology (movement of toxicants in the environment and food chain, effects on various species); and
5. industrial toxicology (deals with the occupational environment).

D. Chemical use classes – includes the toxicological aspects of the development of new chemicals for commercial use

1. agricultural chemicals (pesticides, targeted species);
2. clinical drugs (adverse effects of pharmaceutical agents);
3. drugs of abuse (chemicals taken for psychological effects that cause dependency and toxicity);
4. food additives (food preservatives, facilitate food processing);
5. industrial chemicals (solvents, degreasers, intermediates, etc.);
6. naturally occurring substances (includes phytotoxins, mycotoxins, and inorganic minerals); and
7. combustion products (generated from fuels and other industrial chemicals).

E. Regulatory toxicology – concerned with laws and regulations and their enforcement

1. legal aspects (government agencies); and
2. risk assessment (definition of risk, risk–benefit considerations).

Early History of Toxicology

Obviously, the earliest humans gathered toxicological information through experience, and trial-and-error. Animal venoms and plant poisons eventually were used for killing other animals or humans. Over time, an art of poisoning developed, including the training of professionals. (Much of what follows is based on a chapter written by J.F. Borzelleca in the third edition of A.W. Hayes' *Principles and Methods of Toxicology*, 1994.) The interested reader is encouraged to consult this comprehensive work for more detail.

Poisons, antidotes, and case histories are found in early Egyptian writings (Ebers Papyrus, ~1500 BC); toxic agents were used by Egyptians in the administration of justice. Additional lists of poisons and antidotes appear in early Chinese (Shen Nung, ~2700 BC) and Hindu (the Riga-Veda, ~1500 BC) writings. The contributions of Hippocrates (~400 BC) and Diocles (~350 BC) in ancient Greece described rational methods for the treatment of poisoning. Theophrastus (~350 BC) is said to be the first to recognize the adulteration of food. The Roman physician Celsus' treatise (~40 BC), *De Medicina*, continues Hippocratic teaching and contains a separate section on poisons and antidotes; *De Medicina* gained worldwide importance, since it was the first medical work published (~1500 AD) after the invention of the printing press.

Important writings came from Avicenna of Persia (~1000) and Maimonides, court physician to Saladin and rabbi of Cairo (~200). These texts exerted an enormous influence for nearly 500 years. Finally, Paracelsus (~1525), a Swiss physician, made the important declaration that "all things are poisons...solely the dose determines that a thing is not a poison." This concept is the cornerstone of modern toxicology.

Toxicology was brought to other areas of human endeavor. An Italian physician, Ramazzini (~1700), is credited with bringing toxicology to the workplace with his works on health problems related to the occupational setting. He is considered the founder of occupational medicine. The application of analytical chemistry to food and drug safety was introduced by the works of Accum (~1800) in *A Treatise on Adulterations to Food*, and *Culinary Poisons*. Finally, Orfila's (~1815) classic work on toxicology combined forensic and clinical toxicology with analytical chemistry; it is said to be the first book devoted entirely to toxicology. The father of experimental physiology, C. Bernard, used toxic chemicals (~1850) as laboratory tools to understand mechanisms involved in normal physiological processes. As such, he contributed greatly to the understanding of mechanisms of action of toxic substances.

Modern toxicology, which is over 100 years old, is both an experimental and an applied science. It is a predictive science that has evolved remarkably since the time of Orfila, particularly in the last 60 years. Recent additions to the discipline include safety evaluation and risk assessment. Toxicology will be influenced greatly by expanding knowledge in immunology and genetics in years to come.

See also: Behavioral Toxicology; Developmental Toxicology; Ecotoxicology; Environmental Toxicology; Food Additives; Forensic Toxicology; Information Resources in Toxicology; Molecular Toxicology–Recombinant DNA Technology; Occupational Toxicology; Radiation Toxicology, Ionizing and Nonionizing; Toxicology, Education and Careers; Toxicology, History of.

Further Reading

Albert A (1979) *Selective Toxicity*, 6th edn. New York: Wiley.
Borzelleca JF (1994) History of toxicology. In: Hayes AW (ed.) *Principles and Methods of Toxicology*, 3rd edn., pp. 1–17. New York: Raven Press.
Gallo MA and Doull J (1991) History and scope of toxicology. In: Amdur MO, Doull J and Klaassen CD (eds.)

Casarett and Doull's Toxicology, 4th edn., pp. 3–11. New York: Pergamon.
Hodgson E and Levi PE (1997) *A Textbook of Modern Toxicology*, 2nd edn. New York: Elsevier.
Loomis TA (1968) *Essentials of Toxicology*, 1st edn. Philadelphia, PA: Lea and Febiger.
Lu FC (1996) *Basic Toxicology Fundamentals, Target-Organs, and Risk Assessment,*, 3rd edn. Washington, DC: Taylor and Francis.
Ottoboni MA (1997) *The Dose Makes the Poison*, 2nd edn. Berkeley, CA: Wiley.

Toxicology in the Arts, Culture, and Imagination

Philip Wexler

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Toxicology's merit as an interdisciplinary science with a growing research base and extensive technical literature is undisputed. It has a long history in practice as well. Early man sought to expose himself to food and other substances, both natural and concocted, which would enhance his well-being. Would-be suicide victims aside, he sought to avoid those products which were harmful to him. Dangerous substances did have their uses, though, against enemies and crop pests, or for hunting. Toxicology also has a long-established legal and regulatory framework, aiming at the sensible management and use of chemicals to benefit society while minimizing their harm.

Less appreciated, though, is the extensive role toxicology has played in our collective imagination. There has always been, and always will be, a fascination with the actions of poisons and how people use them, usually for nefarious ends. Poisoning has been seen as tragic, humorous, subversive, and edgy, given one's perspective. The criminal mind has served as a fertile ground for artists and writers throughout the ages. Real and imagined stories about poisons keep us transfixed over the campfire, make us shudder as we read, and chill our bones as we watch movies and theatre. The language of toxicology serves as a rich source of metaphor in our daily lives. Outside the laboratory, away from the regulatory and judicial systems, or the research library, toxicology continues to infiltrate our thoughts.

Literature, Poetry, Drama, and Legend

Because certain stories, real or imagined, have been expressed in a multitude of genres, there is some arbitrariness in this article in classifying a story as literature or art or music if it fits into two or more categories. One will discover, for example, that many of the stories taken up by representational art, as discussed in the next section, could just as well have been treated in this section as part of the verbal domain.

Though historically a king of Babylonia, Gilgamesh has served as a source of legends. The 2000 BC Sumerian *Epic of Gilgamesh*, for example, refers to scorpions as the guardians of hell:

> Those who guard the gate are
> Poison scorpions
> Who terrorize all, whose spells bring death.

Greek mythology is littered with toxic tales. One of the many examples is Achilles' humiliating death toward the end of the Trojan War. Paris let loose a poison arrow which lodged in his heel, the one vulnerable spot on his body. Poison arrows were used extensively by Odysseus and other warriors in the Homeric cycle and legends penned by other classical Greek authors and playwrights.

The Jewish and Christian bibles are splendid sources of toxicological metaphor and allusion. Witness *Job* 6:

> The arrows of the Almighty are in me,
> my spirit drinks in their poison;
> God's terrors are marshaled against me.

And *Jeremiah* 9:

> Therefore, this is what the LORD Almighty, the God of Israel, says: "See, I will make this people eat bitter food and drink poisoned water."

Here is one of the Lord's admonitions to his people in Deuteronomy: "Lest there should be among you man, or woman, or family, or tribe, whose heart turneth away this day from the LORD our God, to go and serve the gods of these nations; lest there should be among you a root that beareth gall and wormwood." Wormwood oil is a potent poison. Wormwood itself, as well as other herbs, is used to distill absinthe, a potent alcoholic beverage, particularly popular in the nineteenth century.

Early literary adaptations of poisoning imagery, before the advent of synthetic pharmaceuticals and industrial chemicals, revolved around food, drink, and poisonous and venomous animals. Chaucer's *Pardoner's Tale* concerns three drunken men on a quest to destroy Death as revenge for a friend who died of the plague. An old man directs them to an

oak tree where he says they can find Death. Surprisingly, they discover, instead, a treasure. The youngest of the three is sent to town for wine for all of them to celebrate. He returns with the wine, poisoned though, in order to murder his companions and keep the treasure for himself. Before he can accomplish his plot, they stab him and he dies. The remaining two celebrate with the poisoned wine and die as well. "Thus ended been thise homicides two,/ And eek the false empoysoner also." Thus, circuitously, they found Death indeed.

William Blake's two poems *The Chimney Sweeper* and *A Poison Tree* are inspired by toxicological imagery. In the latter, anger directed toward a foe grows into a veritable poison tree, cultivated with ill-intentioned care by the poem's persona until it bears a bright shiny apple that seemingly concentrates his wrath. This foe,

> … into my garden stole
> When the night had veiled the pole;
> In the morning glad I see
> My foe outstretched beneath the tree.

Shakespearean drama is rife with daggers, swordplay, and poisonings. Claudius murdered Prince Hamlet's father by pouring poison in his ear. The slew of additional poisonings at the end of the play – Gertrude, Hamlet, Laertes, and Claudius, all are victims – is formidable. Cleopatra's suicide by an asp in Shakespeare's play is one of the many adaptations of this event in literature and art. The Witches' brew of Macbeth with its "Eye of newt and toe of frog, wool of bat and tongue of dog" is anything if not a highly toxic potion.

Romeo intentionally imbibes a fatal draught when he learns, mistakenly, that Juliet is dead:

> Arms, take your last embrace! and, lips, O you
> The doors of breath, seal with a righteous kiss
> A dateless bargain to engrossing death!
> Come, bitter conduct, come, unsavoury guide!
> Thou desperate pilot, now at once run on
> The dashing rocks thy sea-sick weary bark!
> Here's to my love!
>
> *[Drinks.]*
>
> O true apothecary!
> Thy drugs are quick. Thus with a kiss I die.

We have also the Merchant's "If you poison us, do we not die …." King Lear, near the eponymic play's conclusion, already befuddled but sensible, it appears, of the waywardness of his ungrateful daughters, and his own blindness to Cordelia, tells her

> … I pray, weep not:
> If you have poison for me, I will drink it.

Countless more possibilities unfold in Shakespeare if one considers poisoning as a metaphor. One can argue, for example, that Iago poisons Othello's mind, Lady Macbeth may be said to be poisoning her own consciousness, and so on.

In John Keats' (1795–1821) extended reverie, *Ode to a Nightingale*, the poet likens the vivid oneness with nature he seems to achieve through the bird's song to the effects that could be mimicked, though not as successfully, with a designer drink –

> My heart aches, and a drowsy numbness pains
> My sense, as though of hemlock I had drunk,
> Or emptied some dull opiate to the drains
> One minute past, and Lethe-wards had sunk.

Later in the nineteenth century, poisonous plants play a pivotal role in Nathaniel Hawthorne's *Rappaccini's Daughter.* The year 1896, a peak year of productivity for British poet A.E. Housman, saw publication of *Terence This is Stupid Stuff*. It revolves around Mithradates, King of ancient Pontus, who is said to have consumed minute quantities of poison daily in order, presumably, to build up immunity –

> There was a king reigned in the East:
> There, when kings will sit to feast,
> They get their fill before they think
> With poisoned meat and poisoned drink
> He gathered all the springs to birth
> From the many-venomed earth:
> First a little, thence to more,
> He sampled all her killing store;
> And easy, smiling, seasoned sound,
> Sate the king when healths went round.
> They put arsenic in his meat
> And stared aghast to watch him eat;
> They poured strychnine in his cup
> And shook to see him drink it up:
> They shook, they stared as white's their shirt:
> Them it was their poison hurt.
> – I tell the tale that I heard told.
> Mithridates, he died old.

Betrayed by his son, later in life, it is said, Mithradates attempted to take his own life with poison, but did not succeed (because of the above), and ordered a mercenary to kill him in a chemical-free manner.

A.R. Ammons won his second National Book Award in 1993 for his book-length poem, *Garbage*, in which he writes

> toxic waste, poison air, beach goo, eroded
> roads draw nations together, whereas magnanimous
>
> platitude and sweet semblance ease each nation
> back into its comfort or despair …

Andrew Hudgins in the title poem of his 2003 book, *Ecstatic in the Poison*, reminisces about the fog sprayed by DDT trucks when he was a child –

> The white clouds tumbled down our streets
> pursued by spellbound children
> who chased the most distorting clouds,
> ecstatic in the poison.

Poisoning makes good drama. Witness *Arsenic & Old Lace* – a play by Joseph Kesselring that achieved its greatest success in the Frank Capra film version about two elderly matrons who are ultimately less than cordial to certain gentlemen visitors. Aunt Martha is very forthcoming with the recipe for the drinks they supply:

> For a gallon of Elderberry wine, I take one teaspoon full of arsenic, then add half a teaspoon full of strychnine and then just a pinch of cyanide.

The genre of mysteries and its shining lights, Agatha Christie, Dorothy Sayers (*Strong Poison*), Edgar Allen Poe, Arthur Conan Doyle, Raymond Chandler, as well as its mediocre adherents, dip into poisoning plots for their stories. If one is uncertain of what to prepare for dinner, it stands to reason that the only solution is to consult Ebenezer Murgatroyd's *Cooking to Kill; The Poison Cookbook*, a humorous offshoot of the culinary crime genre. And if you are of a literary bent yourself, consider *Deadly Doses: A Writer's Guide to Poisons*, which is aimed at writers who want to incorporate poisons into their literary endeavors. The reader of this how-to text will learn toxicity ratings of poisons, their effects and symptoms, reaction times, antidotes and treatments, all in the interest of making a more accurate and gripping tale.

In *The Poison Belt*, a non-Sherlock Holmes science fiction story, Conan Doyle speculates about a cloud of poisonous gas that will destroy the human race, and the world with it.

Fairy tales and children's literature are other sources that have borrowed from the toxicology lexicon. The fairy tale of the brothers Grimm, *The Poor Boy in the Grave*, concerns a boy who, fixed upon taking his own life because of inadvertently offending his master, feasts on what he falsely believes to be poison. Realizing his mistake he takes another bottle that he is sure is poison. This instead turns out to be strong Hungarian wine. He drinks enough of it, though, to die, conveniently laying himself in a newly dug grave just before his demise. "The dose," as Paracelsus sharply observed, "makes the poison."

In *Snow White*, the evil queen, in a rage, formulates ingredients to poison an apple which she presents to Snow White, causing her to fall into a deathlike sleep. When the bit of apple is removed from her mouth, she is reanimated.

As already noted in the context of Shakespeare, toxicology makes a splendid metaphor. The traditional Five Poisons of Buddhist thought are greed, anger, ignorance, jealousy, and pride. In 2004, during the initial trial of the former Tyco chief executive on charges of grand larceny and more, a juror submitted a note stating that "The atmosphere in the jury room has turned poisonous", and we can be assured that this did not refer to pollution measurable by any analytical instrument. The judge ultimately declared a mistrial.

Much of nonfiction literature has also appropriated terms such as 'toxic' and applied them in novel senses. Consider the books, *Toxic Parents, Toxic Faith, Toxic People, Divorce Poison, The Book of Poisonous Quotes, Toxic Co-Workers, Toxic Emotions at Work, Toxic Work, Toxic Relationships and How to Change Them*, and even *Toxic In-Laws*. We have not begun to exhaust the possibilities.

Not to be neglected, on the verbal front, are jokes and other intentional and unintentional instances of toxicological humor. One variant (substitute pastor, doctor, lawyer, etc., for Rabbi and it still works) of the "My Wife is Poisoning Me" joke goes like this:

> There's this man. He goes to his Rabbi. "Rabbi, I need help. Something terrible is happening to me. I don't know where to turn." "Good grief," answers the Rabbi, "what is the cause of your distress?" "My wife is trying to poison me." Shocked, the Rabbi asks, "Is it possible it's in your imagination?" "No, I'm certain, she wants to poison me." The Rabbi rests his hand on the man's shoulder. "You settle down. I'll get to the root of this. It must be a misunderstanding. Let me talk to her." Several days later the Rabbi pays a visit to the man. "Well, I met with your wife. In fact," he details, rolling his eyes, "we spoke for three entire hours. Would you like my frank advice"? "Yes, please," the man begs, desperate. The Rabbi takes a deep breath, looks him straight in the eye, and says, "Take the poison."

Visual Arts

Just as primitive man learned quickly to determine which natural substances around him were beneficial and which were harmful, these are among the subjects he sought to depict in early art. There has been some speculation that rock paintings in the Sahara Desert dating back as many as 7000–9000 years ago are representations of hallucinogenic mushrooms, perhaps Psilocybe and Amanita. Some of these scenes show such mushrooms arrayed around dancers in ecstatic states.

Representations of the Minoan snake goddess have appeared in many forms, including a famous faience

sculpture from Knossos, Greece, *c.* CE 1600, now part of the collection of the Archaeological Museum in Herakleion. With elaborate floor-length skirts and exposed breasts, she holds a snake in each of her extended arms. Although there seems to be no definitive interpretation of the meaning of such snakes in Minoan religion and culture, one hypothesis suggests that they are, in fact, the poisonous asp viper.

A Magical Stela of dark stone, from the 30th Dynasty of Egypt (CE 360–343), now in the Metropolitan Museum of Art's collections is described in this way:

> On the part below the central figure panel, rows of hieroglyphs record thirteen magic spells to protect against poisonous bites and wounds and to cure the illnesses caused by them … A victim could recite or drink water that had been poured over the magic words and images on the stela.

Indeed, in Egyptian mythology, Selket was the goddess of scorpions and magic. She is typically painted with a scorpion on her head and was said to be a protector from venomous bites.

As in literature, Greek and Roman legends provided much material for art. Hercules in his Second Labor decapitates the Hydra's multiple heads and dips his arrows in the creature's venom. There are various depictions of the beheading, ranging from a *c.* 525 BC painting on a vase in the J. Paul Getty Museum in Malibu to John Singer Sargent's oil on canvas in the Museum of Fine Arts, Boston. Other art shows victims, such as the Centaurs Chiron and Pholus, of the venom-tipped arrows.

Alcohol may be fine in moderation, but in excess it is another story, and sometimes the line between one and the other is blurred. Dionysus, the Greek god of wine (and his Roman counterpart, Bacchus) and his followers, were the subjects of numerous paintings on walls and sarcophagi, as well as of mosaics. Hogarth's print, Gin Lane, on the other hand, leaves little doubt about which end of the virtue or vice spectrum inebriation inhabits.

In medieval times it was thought that the legendary unicorn's single horn could neutralize poisons. Such a scene is portrayed in *The Unicorn is Found* tapestry, one of the famous series completed 1495–1505 in the Netherlands, and now in the Cloisters Collection of the Metropolitan Museum of Art. Serpents were believed to pollute waters with their poisons. In this tapestry the hunters watch as the unicorn cleanses a poisoned stream with his horn.

In Rembrandt van Rijn's *Artemesia*, alternately titled *Sophonisba Receiving the Poison* in the Prado Museum, the robustly figured heroine, theatrically bathed in golden light, is about to accept a goblet of poison from her servant.

There are numerous depictions in art of legends and historic events memorializing poisoning. *The Death of Socrates* by Jacques Louis-David (1787, Oil on Canvas, Metropolitan Museum of Art) shows the philosopher bravely accepting his fate, the cup of hemlock, as he is surrounded by his anguished and grieving followers. In William Henry Margetson's *Cleopatra*, the Egyptian seductress's servants prepare her for death as a basket with figs and snakes sits at her feet.

Although the thrust of this paper considers toxicology as interpreted within the Western tradition, a brief aside to the wealth of artifacts from other cultures is warranted. In Japan, Yotsuya Oiwa was the wife of a masterless samurai known as Tamiya Iemon who is complicit in her poisoning. The poison leaves her face gruesomely disfigured. She goes insane and dies, but returns in assorted forms to wreak vengeance on her unfaithful husband. Her grotesque image returns over and over to haunt Iemon. The story has been the subject of a famous Japanese play and numerous artworks including woodblock prints and netsuke. In Yoshitoshi's print of the story, the face of Oiwa leers from a half-burned paper lantern.

The Five Poisons, unrelated to the Buddhist metaphorical poisons discussed earlier, is a motif consisting of the centipede, lizard, scorpion, snake, and toad. They are incorporated into Chinese folk art, embroidered on clothing, appliquéd to luggage, engraved on amulets, and otherwise used. Customarily this group is not viewed negatively, but serves, on the contrary, as a charm for neutralizing evil.

Moving into the modern realm, there has been an entire genre of psychedelic paintings, most common perhaps in the 1970s, in which artists (or those who claimed to be) created work while under the influence of hallucinogenic agents such as LSD.

Jean Michel Basquiat, very influential in the grafitti movement in the late seventies, burst upon the contemporary art scene in the 1980s. He collaborated with Andy Warhol on a painting in acrylics and oilstick on canvas, littered with many skulls and a few crossbones and announcing 'Poison' and 'Caution'. Basquiat died before reaching the age of 30 from a drug overdose.

Fred Tomaselli recently was featured in a solo museum show, a 10 year retrospective, of his work. Tomaselli has constructed a sizeable body of fairly abstract art combining acrylic, photographs, hallucinogenic plants, synthetic pharmaceuticals, and therapeutic herbs.

In 2002, using Adobe PhotoShop 6, the artist Scott Blake made an intriguing self-portrait from images of

ecstasy pills he downloaded from the Web through the DanceSafe site. DanceSafe promotes health and safety within the rave and nightclub community. Masanta is a young Japanese artist whose work has been exhibited widely, and who uses Photoshop, Illustrator, Macromedia Fontographer, pen, acrylic, and mixed media. One of her constructions, displayed on the virtual Museum of Computer Art, appears to be an orange candelabra not only supporting flames, but in flames itself, with a small female apparition at its center and apex, against a background of turquoise and tiny pearls, and with the word 'Poison' in billowy pink letters across the lower foreground.

Video and Cartoons

Arsenic and Old Lace was already mentioned above among the literary arts as a famous toxicology-oriented play. It found, of course, another life in the memorable 1944 film version directed by Frank Capra. Toxic themes have been adopted by many movies.

D.O.A., a 1950 film noir starring Edmund O'Brien, concerns a man who's been poisoned and has only a few days to live. He needs to find out who is trying kill him, and why.

Godzilla, or more properly *Gojira* (1954), was very influential in Asian and monster cinema genres. Godzilla is said to have been an ancient dinosaur of sorts whose underwater habitat was disrupted by nuclear testing and subsequently became irradiated himself, the outcome of a toxicological experiment gone awry.

The Young Poisoner's Handbook, inspired by an actual mass murderer, is a dark comedy set in England in the 1960s. It concerns a young teenager who is a budding chemist and proclaims, "I want to be the greatest poisoner the world has ever seen."

The series of movies beginning with *The Toxic Avenger* is the brainchild of Troma Productions. These raunchy films feature the mop boy at a local health club who falls into a vat of hazardous chemicals, making him hideously deformed and at the same time bestowing upon him superhuman powers.

The winner of numerous awards in 2003, Bill Domonkos' short film, *The Fine Art of Poisoning*, uses animation and music by Jill Tracy to layer nightmare visions in the creation of a strange, unsettling world.

Only slightly touched upon here are the countless literary works, films, plays, and other artworks that consider addictions of one kind or another – alcoholism, drug abuse, tobacco use. Think of the decades in which holding, lighting, smoking, or caressing a cigarette was standard fare, even considered romantic, sexy, and cool, in the movies. This use of cigarette as prop may be less pervasive in contemporary works, but it has hardly disappeared. Consider Otto Preminger's 1955 film, the *Man with the Golden Arms*, starring Frank Sinatra in a powerful look at drug addiction, and its many more explicit successors. There is also a veritable cornucopia of material on alcoholism – for example, *Days of Wine and Roses* with Jack Lemmon and Lee Remick from 1958, *Leaving Las Vegas*, released in 1995 and featuring Nicholas Cage and Elisabeth Shue, and everything earlier, in between the two, and since.

Cartoons and animation, too long relegated to kiddy fare, have come into their own and been recognized on their own artistic merit. There is a surfeit of cartoons exploiting issues related to the environment, hazardous chemicals, toxic waste, etc. Many websites compile and offer access to these, which are typically copyright. CartoonStock is one among many.

In the 1935 Mickey Mouse short, *Mickey's Garden*, our protagonist and Pluto attempt to bug-proof their vegetable garden. Exposure to insecticide causes the pair to hallucinate that they shrink down to the size of the bugs.

Among the many villains encountered by Batman was Poison Ivy, formerly a female botanist who, because of experiments gone awry, developed a deadly touch and simultaneously became immune to all poisons. She is a specialist in the use of plant toxins, may use a cross-bow and vine whip and, sometimes, poisoned darts. Poison perfumes and lipstick are also in her arsenal. Uma Thurman played the role in the nonanimated and critically panned film, *Batman and Robin*.

Eric Pigor's Toxic Toons incorporates poisons into generally ghoulish, grotesque, and gross (and he would surely be proud to have them described as such) images and animations. In a similar vein, Jacob Wexler's celebrated Ruin Dog single-panel cartoon (**Figure 1** bottom panel) is symbolic of toxicology not as a science but as a poster child for its most depraved practitioners and victims.

Television, especially series, and made for TV movies have employed countless poisoning motifs. The genres are typically mysteries or shows featuring detectives or police, or set in the courtroom and hospital.

No discussion on entertainment today would be adequate if it did not mention video games, whether via computer or console. By some accounts, television viewing hours are declining considerably, with much of the loss applied to a gain in video game usage, especially among young men. Although hand-to-hand combat, weapons that shoot, detonate,

Figure 1 Toxicological iconography – traditional (skull and crossbones/JollyRoger) and contemporary (ruin dog).

slash, pierce, radiate, or burn are the overwhelming favorites, poisons are not totally absent. Poison Claws and Poison Rods, for example, are among the cornucopia of weapons available to players in *Final Fantasy 11*, part of a role-playing game series extraordinaire. In *Everquest*, rogues can use blinding poisons, dizzy poisons, feeble mind poisons, flesh rot poisons, system shock poisons, etc.

The long awaited and exemplary first-person shooter, Half-life 2, for PC, released in late 2004 brings the headcrabs, evil creatures which gain control of others by attaching themselves to their head. A new variant, the black headcrab, can deliver a potent poison. It is lethal but will drastically reduce a victim's health and potentially turn him a poison zombie. Socrates must surely be turning over in his grave.

Music

Can music be poisonous? Perhaps. There is a greater challenge in trying to find kinships between music, especially music which is not vocal or at least programmatic, and toxicology. Music not designed to tell a story is referred to as 'absolute' music, and although one can try to make a case that a Beethoven symphony or a Hindemeth sonata is rife with toxicological tendencies, such connections can be far-fetched and are, regardless, a matter of personal interpretation. A nonvocal programmatic piece such as *Mars: The Bringer of War*, a segment of Gustav Holst's suite, *The Planets*, while intended by the composer to be suggestive of the battlefield, leaves it to the imagination to determine what the implements of destruction may be – rifles, tanks, poison gas?

Music that explicitly tells a story or has a message, by virtue of its lyrics, is a more reliable source of toxicological lore and legend. The high drama of much of the standard nineteenth century opera fare opens the floodgates of poisonous mischief. Consider that Donizetti composed an entire opera based on *Lucrezia Borgia*. Major characters in Verdi's operas *Nabucco*, *Simon Boccanegra*, and *Luisa Miller* all succumb to poison. In the same composer's *Il Trovatore*, the heroine Leonora promises to give herself to her enemy Count Luna if he will release her true love, Manrico. Leonora takes poison in order not to have to fulfill her pledge.

The legend of *Tristan & Isolde* has been variously interpreted, probably to greatest musical acclaim in Wagner's opera of the same name. A key plot element revolves around what is mistakenly believed to be poison. Isolde, in love with Tristan but spurned by him, and unwilling to be part of an arranged marriage with his uncle, conspires to poison herself and her would-be lover. Her maid substitutes a love potion for the poison and the rest is history, not to mention great drama and music.

Even ballet has not been immune from toxic influences. *La Bayadere*, with music by Leon Minkus, and choreographed by Lucien Petipa in its 1877 premiere, involves a love triangle. Nikiya, the temple dancer and abandoned third of the triangle, succumbs to the bite of a poisonous snake sent to her in a basket of flowers by her rival, Gamzatti, and the latter's prospective father-in-law, the Rajah.

In 1910, Calvin Lee Woolsey, during ragtime's heyday, composed, in a minor key, the *Poison Rag*. Herbert Ingraham is responsible for the *Poison Ivy Rag* in 1908. It is easy to hear the playful piano trill suggestive of itching in Ingraham's rag.

Fast forwarding to 1959, we reach the Coaster's hit, *Poison Ivy*:

> Measles make you bumpy
> And mumps'll make you lumpy
> And chicken pox'll make you jump and twitch
> A common cold'll fool ya
> And whooping cough'll cool ya
> But poison ivy, Lord'll make you itch!!

The Rolling Stones also recorded this several years later.

The grisly black humor of Stephen Sondheim's *Sweeny Todd, the Demon Barber of Fleet Street*, while not explicitly incorporating poisoning, certainly captures its spirit. One can envision considerable offstage food poisoning by minimal stretching of the lyrics of *The Worst Pies in London* and *A Little Priest*.

Among the many popular songs with toxicology leanings are *Poison in the Well* (10 000 Maniacs), *Poison* (Alice Cooper), *Church of the Poison Mind* (Culture Club), *Poison* (Laurie Anderson), *Toxic* (Britney Spears, in which she sings "I'm slipping under/With a taste of poison paradise/I'm addicted to you/Don't you know that you're toxic/And I love what you do"), and *Sweet Toxic Love* (Boy George).

Toxic Audio, the a capella group, has given new meaning to the flexibility and versatility of the human voice. Their production, *Loudmouth*, played on Broadway in 2004. One suspects that the 'toxic' in their name refers more to their intoxicatingly refreshing sound than to anything negative about their music.

The heavy-metal band Poison, peaked in the late 1980s and early 1990s. The band named System of a Down's 2001 album is titled *Toxicity* –

> More wood for their fires, loud neighbors,
> Flashlight reveries caught in the headlights of a truck,
> Eating seeds as a past time activity,
> The Toxicity of our city, of our city …

Dr. Carl Winter, a respected extension toxicologist with the University of California at Davis and director of its FoodSafe program has another, somewhat related musical life. He parodies popular songs, rewriting lyrics, composing and recording musical arrangements, and producing the music, and it is all with a toxic twist. Among his 'greatest hits' are *I Sprayed it on the Grapevine* to the tune of *I Heard it through the Grapevine*, *Rat Number 49* to the tune of *Love Potion Number 9*, and *You Better Wash your Hands* to the tune of *I Want to Hold Your Hand*.

More Cultural Miscellany

The perfume industry may seem an unlikely province to inspire the toxic imagination, but the market is ripe. Witness Christian Dior. Its women's fragrance, Poison, created in 1985, is a blend of amber, honey, berries, and spices. The year 1994 saw a follow-up with Tendre Poison, combining florals, mandarin, vanilla, and sandalwood. Hypnotic Poison, as an eau de toilette spray, was launched in 1998. It blends bitter almond, caraway, jasmine, moss, wood, and vanilla. Advertising copy has depicted it as "Mysterious and mesmerizing, extravagant and bewitching, audacious and profoundly feminine, the fragrance is an unsettling harmony, a fusion of contrasting olfactory facets. Recommended for romantic use." It has also been described as 'temptation in a bottle', 'a magic potion for modern times', and 'disturbing, sensual, and bewitching, to take you beyond impulse and beyond fantasy. ' It may seem ironic to identify anything suggestive of poison with romance, but in the context of perfume, there is an allure to danger. As an aside, there is also a Dior purse spray called Addict. One wonders that a scent called Risk has not yet been developed. Versions of these various 'Poison' fragrances come as lotions, shower gel, and in other forms.

Alcoholic beverages, in immoderate doses, are well-documented toxic agents. Drinkers often, of course, perceive the effects that they yield as beneficial. Names of straight and mixed drinks vary from the buoyant (and even erotic) to the rather diabolical. Consider the following mixed drinks – Choke and Puke (a combination of Jack Daniel's, Jose Cuervo Gold, and gin, with grenadine as the mixer), Cocaine Shooter (with blackberry brandy, vodka, and grapefruit juice as the mixer), and Death Wish (a combination of Wild Turkey 101, peppermint schnapps, and 151 Rum, also using grenadine as the mixer). The potent Icelandic potato-based vodka known as Brennevin used to be called Black Death and had an ominous-looking skull on its label. Armida Winey, of Sonoma County throws its hat into the ring of beverages influenced by the lure of toxicology with a Zinfandel cleverly named 'Poizin', described as 'the wine to die for'.

Street names of abused drugs are a testament to the verbal creativity of the addicted. Flamethrowers, for example, are cigarettes laced with cocaine and heroin. Heroin itself is the subject of many pseudonyms – while most of them are derivative of the drug's potency (e.g., thunder, red eagle, raw fusion, Rambo), some have clear negative connotations – poison, dead on arrival, brain damage.

The graphic symbol that seems to be universally understood to apply to poisons is the skull and crossbones (**Figure 1**, top panel). Traditionally associated with European and American pirate flags and known in English as the Jolly Roger, it has long symbolized death and signified that those who dare approach the object or persons associated with it had better beware. Beginning in the late nineteenth century, the skull and crossbones was regularly embossed on glass bottles of poisons. This image was also appropriated by one of the branches of the Nazi SS.

The Pittsburgh Poison Center created the graphically vivid Mr. Yuk symbol to educate the public about poison prevention. The round sticker currently has a frowning green face with its tongue out and includes the US national toll-free poison help phone number. Millions upon millions of these have been distributed worldwide through the years. Curiously enough, certain research has shown that these stickers do not necessarily serve as a deterrent to poisoning but may have the opposite effect, attracting children, in particular, to the products on which they are placed, and some poisoning centers have, therefore, stopped distributing them.

Public health posters have been used by various organizations and in a variety of settings to influence behavior conducive to a healthy society. The International Programme on Chemical Safety, for instance, created several posters in the 1980s related to environmental health. One, showing children in the process of opening a bottle of insectide, was designed to promote poison prevention. Another, also utilizing children, this time eating ice cream and an apple, states "Food Additives and Pesticides Should be Used with Care."

To delve more deeply into the linguistic ramifications of 'toxicology' and 'poison', or indeed of any words, one would do well to consult The Rosetta Edition of Webster's Online Dictionary. In it, one would discover that 'ilmu racun' means toxicology in Indonesian, that 54 6F 78 69 63 6F 6C 6F 67 79 is hexadecimal for toxicology, that some rhyming words are gynecology, mycology, and oncology, and that there are an estimated 300 searches executed per

day on the word 'toxicology' across the major English-language search engines.

Conclusion

This entry has barely scratched the surface of the ways in which toxicology, often without our realizing it, continues to hold a spell over us. Enter the word 'toxic' or 'poison' or some such term on Google or another Web search engine. It is likely you will not be surprised by the amount of retrieval, but you may be by the proportion of sites that use the word purely in a figurative and sometimes self-contradictory sense. Toxicology lives in fact and as metaphor. The terminology and spirit of toxicology and poisoning has infiltrated our own vocabulary and the way we view the world. Clearly something about it will not let go of our psyches, so we might as well just roll with the (poison) punches and enjoy it.

See also: Ancient Warfare and Toxicology; Notorious Poisoners and Poisoning Cases; Hemlock, Poison; Toxicology, History of.

Further Reading

Martinetz D and Lohs K (1987) *Poison: Sorcery and Science, Friend and Foe*. Germany: Edition Leipzig.
Mayor A (2003) *Greek FirePoison Arrows and Scorpion Bombs: Biological and Chemical Warfare in the Ancient World*. Woodstock, NY: Overlook Press.
Mithrada: Newsletter of the Toxicological History Society.

Relevant Websites

http://www.nlm.nih.gov – Visual Culture and Public Health Posters (from the National Library of Medicine).
http://arthursclassicnovels.com – Doyle C. *The Poison Belt* a non-Sherlock Holmes science fiction story.
http://www.dancesafe.org – DanceSafe website.
http://www.cartoonstock.com – CartoonStock website.
http://www.toxictoons.com – Eric Pigor's Toxic Toons website.
http://www.websters-online-dictionary.org – The Rosetta Edition of Webster's Online Dictionary.
http://moca.virtual.museum – Virtual Museum of Computer Art.
http://foodsafe.ucdavis.edu – See link to the page containing details of the music of Dr. Carl Winter, a respected extension toxicologist with the University of California at Davis.

Toxicology, Education and Careers

Susan J Borghoff

Toxicology and Education

Toxicology is the study of harmful effects of agents on people, animals, and other living organisms. One aspect of toxicology is to evaluate the likelihood that adverse effects will occur under specific chemical exposure scenarios; referred to as risk assessment. Toxicology is a combination of biology and chemistry, with elements of physical and computational sciences, that leads to a wide variety of career opportunities. The field of toxicology provides the excitement of science and research while contributing to the well-being of current and future generations.

The objective of this entry is to provide information to individuals considering a career in toxicology and some guidance and resources as to the education and training that is required. Various careers in toxicology will be discussed in the following section and opportunities for education and training outlined.

Much of the information provided in this section was obtained from the book *Resource Guide to Careers in Toxicology* developed by the Society of Toxicology. A complete reference for this guide is presented in the Further Reading section. A copy of this *Resource Guide* can be obtained from the Society of Toxicology. Individuals serious about a career in toxicology are encouraged to obtain a copy.

Why Consider a Career in Toxicology?

Challenges

Chemicals are an essential component of the high standard of living we enjoy. The challenge to toxicologists is to ensure that products or by-products of modern living are not endangering our health or environment. With a career in toxicology the individual can contribute toward finding solutions to important challenges such as protecting public health and the environment.

Opportunities

A wide variety of career opportunities exists in toxicology. Toxicologists participate in basic research

studying mechanisms by which chemicals exert their toxicological or cancer-causing effects. Research toxicologists use the most advanced techniques in molecular biology, chemistry, and the biomedical sciences. Many toxicologists work in the chemical, pharmaceutical, and consumer products industries to test and ensure that their products and workplaces are safe. There are also many toxicologists that work within government at the state and federal levels to develop and enforce laws to ensure that chemicals are produced, used, and disposed of safely. Toxicologists are also involved with monitoring both water and air for levels of specific chemicals and biological agents known to cause adverse health effects to people and the environment. In academia, toxicologists train future toxicologists as well as conduct research to understand the mechanisms by which chemicals cause toxic effects in living organisms. Clinical toxicologists help to diagnose patients with diseases caused by toxic substances and often work at hospitals or Poison Control Centers. Forensic toxicologists help establish the cause of death or identify important toxicity clues that can be used to solve a crime, whereas the occupational toxicologist conducts studies to understand conditions of chemical exposure or work practices that may place the worker at unacceptable risk.

Attractive Salaries and Professional Advancement

There continues to be a high demand for toxicologists within all sectors of employment – government, industry, and academia. Salaries are especially competitive for the advanced trained toxicologist. Specific information on salaries for toxicologists in various workplace settings and with various levels of experience and education can be found in a survey published by Gad Consulting Services in Raleigh, NC. The Fifth Triennial Toxicology Salary Survey is currently posted on the Society of Toxicology website (see Relevant Websites section). A reference for this survey can be found in the Further Reading section.

What Do Toxicologists Do?

Research

Research in toxicology is conducted at basic and applied levels. Basic research may involve studying the biochemical or molecular mechanism by which a chemical causes an adverse effect on various cellular processes. Knowledge gained through toxicology has improved our fundamental understanding of basic life processes. Applied research is more directed and is expected to yield direct social or commercial benefit. Examples of applied research are studies to identify chemicals that selectively kill certain pests or studies to determine whether a particular industrial process is responsible for a specific disease identified in a population of workers. Toxicologists working in applied areas also conduct studies directly related to determining whether or not a chemical is toxic to laboratory animals and by inference, toxic to people.

Research in toxicology is generally conducted in various specialty areas such as carcinogenesis, reproductive and developmental toxicology, neurotoxicology, immunotoxicology, respiratory toxicology, dermal toxicology, endocrine or genetic toxicology. The specialty areas may also focus on various organ systems such as the liver, kidney, eye, skin, or on different species of plants or animals. Since researchers are studying the effects of substances on living organisms, they work with various systems ranging from whole organisms (*in vivo*) to isolated cell suspensions or cell cultures (*in vitro*), to imaginary systems based on computer simulation or modeling of living organisms (*in silico*).

Product Safety Evaluation

Drugs, agricultural products, and other chemicals are introduced into society every day. Toxicologists in product safety evaluation are continuously developing better ways to evaluate the potential adverse effects of chemicals and physical agents and to determine the dose at which adverse responses occur. Many industries employ toxicologists to evaluate the safety of their products. For drugs, food additives, cosmetics, agricultural chemicals and other classes of chemicals, federal laws require that the manufacturer provide adequate testing of the product before it is approved for use. Tests to determine whether a chemical has the potential to cause cancer, birth defects, reproductive effects, neurological toxicity, or other adverse effects are commonly conducted by the manufacturer. Toxicologists involved in product safety evaluation have the responsibility to ensure that these tests are designed, conducted, and interpreted in a scientifically sound manner. The information gathered from these studies is reviewed by toxicologists in various regulatory agencies such as the Food and Drug Administration or the United States Environmental Protection Agency to ensure that the products will not present an unreasonable risk to human health or the environment.

Teaching

Toxicologists employed in colleges and universities are frequently involved in teaching courses in toxicology. Many colleges and universities are developing new courses at both the undergraduate and graduate

Table 1 Partial listing of academic institutions that support training programs in toxicology at the graduate level (grouped by geographical location)

Mid-Atlantic
Clemson University
Duke University
North Carolina State University
University of Kentucky
University of Louisville
University of North Carolina at Chapel Hill
Vanderbilt University
Virginia Commonwealth University
Virginia-Maryland Regional College of Veterinary Medicine
North central
Indiana University
Iowa State University
Michigan State University
Purdue University
University of Cincinnati
University of Illinois at Urbana-Champaign
University of Kansas Medical Center
University of Michigan
University of Nebraska
University of Wisconsin-Madison
Wayne State University
Wright State University
Northeast
Dartmouth College
Johns Hopkins University School of Hygiene and Public Health
Massachusetts Institute of Technology
New York University
Northeastern University
Rutgers University
St. John's University
State University of New York at Buffalo
University of Albany
University of Connecticut
University of Maryland
University of Pittsburgh Graduate School of Public Health
University of Rochester School of Medicine and Dentistry
University of the Sciences in Philadelphia
Northwest
Oregon State University
University of Washington
South central
Louisiana State University Medical Center
Mississippi State University
Texas A&M University
Texas Tech University
University of Arkansas for Medical Sciences
University of Mississippi
University of Oklahoma Health Sciences Center
University of Texas at Austin
University of Texas Health Science Center of Houston
University of Texas Medical Branch at Galveston
Southeast
Florida A&M University
University of Alabama at Birmingham
University of Florida
University of Georgia
Southwest
Colorado State University
San Diego State University
University of Arizona
University of California, Berkeley
University of California, Davis
University of California, Irvine
University of California, Los Angeles
University of California, Riverside
University of Colorado Health Sciences Center
University of New Mexico
University of Utah
Utah State University

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levels to provide students with a background in the science of toxicology. A partial list of Universities that support toxicology training programs at the graduate level is presented in **Table 1**. Details of each of these programs can be obtained through the online version of the fifth edition of the *Resource Guide to Careers in Toxicology*. Many other academic institutions do not have a specific graduate program in toxicology but employ toxicologists to participate in curriculum development and teaching in more basic programs such as chemistry and biology. Thus, opportunities exist to teach toxicology in small colleges as well as major universities.

Public Service and Regulatory Affairs

There has been tremendous growth in public awareness of chemical hazards over the past two decades which has resulted in the passage of many laws governing the production, use, and disposal of chemicals. Many local, state, and federal agencies employ toxicologists to assist in the development and enforcement of their laws. An increasingly important area of toxicology is public communication of chemical risks. Toxicologists employed by regulatory agencies may often be called on to examine the scientific basis for regulatory actions or to assist in communicating to the public the reasons regulatory actions are or are not taken in particular situations. There are many private consulting firms with expanding expertise in toxicology that can now provide such services to local and state health departments, public utilities, and private industries. Thus, many employment opportunities in the private sector are available to the toxicologist interested in assisting public agencies and private industries in resolving many public health and environmental problems.

Clinical and Forensic Toxicology

Clinical toxicologists are health professionals concerned with disease caused by exposure to toxic agents. Generally, clinical toxicologists are physicians, pharmacologists (e.g., individuals with a Doctor of Pharmacology degree), and veterinarians who receive specialized clinical training in toxicology. These individuals are engaged in the diagnosis and treatment of poisoned patients. Poisoning may result from accidental, deliberate, environmental, or occupational exposure to a toxicant. Forensic toxicologists interact with clinical toxicologists to establish analytical chemical methods for the detection of toxic agents in tissue samples from poisoned patients. Research performed by clinical and forensic toxicologists has led to the recognition of new chemical hazards and the development of novel therapies for poisoning. Clinical and forensic toxicologists may be found in academia (medical centers), industry, and other places in which health professionals are employed.

Occupational Toxicology

Occupational toxicologists conduct studies to understand the conditions of chemical exposure and develop work practices that reduce health risks to the worker. They work in all sectors: industry, academia, and government. Their efforts are focused on obtaining knowledge of the relationship between workplace exposure to a chemical, and the health effects that are of concern to workers.

Who Employs Toxicologists?

Industry

The 'Job Market Survey' reported on the SOT website (see Relevant Websites section), reveals that in North America, the chemical, pharmaceutical, and support industries account for ~47% of the toxicologists employed. Product development, product safety evaluation, and regulatory compliance generate a large job market for toxicologists. These industries employ toxicologists trained at all levels of education, including those holding bachelor, master, and doctoral degrees. Many companies have their own research programs in product safety evaluation, whereas others may contract their work to specific organizations that specialize in contracted research studies.

Academia

Academic institutions account for ~21% of all employed toxicologists. Most have advanced degrees and are conducting basic research. There has been an increase in the number of programs in toxicology at many academic institutions because of a need for toxicologists with doctoral-level training. Most of these opportunities are within Medical Schools and Schools of Public Health at major universities. Increasingly, smaller colleges are beginning to employ toxicologists to teach toxicology within their basic biology, chemistry, and engineering programs.

Government

The government employs ~15% of toxicologists. Although most government jobs are with federal regulatory agencies, many states employ toxicologists with masters or doctoral degrees. While most of the toxicologists employed by the federal government are involved in the development and enforcement of laws related to the toxicity of materials, a number of federal agencies employ toxicologists to conduct both basic and applied research in toxicology.

Consulting Firms

The professional service industry is a growing employer of toxicologists and currently accounts for ~15% of toxicologists. Many graduates of baccalaureate and master's programs in toxicology are finding employment with consulting firms. In the consulting field, experienced individuals provide professional guidance and advice to local public agencies, industries, and attorneys involved in problems with toxic chemicals. Consulting is a rapidly growing activity for the experienced toxicologist.

Research Foundations

Private nonprofit research foundations provide opportunities for research in toxicology to ~4% of the toxicologists. Numerous public and private research foundations employ toxicologists to conduct research on specific problems of industrial or public concern. Toxicologists at all levels of education might find employment with these research foundations.

Preparing for a Career in Toxicology

For those individuals who are in the midst of their college education, careful planning of undergraduate courses will enhance graduate education opportunities in toxicology and other biomedical sciences. For those who have already received an advanced degree such as a PhD, an MD, or a DVM in a biomedical science other than toxicology, careers can be focused toward toxicology through postdoctoral clinical or research training.

Undergraduate and Graduate Training

Planning Depending on career aspirations, a bachelor's degree may not be sufficient for achieving career

goals. Although there are some employment opportunities in toxicology for those with a bachelor's degree, the breadth of career choices and opportunities for advancement are much greater for those with post baccalaureate degrees. Acceptance into graduate programs in toxicology generally requires a strong academic record and evidence of research and/or leadership abilities. Most graduate toxicology programs have specific prerequisites for admission. The primary requirement is a baccalaureate degree in a relevant field of study such as biology, chemistry, environmental health, or other science-related field. Persons graduating with these degrees can seek employment at the technical support level at many research institutions. Additional upper level courses in biochemistry and physiology will often increase the competitive advantage for graduate school admissions. As the ability to be an effective communicator becomes increasingly important for toxicologists, course work in scientific writing and public speaking is also useful. Performance on the Graduate Record Examination (GRE) is often evaluated by graduate admissions committees and the exam should be prepared for in advance. Many programs require GRE scores on both the General Test and on the Subject Test if it is given in an undergraduate major such as biology or biochemistry. The GRE should be taken at least 5 months prior to the time one plans to begin graduate study. Individual graduate programs should be consulted in advance to determine specific admission requirements.

In addition to a strong academic record, demonstration of basic laboratory research skills enhances the chance of admission. Laboratory courses in chemistry and biology are an important part of an undergraduate education and help develop research skills. Cooperative work-study programs enhance those skills by placing students in a research setting during the semester. Summer internships in a research laboratory are another approach to enhancing laboratory skills. Research internships provide interested undergraduate science majors with a stimulating research experience in toxicology. These internships are available in academic and industrial research laboratories across the country. More information on research internships in toxicology can be obtained by contacting the Society of Toxicology or searching the Peterson's Guide (see Relevant Websites section).

Selection of an Appropriate Toxicology Program Identifying a graduate training program and mentor most appropriate for a particular individual requires some advance planning. First, individuals should establish a potential career plan. By considering the various subspecialties in toxicology such as neurotoxicology, chemical carcinogenesis, teratology, inhalation toxicology, computational modeling and risk assessment, a specific field of research that is of particular interest to the student can be identified. Although such a choice early in the education process does not commit one to this direction, careful assessment helps in deciding which programs are most likely to meet your needs. Talking with toxicologists in local universities, industries, and governmental agencies is helpful in selecting a training program and in deciding on a future career direction.

The admission requirements of the graduate program should be identified well in advance since these requirements must be met prior to the time of beginning the program. Requirements vary among programs and from the general requirements described previously. Details of the specific requirements of toxicology graduate programs can be obtained by referring to the *Resource Guide to Careers in Toxicology*, which is available on the SOT website.

Financial Assistance University financial assistance is often available through research and teaching assistantships, fellowships, traineeships, and grants. Inquires should be made to the prospective institution, program, and mentor as to the availability of grants and financial aid. The National Institutes of Health, other federal institutions such as the Environmental Protection Agency, private foundations, and the Society of Toxicology are all potential sources of financial support.

Resource Guide to Careers in Toxicology The *Resource Guide to Careers in Toxicology* contains descriptions of a large number of very diverse academic programs in toxicology located throughout the United States (**Table 1**). Geographic considerations may be important to some individuals and may substantially limit the number of potential toxicology programs of interest to those individuals. Review of this document in the early stages of planning a career in toxicology is one of the most important steps that individuals can take in planning their toxicology education. The listing for each toxicology program includes program website address, degrees offered, areas of program strengths and contact information. The online version of the *Resource Guide* has links to these listed programs. Most of the websites for toxicology departments include a list of faculty and a synopsis of their research interests.

When a specific program website is accessed there will be a description of the program along with an outline of the prerequisites for admission. This is a very important section and provides clear direction as to the types of college courses needed to be accepted

into toxicology graduate programs. It is also useful to know at an early point other information required by the toxicology program. For example, most programs require official college transcripts, GRE scores, a letter of intent, and letters of recommendations. The letter of intent describes why the individual wants to be admitted into the graduate program and general career goals. Recommendations are generally from individuals who know the applicant on a professional or academic level. Examples of appropriate references are teachers, advisors, or employers.

The final section in each program description is the curriculum. The description of the curriculum includes the degrees that are offered by the particular toxicology program, the areas of specialization for the degrees, required courses, optional course work, and specific dissertation requirements. The graduate curriculum for a doctorate in toxicology often includes courses in biochemistry, physiology, anatomy, histology, pathology, pharmacology, and statistics. The academic program can also include areas such as analytical methods, carcinogenesis, mutagenesis, teratogenesis, comparative toxicology, molecular mechanisms of toxicology, and organ-specific toxicity. Some programs may also include course work in such fields as statistics, computer science, computational modeling, immunology, and pharmacokinetics.

Because the doctorate in toxicology is a scholarly degree, the student is required to conduct a program of original research that extends over a period of 2 or more years. Part of this research requirement is the completion of a dissertation. This document is written by the student and includes an introduction or literature survey, a statement of the hypothesis underlying the dissertation, methods, results (including figures, graphs, and tables), and a discussion. By conducting original research, the student can understand and experience the application of observation and analysis to specific problems in toxicology. In keeping with the tradition of the doctorate degree, defense of the graduate research thesis is expected. The final section of the program description provides the name and address of a person who can be contacted for more information and applications for admission and financial assistance.

Postdoctoral Training

Graduate students who are interested in a career at an advanced level in toxicology (i.e., conducting basic research, leading research groups and projects, or teaching) typically need to go through some additional postdoctoral training. This training may last from 2 to 4 years beyond the doctoral degree, and typically consists of an in-depth, independent research project. Most institutions that offer a doctoral degree in toxicology or support advanced research in toxicology have postdoctoral programs. These programs are highly tailored to the individual researcher and his/her postdoctoral mentor.

Even students who have not gone through a graduate program in toxicology can enter into a career in toxicology at this junction. Since a postdoctoral fellowship usually has highly specific requirements, students with specialized training in fields such as molecular biology, genetics, computational modeling, chemical engineering, medicine, and many others may be able to find a toxicology-related research topic that requires their specialty.

See also: Academy of Toxicological Sciences; American Academy of Clinical Toxicology; American Board of Toxicology; American College of Medical Toxicology; American College of Toxicology; European Society of Toxicology; Information Resources in Toxicology; International Union of Toxicology; National Center for Toxicological Research; Society for Environmental Toxicology and Chemistry; Society of Toxicology; Toxicology Forum; Toxicology in the Arts, Culture, and Imagination.

Further Reading

Gad SC (2002) *Fifth Triennial Toxicology Salary Survey.* Currently listed on the Society of Toxicology's website.

Society of Toxicology. *Resource Guide to Careers in Toxicology*, 4th edn. Reston, VA: Society of Toxicology. (Available from the Society of Toxicology Offices, Society of Toxicology, 1821 Michael Faraday Drive, Suite 300, Reston, VA 20190-5332, USA. Tel: +1-703-438-3115.)

Relevant Websites

http://www.toxicology.org – Society of Toxicology website.
http://www.petersons.com – Peterson's Guide.

Toxicology, History of

Katherine D Watson

> All substances are poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy.
>
> Paracelsus

The science dealing with the harmful effects of chemical agents on biological systems is called toxicology, from the Greek word *toxikon*, a bow (to shoot poisoned arrows) or a poison in which to dip arrowheads. A poison is generally defined as a substance that is capable of destroying life or causing illness when introduced into, or absorbed by, a living system in small quantities. Since 1900, toxicology has undergone continuous expansion and development by assimilating knowledge and techniques from many branches of the physical and biological sciences. Historically, attempts to both kill and cure with chemically active preparations (poisons and drugs) have led to the evolution of toxicology, and today it is a discipline of diverse application and widespread importance.

It is likely that the history of toxicology is as old as the history of the human race: early humans must have learned to discriminate between things that were good to eat and those that were not. In exploring their environment and searching for food, they observed the healing or harmful effects of plants and minerals, and that the bites of certain insects and reptiles caused illness or death. It was a natural progression to use injurious substances for hunting, in warfare, and for homicide. Arrow poisons were developed by ancient peoples in all parts of the world (with the possible exception of Australia and New Zealand), and many are still in use. Among the best known are the 'calabash curares' (derived mainly from varieties of *Strychnos* in South America), reptile poisons (venoms) from toads and salamanders in Central and South America, and ouabain, from African varieties of *Acocanthera* and *Strophanthus*.

Toxicology in the Ancient World

The earliest use of arrow poisons probably occurred in the Mesolithic Age, when arrows first began to appear. It is possible that Masai hunters who lived in Kenya 18 000 years ago may have used poison; evidence from other sites in Africa indicate later use (3000–1700 BC), and in ancient Egypt and Nubia poisoned arrows appear to have been used during the period 3100–300 BC.

In China, arrow poisons have been known to the Han and other peoples for at least 2500 years. They were used for both hunting and warfare, and documentary evidence indicates that the principal source of poison was *Aconitum*, the tubers of which yield aconitine. The same poison was also used in ancient India, where it was called visha and derived from a plant known as Bish. The hymns of the *Rg Veda* and *Atharva Veda* (1200–900 BC) show that poisoned arrows were used in war, and that the tubers of *Aconitum* were the major poison source. Later Buddhist and Sanskrit writings indicate the continued use of poisoned arrows and reveal that a second source of poison was decomposing snakes.

Among the peoples of the ancient Middle and Near East, the Egyptians, Assyrians, Sumerians, and Hebrews all had some knowledge of poisons, from which they developed a primitive pharmacology. Much of their experience was bound up with mysticism and the supernatural, and many details remain unclear. The Bible, where references are mostly to venoms (as in the Midrash and Talmud), does not contain a list of poisons or allude to their deliberate use. The Hebrew people most likely acquired information about poisons in Egypt, where they established a vibrant community after the destruction of Jerusalem in the sixth century BC.

Egyptian knowledge of poisons appears to have been highly advanced. The first pharaoh (or king), Menes, is said to have cultivated and studied poisonous and medicinal plants in about 3000 BC. Following his reign, information about animal, vegetable, and mineral poisons was accumulated in Egypt. The discovery in 1872 of the Papyrus Ebers, written about 1550 BC, revealed the extent of this knowledge. It is a compilation of medical prescriptions derived from much earlier sources and includes 829 prescriptions, of which 72% are quantified.

The text lists many possibly active drugs, including: sulfate, oxide, and other salts of lead used as astringents and demulcents; pomegranate and acanthus pith as vermifuges; sulfate and acetate of copper; magnesia, lime, soda, iron, and niter; oxide of antimony, sulfide of mercury; peppermint, fennel, absinth, thyme, cassia, coriander, carraway, juniper, cedar wood oil, turpentine, and many other essential oils; gentian and other bitters; mandrake, hyoscyamus, opium with other hypnotics and anodynes; linseed, castor oil, squills, colchicum, mustard, onion, nasturtium, tamarisk, frankincense, myrrh, and yeast.

Toxicology in Ancient Greece

The literature of ancient Greece contains many references to poisons and their use, none more famous than Plato's account of the death of Socrates. Condemned to death for impiety and corruption of youth, the Athenian philosopher swallowed a fatal dose of hemlock in 399 BC. This was the state method of execution, the poison being derived from the tubers of *Conium maculatum* (the 'spotted hemlock' or 'poison hemlock'); for quicker effect, it may have been mixed with opium. Other poisonous plants known to the Greeks included aconite, hellebore, mandrake, and henbane. The writings attributed to Hippocrates (460 to *c.* 375–350 BC), the 'father of medicine', included ~400 drugs of mainly plant origin and contained suggestions for managing poisoned patients primarily by limiting the absorption of toxic agents. During the reign of Attalus III (138–133 BC), poisonous plants were cultivated and used in experiments on condemned prisoners.

Rulers lived in fear of poison, and Mithridates VI Eupator, king of Pontus from 120 to 63 BC, spent years searching for a universal antidote to all poisons; he has been called the first experimental toxicologist. After investigating individual venoms, poisons, and antidotes, he combined all of the effective substances into one antidote, which he took daily to obtain, reportedly successfully, immunity to poison. His formula, called Mithridatium, survived in various forms until the nineteenth century. A variant derived from poisonous reptiles became known as theriac, and was equally long-lived in European pharmacopoeias.

Theriac became famous as a result of its association with the earliest extant work on poisonous animals, the *Theriaca* of Nicander of Colophon (second century BC). His poems describe venomous animals (snakes, scorpions, spiders, insects, and myriapods) and their bites as well as poisonous plants and prescribe specific remedies to counteract the effects of these poisons. Nicander's work was widely influential: successive Greek and Roman authors took much of their information on toxicology from him; he was read and cited for many centuries.

Following the work of Nicander, which included the beginnings of a scheme for identifying toxic agents by means of the symptoms they produce in human victims, a system of toxicology was developed between the second century BC and the first century AD. The Roman naturalist and historian Pliny the Elder (23/24–79 AD) described the biological effects of poisonous plants and animals in his *Historia Naturalis*. A contemporary, Pedanius Dioscorides, developed a classification scheme for poisons based on their origin (animal, vegetable, mineral) that remains convenient to this day. Dioscorides studied the medicinal properties of plants and minerals, and provided descriptions of ~600 plants and 1000 simple drugs, with the diseases they might cure, in his *Materia Medica*, the leading text in pharmacology for 16 centuries.

Mineral poisons were also well known in the ancient world. In particular, the ores and compounds of arsenic, antimony, copper, mercury, and lead were familiar to many cultures. Pseudo-Dioscorides detailed the poisonous effects of arsenic (meaning sometimes the sulfide, sometimes the white oxide), litharge (red lead or lead oxide), cinnabar (mercuric sulfide), and white lead (lead acetate). Hippocrates, Nicander, Dioscorides, Galen, and Paul of Aegina wrote clinical accounts of lead poisoning, of which there were occasional epidemics, and miners were known to be at risk from the fumes created by smelting processes.

The chronicles of ancient Greece contain few references to criminal poisoning, but the fact that Hippocrates required his students to swear that they would 'give no deadly medicine to anyone if asked, nor suggest any such counsel' implies that it existed. Suicide by poison was fairly common; the state gave permission and provided a lethal dose of hemlock. In the Roman Republic, however, criminal poisoning reached epidemic proportions as documented by Livy (59 BC–17 AD) in his *History of Rome*.

At the time of the civil wars in Rome, poisoning had become so common that the dictator Sulla issued the *Lex Cornelia* in 82 BC. This was the first legislative attempt to prevent poisoning, and it carried harsh penalties: banishment and confiscation of property if an offender was of noble birth, exposure to wild animals if of low status. Later interpretations extended the law to careless preparers of drugs. Despite this edict, however, homicidal poisoning continued to plague Rome, where a class of professional poisoners arose and practiced their skills with impunity. During the first century AD, the worst offenders were members of the ruling family, particularly Nero and his mother Agrippina, who used a variety of poisons, probably aconite, henbane, belladonna, arsenic, and poisonous fungi.

Islamic Toxicology

The death of Galen (~216 AD) marked the beginning of the transition of Western (i.e., Greek) medicine into monastic medical practice that resided in the hands of monks and was a part of their divine mission. As a result, the study of toxicology as a system of knowledge came to a halt in the Christian world

and did not reappear until the rise of the school of Salerno in twelfth-century Italy. Following the rise of Islam in the seventh century, scholarship shifted to Muslim centers, where Arab and Persian physicians dominated medical learning. They discovered Greek medicine through translations made from Byzantine manuscripts.

Several Indian medical texts containing information about poisons were available in translation and, together with the works of Greek authors, became key sources of information for Arab toxicologists. The most complete Arabic works on toxicology still extant are the *Book on Poisons* of ibn Jabir, the *Paradise of Wisdom* of al-Tabari, and the *Book on Poisons* of ibn Wahshiya – all dating to the ninth century AD. The *Canon* of ibn Sina, or Avicenna, and the *Treatise on Poisons and Their Antidotes* of Moses Maimonides (1135–1204) were particularly well known in medieval European universities and medical schools where works written in Greek and Arabic were made available in Latin translation after the eleventh century.

The physicians and alchemists of the Islamic world were the first to note the toxic properties of corrosive sublimate (mercuric chloride), and ibn Sina described the foul odor exhaled by victims of mercury poisoning. The replacement of arsenic trisulfide by white arsenic (arsenic trioxide) in poisonous preparations had a profound influence on the history of toxicology, as it became one of the most versatile and widely used poisons ever known. The medical works of Maimonides, a Jewish philosopher and physician in the service of the Sultan of Egypt, are still seen as modern in their approach to illness. The first part of his book on poisons described the effects of the bites of snakes and other animals, while the second part addressed poisoning with vegetable and mineral substances. He included advice on treating poisoned patients: he advised drawing animal poisons from the wound (sucking, cupping glasses, plasters) and employing antidotes (including theriac and Mithridatium); to treat poisoning by vegetable and mineral substances, he suggested inducing vomiting and purging. Some of his suggestions – suitable diet, keeping the patient awake, applying sedatives to the affected spot or internally – hold true today. The compositions of some of his medicinal recipes and their use according to the age of the patient are also relevant today.

Toxicology in the Middle Ages and Renaissance

One century later, Petrus of Abano (1250–1316), wrote *De Venenis* based on Greek and Arabic works. In it, he classified poisons as vegetable, mineral, and animal, and listed all known poisonous agents with their symptoms and treatment. He also suggested methods for avoiding the ingestion of poison and for neutralizing it if taken. Poison was frequently used for murder and political assassination in Italy in the later Middle Ages and Renaissance. Schools of poisoning arose in Rome, Naples, and Florence. In Venice, the records of the infamous Council of Ten listed the names of intended victims and the fees paid to poisoners for their services. By the seventeenth century, the activities of Italian poisoners had been redirected from political toward social, marital, and financial objectives. In Naples, Giulia Toffana (*c.* 1635–1719) sold arsenical solutions and supposedly poisoned over 600 people; in Rome, Hieronyma Spara conducted a similarly lucrative business (*c.* 1659), her clients being primarily young married women. Both were executed for their crimes.

Italian refinements to the 'art' of poisoning are said to have been introduced to France by Catherine de Medici in the sixteenth century. Favored poisons included arsenic mixed with the decomposition products of an animal to which it had been administered (corrosive sublimate was sometimes substituted), cantharides, and mixtures of arsenic, aconite, belladonna, and opium. Poisoning became a public menace, and in 1662 Louis XIV issued a decree forbidding apothecaries to sell poisons to anyone unknown to them and requiring purchasers to sign a register. A series of scandals soon brought about the downfall of professional poisoners. In 1679, the Chambre Ardente was appointed to investigate suspected poisoning cases, and within 3 years it had brought charges against 442 people. Of those executed, the most notorious was Catherine Deshayes, known as La Voisin: she was convicted of many murders, including those of 2000 infants.

The 'Affaire des Poisons' represented the culmination of the professional poisoners in France, but the fact that the crimes were brought to light owed more to the use of torture to extract confessions than to the ability of doctors or chemists to detect and identify poisons. It was not until the nineteenth century that experimental toxicology developed sufficiently to make such identification possible, but the foundations of this progress were laid much earlier, during the sixteenth century. The key figure in the change from reliance on traditional lore to reliance on objective investigation in science and medicine was Paracelsus (1493–1541), a controversial but influential physician, alchemist, and scientist. Although his science was mixed with mysticism and astrology, his contributions to medicine were revolutionary. Paracelsus rejected the medical theories of the

Greco-Arabic classics, insisted on the value of experimentation (including the use of animal tests), and developed the idea that minerals and chemicals could have medicinal applications (iatrochemistry). His use of mercury preparations in the treatment of syphilis led to accusations of poisoning, to which Paracelsus replied by writing the *Third Defense*. It contains the following important statement:

What is there that is not poison? All things are poison and nothing (is) without poison. Solely the dose determines that a thing is not a poison.

Consequently, toxicologists give credit to Paracelsus for this basic tenet of toxicology, dose-dependency.

Toxicology in the Eighteenth and Nineteenth Centuries

Another concept originated by Paracelsus, that chemicals have effects on specific organs of the body (target-organ toxicity), was developed by Felice Fontana (1730–1805). In his experimental studies of the venom of the European viper, Fontana discovered that the symptoms of poisoning caused by a bite were attributable to the direct action of venom on the blood. His findings contributed to the ongoing debate about whether drugs and poisons acted through the nerves, or by a process of absorption and transport in the blood. This debate stimulated chemical and physiological research throughout the seventeenth and eighteenth centuries. Together with advances in the analytical chemistry of animal and plant substances, and a mounting acceptance of animal experimentation, this contributed to the development of experimental toxicology as a distinct scientific discipline during the nineteenth century.

François Magendie (1783–1855), the first great experimental physiologist of the nineteenth century, laid the foundation for the systematic study of the mechanisms by which poisons act in the body with his investigation of the Javanese arrow poison *Upas tieuté*, later shown to contain strychnine. His pupil, Claude Bernard (1813–78), studied the nature of the action of curare on neuromuscular transmissions, effectively using a poison as an instrument for resolving important physiological problems. In addition, Bernard suggested that carbon monoxide poisoning occurs as a result of tissue asphyxiation caused by an irreversible combination with hemoglobin, preventing the effective transport of oxygen to body tissues. Another of Magendie's students, James Blake (1815–93) performed research on the relationship between the chemical structure of a drug and its biological activity, supporting the concept of target-organ toxicity. Additional research on structure–activity relationships was conducted in Britain, perhaps the most sophisticated being that of Alexander Crum Brown (1838–1922) and Thomas Fraser (1841–1920) on organic alkaloids. The successes of the experimental method in physiology, combined with advances in analytical chemistry, stimulated the development of pharmacology. The complementary nature of toxicological and pharmacological research during the nineteenth century was embodied in the work of the Germans Rudolf Kobert (1854–1918), who studied the digitalis glycosides and the ergot alkaloids, and Louis Lewin (1850–1929), an expert on narcotics, alcohols, poisonous gases, and arrow poisons.

The chemical approach to the study of poisons was pioneered by a man long considered the founder of modern toxicology, Mathieu Joseph Bonaventura Orfila (1787–1853). Orfila put toxicology on a firm quantitative basis by introducing new, primarily chemical, experimental methods for proving lethal intoxications – replacing diagnoses made solely on the basis of observed features. A trained chemist and physician, he performed experiments on thousands of dogs, the basis for his monumental work: *Traité des poisons tirés des règnes minéral*, *végétal et animal*, *ou toxicologie générale*, *considérée sous les rapports de la physiologie*, *de la pathologie et de la médecine légale*, published in 1814–15. The book examined the physiological and pathological effects of poisons, the symptoms of poisoning, antidotes, the chemical properties of poisons, and analytical methods for detecting them. This was the first systematic attempt to correlate chemical and biological information concerning known poisons and was unique in combining the use of postmortem examination with analytical chemistry.

As the leading medicolegal expert of his time, Orfila made considerable contributions to legal (forensic) medicine such as the discovery that poisons are absorbed from the gastrointestinal tract and accumulate in tissues specific to each poison. Previously, a chemist or a physician who found nothing in the stomach would not have examined the other organs of the body. In Britain, the development of forensic toxicology was stimulated by one of Orfila's pupils, (Sir) Robert Christison (1797–1882), who wrote *A Treatise on Poisons in relation to medical jurisprudence*, *physiology and the practice of physic* – the first textbook of its kind written in English. He regarded toxicology as the principal branch of medical jurisprudence, its object being to unite evidence from four sources (pathology, chemistry, physiology, and visible symptoms) to detect crime.

The works of Orfila and Christison, which were widely read and translated, laid the foundation for the development of forensic toxicology in the

nineteenth century. Orfila was the first (1839) to extract arsenic from human organs other than gastrointestinal tissue; in 1840, his analysis of organ samples resulted in the conviction of Marie Lafarge for the murder of her husband. The method used was based upon Scheele's discovery (1775) that when zinc and acid act on arsenic salts, a gaseous compound (arsine) is evolved, that, when burned, deposits metallic arsenic. This qualitative procedure was modified by Berzelius to permit quantitative evaluation of metals. Three years later, Fresenius and von Babo devised a method for quantitating all mineral poisons, using wet ashing with chlorine. Other quantitative methods were developed soon afterward.

Newer methods of chemical analysis led to the isolation of the major alkaloids from crude drug preparations. By 1833, aconitine, atropine, codeine, hyoscyamine, morphine, nicotine, and strychnine had been isolated from plants. Color tests for alkaloids were developed between 1861 and 1882; by 1890 quantitative analysis methods became available. Physiological tests for alkaloids, particularly strychnine, first used in 1856, were employed well into the twentieth century. Tests for alcohol, devised by Lieben (iodoform crystal test, 1870) and others, were later perfected for the quantitative analysis of alcohol in body fluids and tissues. Qualitative tests for carbon monoxide in the blood were developed about this time and in 1880, Fodor developed a palladium chloride reduction method to quantitate carbon monoxide in blood.

Textbooks of forensic medicine and toxicology proliferated throughout the nineteenth century. In Britain, the work of Christison was complemented by that of Alfred Swaine Taylor (1806–80), an eminent medico-legal expert who wrote texts that incorporated legal precedents and judicial rulings. These became standard references for over a century; the most recent, thirteenth edition, of *The Principles and Practice of Medical Jurisprudence* appeared in 1984. In 1848, O.H. Costill wrote the first book in the United States pertaining to the symptoms and treatment of poisoning: *A Practical Treatise on Poisons*. In 1867, Theodore Wormley (1826–97) published the first American text devoted exclusively to the experimental detection of poisons in organic mixtures, *The Microchemistry of Poisons*. Soon after, John Reese produced a similar book (*Manual of Toxicology*, 1874), which he followed up a decade later with *A Text Book of Medical Jurisprudence and Toxicology* (1884). During the late nineteenth and early twentieth century, a great amount of toxicological data was presented in the thorough textbooks of German scientists, particularly Kobert (*Compendium der praktischen Toxikologie*, 1887) and Lewin (*Gifte und Vergiftungen*, 1929). Lewin is especially remembered as the author of a toxicologist's view of world history: *Die Gifte in der Weltgeschichte* (1920).

Toxicology in the Twentieth Century

The early part of the twentieth century marked the beginning of the development of the modern science of toxicology. However, the most rapid growth of the discipline occurred after the Second World War, as the production of organic molecules for use as drugs, pesticides, and industrial chemicals began to increase at an exponential rate. Today, toxicology is concerned with the many chemicals that may cause toxicity in the outdoor, indoor, and occupational environments. Modern toxicology utilizes skills and knowledge derived from pathology, pharmacology, physiology, biochemistry, chemistry, and statistics to quantitate the effects of chemicals on living tissue.

Research on anesthetic gases during the nineteenth century facilitated the development and use of poisonous war gases in the twentieth. This led to attempts to counteract the effects of chemical warfare agents and other toxic compounds, particularly arsenicals, introduced by Paul Ehrlich (1854–1915) for the treatment of syphilis. This resulted in the synthesis of the first specific chemical antidote, British anti-Lewisite (BAL), in 1945 by R.A. Peters, L.A. Stocken, and R.H.S. Thompson in Oxford. Studies on the mechanistic bases for toxicity were applied to the synthesis of effective insecticides. For example, during the 1940s, the Swiss chemist Paul Müller discovered a compound, now known as DDT, that poisons insects on contact.

With the increasing use of synthetic drugs and chemicals, toxicology assumed an important role in public health: the protection of workers and the public from the adverse effects of chemical exposure. In Britain, the systematic application of scientific techniques to the detection and control of food and drug adulteration arose largely as a result of the work of the Society of Public Analysts. In the United States, concerns about the adulteration of foods led to the passage of the Food and Drug Act in 1906. This law, created under the impetus of H.W. Wiley (1844–1930), the head of the Bureau of Chemistry of the US Department of Agriculture, influenced food safety legislation worldwide. Since that time numerous laws, in the United States and elsewhere, have been established to minimize public encounters with harmful chemicals in the environment and consumer products. To carry out these laws, toxicologists are needed to provide accurate safety assessments of new and existing chemicals; particularly to establish the

dose–response relationship for both short- and long-term toxicity.

The principal method used for assessing the safety of drugs, pesticides, food additives, and other chemicals is animal testing, which can usually reveal the range of potential toxic effects. Over the past 50 years testing guidelines have been developed and modified. For example, the thalidomide catastrophe of 1961 led to significant changes in existing tests for reproductive and developmental effects. It is recognized that laboratory studies cannot always establish the full toxicity of the test agent in humans, and safety factors are used to compensate for limitations in testing protocols and the possible differences in response between humans and the test species. In addition, increasing social disquiet in regard to animal testing and animal welfare has led to the development of alternative methods in toxicology, particularly *in vitro* assays, which can sometimes serve as substitutes for live animal testing.

Modern toxicology may be divided into six principal areas of application: regulatory, occupational, environmental, clinical, forensic, and analytical. In the United States, the eminent regulatory toxicologist Arnold J. Lehman (1900–79) was instrumental in strengthening the commitment of the Food and Drug Administration (FDA) to toxicology. In 1955, he and his staff at the FDA published *Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics,* the first agency guidelines for toxicological studies. These guidelines have been very influential in the subsequent approaches adopted by agencies in the United States and elsewhere to assess and manage risks from chemicals in the environment and in foods and consumer products. Lehman's statement that "anyone can become a toxicologist in two easy lessons, each of which takes 10 years" has achieved the status of an adage among practicing scientists.

Occupational and environmental toxicologists study and monitor the causes, conditions, and effects of exposure to chemicals in the workplace and environment. In some cases, the same chemical may show toxicity in both the industrial and environmental setting: for example, lead and other heavy metals. In addition, toxicologists have shown that some chemicals that have beneficial effects on human health, for example, DDT, may also have adverse effects ecologically. Public awareness of this dichotomy was heightened as a result of the publication of *Silent Spring* in 1962. The author, Rachel Carson, touched off a heated debate about the links between industrialization and pollution when she claimed that "we have put poisonous and biologically potent chemicals indiscriminately into the hands of persons largely or wholly ignorant of their potentials for harm." Although highly controversial, the book strongly stimulated the study of chemical effects on ecosystems and the development of more stringent regulation of environmental contaminants.

When cases of intoxication occur, the need for clinical toxicology becomes apparent: physicians are expected to make a correct diagnosis and implement appropriate treatment, which may involve delaying the absorption of the poison and/or enhancing its elimination. Today, accidental and intentional self-poisoning contribute significantly to morbidity and mortality in many countries, most as a result of household chemicals, drugs, pesticides, solvents, and carbon monoxide. The establishment of poison control centers, the first of which opened in Chicago in 1953, has facilitated the compilation of information on the ingredients of pharmaceuticals and other industrial products and their toxicity, and has led to the creation of sophisticated information distribution systems. The ultimate aim is to quickly and accurately supply information to aid the diagnosis, treatment, and prevention of poisoning. Similar centers have been set up in many other countries.

Since poisons continue to be significant causes of death and disease, forensic and analytical toxicology remain important sciences. Both employ some of the same methods and techniques but for different ends. Forensic toxicology is concerned with intentional and accidental poisonings in relation to law, while analytical toxicology deals with the detection, identification, and measurement of poisons and their metabolites in both biological and environmental matrices. Before the advent of spectroscopic and chromatographic methods in the early 1950s, chemical techniques for separating and identifying the increasing number of synthetic chemicals were time consuming and lacking in sensitivity. Analytical capabilities have increased greatly in recent decades, permitting rapid tests for a variety of compounds. For example, gas and high-performance liquid chromatography, together with immunoassay techniques, now allow quantitation of most organic drugs. Low concentrations of metals can be quantitated using mass spectrometric, electrochemical, radiochemical, and spectrophotometric methods.

During the past three decades, toxicology research has been increasingly devoted to a quantitative assessment of the probable health risks posed by chemicals to which humans might be exposed. In a society that is increasingly risk averse, toxicological information is heavily relied upon by regulatory agencies for prioritizing and managing environmental health concerns. Environmental regulations and the associated

risk assessments have, in effect, become the driving force behind the practice of toxicology in the United States and, more recently, in the European Union. Toxicity testing requirements are in fact remarkably similar across international boundaries.

Advances in risk assessment depend on a growing foundation of scientific knowledge, particularly increased understanding of toxicology at the molecular level. This has resulted from advances in molecular biology such as methods for the sequencing of nucleic acids as well as biochemical (enzyme-oriented) approaches to the study of the metabolism of drugs and environmental toxicants. These advances have contributed to a better understanding of the nature, site, and mechanism of action of toxicants. Once the mechanism of toxicity of a compound is understood, it may be possible to design a replacement chemical that retains desirable properties but is less toxic. The role played by genes in metabolic activation and detoxification constitutes another leading area of research in modern toxicology.

Currently, a great deal of attention is being focused on two new areas of toxicology – toxicogenetics and toxicogenomics. Toxicogenetics is the study of variations in human heritable make-up related to differences in susceptibility to the adverse effects of exogenous agents. Research on toxicogenomics, on the contrary, focuses on changes in gene expression resulting from exposure to xenobiotics. It is expected that advances in these complementary areas of study will lead to greater ability to make individual-based rather than population-based predictions of adverse effects in humans as a consequence of exposures to potentially toxic substances. Such predictions will reduce the need to utilize arbitrary safety factors in risk assessment and risk management.

The development of toxicology as a recognized scientific discipline has proceeded at a rapid pace since the end of the Second World War. One of the most important consequences has been the establishment of training programs and the founding of scientific journals and societies. Graduate education in North America and Western Europe reflects the multidisciplinary nature of toxicology, as it is administered by a variety of university departments, including medicine (human and veterinary), pharmacy, pharmacology, and chemistry. The modern toxicologist is thus a specialist in one or more branches of the field, as it becomes increasingly difficult for one individual to be qualified in all aspects of the science. This specialization is mirrored in the very many national and international organizations and journals that are dedicated to toxicology and related subjects. Toxicologists remain united, however, in their ultimate objective, which is understanding the basis of the morbidity and mortality that occurs in humans and other living systems as a result of exposures to toxic substances.

See also: Animals, Poisonous and Venomous; BAL (British Antilewisite); Food and Drug Administration, US; Toxicology.

Further Reading

Borzelleca JF (1994) History of toxicology. In: Hayes AW (ed.) *Principles and Methods of Toxicology*, 3rd edn., pp. 1–17. New York: Raven Press.

Gallo MA (2001) History and scope of toxicology. In: Klaassen CD (ed.) *Casarett and Doull's Toxicology: The Basic Science of Poisons*, 6th edn., pp. 3–10. New York: McGraw-Hill.

Lewin L (2000) *Die Gifte in der Weltgeschichte: Toxikologische allgemeinverständliche Untersuchungen der historischen Quellen*. Cologne: Parkland Verlag (Reprint of 1920 original, published by Springer-Verlag, Berlin).

Touwaide A (1992) Studies in the history of medicine concerning toxicology after 1970. *Newsletter Society of Ancient Medicine* 20: 8–33.

Watson KD, Wexler P, and Everitt JM (2000) History. In: Wexler P (ed.) *Information Resources in Toxicology*, 3rd edn., pp. 1–25. San Diego: Academic Press.

Wax PM (1998) History. In: Goldfrank LR, Flomenbaum NE, Lewin NA, Weisman RS, Howland MA, and Hoffman RS (eds.) *Goldfrank's Toxicologic Emergencies*, 6th edn., pp. 1–18. Stamford, CT: Appleton & Lange.

Toxicology, Intuitive

Pertti J Hakkinen

Humans have always been intuitive toxicologists via the use of the senses of sight, taste, and smell to try to detect harmful or unsafe food, water, and air. Relying only on these senses is inadequate to assess all potential dangers, and complicating matters is that even the best scientific approaches used in human risk assessment depend on extrapolations and judgments when assessing animal and other toxicology data. This has led to the study of the intuitive elements of expert and public risk judgments involved with

exposure assessment, toxicology, and risk assessment, usually called the study of 'intuitive toxicology'.

The studies of intuitive toxicology have surveyed toxicologists (e.g., members of the Society of Toxicology) and others about a wide range of attitudes, beliefs, and perceptions regarding risks from chemicals. These have included basic concepts, assumptions, and interpretations related to the effects of chemical concentration, dose, and exposure on risk, and the value of animal studies for predicting the effects of chemicals on humans. The chemicals covered in these studies have included those used in pesticides, food additives, industrial chemicals, household cleaning agents, and prescription and nonprescription drugs.

Two statements studied repeatedly in intuitive toxicology studies are:

- Would you agree or disagree that the way an animal reacts to a chemical is a reliable predictor of how a human would react to it?
- If a scientific study produces evidence that a chemical causes cancer in animals, then we can be reasonably sure that the chemical will cause cancer in humans.

Noteworthy findings from studies of intuitive toxicology in the United States, Canada, and the United Kingdom include:

- The public is more likely than toxicologists to think chemicals pose greater risks.
- The public finds it difficult to understand the concept of dose–response relationships.
- The public is much more likely than toxicologists to think the results of animal carcinogenicity studies can be applied to humans.
- Much disagreement between toxicologists about how to interpret various results, for example, a study in the United States noted, "among the most important findings in this study was…the high percentage of toxicologists who doubted the validity of the animal and bacterial studies that form the backbone of their science."
- Fewer toxicologists in industry than in university or government jobs agreed that animal carcinogens could reasonably be expected to cause cancer in humans.
- Technical judgments of toxicologists were also found to be associated with factors such as gender, age, and the level of agreement with various 'worldview' statements.

The demographic information for experts gathered in these studies has included the highest academic degree earned, fields of study, age, sex, race, health, organizational affiliation, and current position at work. The demographic information for the lay public gathered as part of the studies has included education, age, sex, marital status, race, children, health, present employment status, career, and annual household income.

As highlighted above, studies of intuitive toxicology have yielded a number of intriguing findings, and have highlighted some important risk communication and other challenges. Large differences in responses to intuitive toxicology questions and statements can exist between toxicologists and laypeople. Further, meaningful differences exist between toxicologists working in industry, academia, and government, including sharp divisions in their opinions about the ability to predict a chemical's effect on human health when basing the prediction on results from animal studies. Although these studies have identified misconceptions that experts should try to clarify for the public, the results also suggest that disagreement among experts, especially as perceived by the news media and the public, can play a key role in controversies over chemical risks.

Further Reading

Hakkinen PJ (1993) Commentary about intuitive toxicology: Expert and lay judgments of chemical risks. *Comments on Toxicology* 4: 505–509.

Kraus N, Malfors T, and Slovic P (1992) Intuitive toxicology: A comparison of expert and lay judgements of chemical risks. *Risk Analysis* 12: 215–232.

Slovic P (1999) Trust, emotion, sex, politics, and science: Surveying the risk-assessment battlefield. *Risk Analysis* 19: 689–701.

Slovic P, Malmfors T, and Krewsky (1995) Intuitive toxicology. II. Expert and lay judgements of chemical risks in Canada. *Risk Analysis* 10: 661–675.

Slovic P, Malmfors T, Mertz CK, Neil N, and Purchase IFH (1997) Purchase, evaluating chemical risks: Results of a survey of the British Toxicology Society. *Human & Experimental Toxicology* 16: 289–304.

Trans Fatty Acids

Pertti J Hakkinen

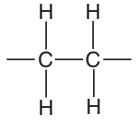
Trans fatty acids, also called trans fat, occur in foods when manufacturers use hydrogenation, a process in which hydrogen is added to vegetable oil, to turn the oil into a more solid fat. Trans fat is often found in the same foods as saturated fat, including vegetable shortening, some margarines, crackers, candies, cookies, snack foods, fried foods, baked goods, salad dressings, and other processed foods. In addition, a small amount of trans fat is found naturally, primarily in some animal-based foods.

The Types of Fatty Acids

There are three main types of fatty acids: saturated, monounsaturated, and polyunsaturated. All fatty acids are chains of carbon atoms with hydrogen atoms attached to the carbon atoms. A saturated fatty acid has the maximum possible number of hydrogen atoms attached to every carbon atom. Fatty acids missing a pair of hydrogen atoms in the middle of a chain, leaving two carbon atoms connected by a double bond rather than a single bond, are 'unsaturated'. A fatty acid with one double bond is called 'monounsaturated' and fatty acids having more than one gap are called 'polyunsaturated'.

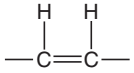
The fat in foods contains a mixture of saturated, monounsaturated and polyunsaturated fatty acids. In foods of animal origin, a large proportion of fatty acids are saturated. In contrast, in foods of plant origin and some seafood, a large proportion of the fatty acids are monounsaturated and polyunsaturated. The structures of saturated and unsaturated chemical bonds are shown below.

Saturated fat
(i.e., saturated fatty acid)



Carbon–Carbon
single bond

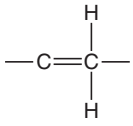
Unsaturated fat
(i.e., unsaturated fatty acid)



Carbon–Carbon
double bond

The hydrogenation used to make trans fats increases the shelf life and flavor stability of foods containing these fats. Usually, the hydrogen atoms at a double bond are positioned on the same side of the carbon chain. However, partial hydrogenation reconfigures some double bonds and the hydrogen atoms end up on different sides of the chain. This type of configuration is called 'trans' ('across' in Latin). The structure of a trans unsaturated chemical bond is shown below.

Trans fat
(i.e., *trans* fatty acids)



Hydrogen atoms are on opposite sides of the chain of carbon atoms at the carbon–carbon double bond.

the Food and Drug Administration's (FDA's) regulatory chemical definition for trans fatty acids is all unsaturated fatty acids that contain one or more isolated (i.e., nonconjugated) double bonds in a trans configuration. Under the Agency's definition, conjugated linoleic acid would be excluded from the definition of trans fat.

The Health Impact

Scientific reports have confirmed the relationship between trans fat and an increased risk of coronary heart disease. Trans fat, like saturated fat and dietary cholesterol, can raise low-density lipoprotein (LDL or 'bad' cholesterol) levels in the blood. An elevated LDL cholesterol level increases the risk of developing coronary heart disease. Unlike saturated fat, trans fat also lowers high-density lipoprotein (HDL or 'good') cholesterol in the blood.

US Food and Drug Administration Actions

The Food, Drug, and Cosmetic Act (FD&C Act) provides the FDA with statutory authority to require food and nutrition labeling. Two sections of the FD&C Act provide the legal authority, including Section 403(a) requiring foods to be adequately labeled and that material facts be disclosed to consumers, and Section 403(q) (2) (A) giving the authority to require additional nutrients to be included in nutrition labeling if such information will "assist consumers to maintain healthy dietary practices." A goal of the Nutrition Facts panel part of labeling is to provide consumers with more information to make

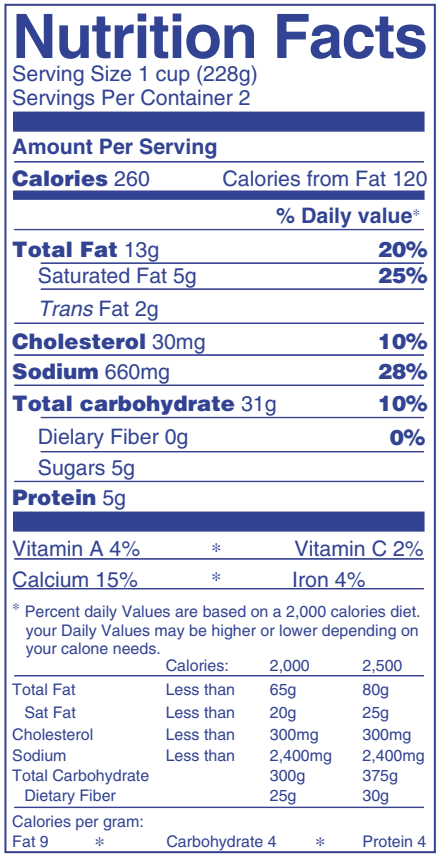
healthier food choices that could lower their consumption of trans fat as part of a heart-healthy diet.

When the 1993 Nutrition Labeling and Education Act regulations were finalized, FDA did not require trans fat to be listed on the Nutrition Facts panel because the scientific evidence was not conclusive about the relationship between trans fat intake and increased blood cholesterol levels. In 1994, the Center for Science in the Public Interest, a consumer advocacy organization, filed a petition (amended in July 1998) with FDA requesting that the agency take steps to require trans fat to be listed on nutrition labels. In response to that petition, FDA issued a proposed rule in the Federal Register on November 17, 1999, proposing to amend the regulations to require that trans fat be listed on nutrition labels. In response to comments and evolving science, FDA reopened the comment period on December 5, 2000 and November 15, 2002. FDA received over 1650 letters in response to the November 1999 proposal, over 45 letters in response to the December 5, 2000 notice reopening the comment period, and over 25 letters in response to the November 15, 2002 proposal and notice to reopen the comment period.

FDA reviewed the scientific evidence and recommendations of various scientific bodies, including the Institute of Medicine, National Academies of Science, an expert panel for the National Cholesterol Education Program, and the Advisory Committee on the Dietary Guidelines for Americans 2000. On July 9, 2003, the FDA issued a regulation requiring manufacturers to list trans fatty acids on the Nutrition Facts panel of the labels of foods and some dietary supplements. The new requirement will mean that manufacturers of most conventional foods and some dietary supplements will have to list in the Nutrition Facts panel the trans fat content of the product, in addition to the information about its overall fat content and saturated fat content. Dietary supplement manufacturers will need to list trans fat, as well as saturated fat and cholesterol, on the Supplement Facts panel when their products contain more than trace amounts (0.5 g) of trans fat. Examples of dietary supplements that may contain trans fat are energy and nutrition bars.

The new information is the first significant change on the Nutrition Facts panel of the labeling since it was established in 1993. Food manufacturers have until January 1, 2006, to list trans fat on the nutrition label. The FDA estimates that by 3 years after that date, trans fat labeling will have prevented from 600 to 1200 cases of coronary heart disease and 250–500 deaths each year, and that the changes in regulations will save between US$900 million and $1.8 billion each year in medical costs, lost productivity and pain and suffering. However, while the relationship between trans fat and an increased risk of coronary heart disease has been confirmed, no studies, reports, or expert panels have provided a reference value for trans fat or any other information that the FDA believed to be sufficient to establish a daily reference value. Thus, FDA does not intend to include a percent daily value (%DV) in the Nutrition Facts panel.

The FDA final rule on trans fat requires that the amount of trans fat in a serving be listed on a separate line under saturated fat on the Nutrition Facts panel (see below). However, trans fat does not have to be listed if the total fat in a food is less than 0.5 g per serving and no claims are made about fat, fatty acids, or cholesterol content. If it is not listed, a footnote will be added stating that the food is "not a significant source of trans fat." Further, food manufacturers are allowed to list amounts of trans fat with less than 0.5 g as 0 (zero) on the Nutrition Facts panel. As a result, consumers could see products that list 0 g trans fat on the label, while the ingredient list will have 'shortening', 'partially hydrogenated vegetable oil', or 'hydrogenated vegetable oil' on it. This means the food contains very small amounts (less than 0.5 g) of trans fat per serving.



Nutrition Facts

Serving Size 1 cup (228g)
Servings Per Container 2

Amount Per Serving

Calories 260 Calories from Fat 120

% Daily value*

Total Fat 13g **20%**
Saturated Fat 5g **25%**
Trans Fat 2g
Cholesterol 30mg **10%**
Sodium 660mg **28%**
Total carbohydrate 31g **10%**
Dielary Fiber 0g **0%**
Sugars 5g
Protein 5g

Vitamin A 4% * Vitamin C 2%
Calcium 15% * Iron 4%

* Percent daily Values are based on a 2,000 calories diet. your Daily Values may be higher or lower depending on your calone needs.

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Sat Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 * Carbohydrate 4 * Protein 4

In addition, in partnership with the (US) National Heart, Lung and Blood Institute of the National Institutes of Health, health and consumer organizations, and trade associations, FDA wants to educate consumers on the importance of lowering their intake of saturated and trans fats by developing consumer education materials for its nutrition and food labeling website. Further, FDA is interested in establishing new nutrient content and health claims about trans fat, as well as possibly having footnote or disclosure statements on the label that could enhance consumer's understanding about saturated fat, trans fat, and cholesterol in order to help them make heart-healthy food choices. Finally, FDA plans to conduct a study on whether nutrient content claims could be made for products with either 'reduced' or 'zero' trans fatty acids in a way that would not mislead consumers when there are significant amounts of saturated fat present.

See also: Food and Drug Administration, US.

Further Reading

Kris-Etherton PM and Etherton TD (2003) The impact of the changing acid profile of fats on diet assessment and health. *Journal of Food Composition and Analysis* 16: 373–378.

Relevant Websites

http://www.cfsan.fda.gov – US Food and Drug Administration Federal Register Final Rule: *Trans* Fatty Acids in Nutrition Labeling, Nutrient Content Claims, and Health Claims. Guidance on How to Understand and Use the Nutrition Facts Panel on Food Labels, July 11, 2003.

http://www.fda.gov – US Food and Drug Administration. FDA Acts to Provide Better Information to Consumers on Trans Fats.

Transgenic Animals

Kartik Shankar and Harihara M Mehendale

Understanding the molecular mechanisms of toxicity is a central part of advancing the processes of drug development and regulatory decision-making. Due to the complex nature of the varied interactions that leads to eventual toxic outcome, it is a daunting challenge in most cases to identify the role of a single gene (or signaling thereof) in mediating an adverse outcome. Genetic manipulations of organisms have been a powerful and staple technique in the biosciences for almost two decades. Mutant *Drosophila* lacking specific genes have been employed to identify the roles of specific genes. However, the use of these techniques in rodent models to identify mechanisms of toxicity is relatively new. Currently, transgenic rodents (specially mice) are being used for a number of reasons including, understanding roles of specific metabolizing enzymes, receptors, transcription factors, regulatory sequences of promoters to just name a few. Although transgenic manipulations of rodents can broadly involve a large number of genetic manipulations the two most commonly used manipulations by far are deletion of a gene (gene knockout or 'null') or expression (or overexpression) of a particular gene. The animal model of choice in biomedicine remains the mouse (*Mus musculus*).

Creating a Knockout Animal

The cursory protocol of creating a gene knockout mouse for a particular gene begins with creating a gene-targeting construct that is transfected into embryonic stem (ES) cells to silence a particular gene. ES cells that have successfully included the recombination are injected into a blastocyst, which is implanted in a pseudo-pregnant female. After a series of back and self-crosses, a mouse homozygous for the mutation is generated. A variety of methods exist to selectively target genes including targeted gene deletion, gene trapping, and the Cre/*lox*P recombinase system.

A protocol of creating a gene knockout mouse is given below:

1. Prepare gene-targeting construct.
2. Transfect embryonic stem cells.
3. Select for transfectants.
4. Confirm recombination.
5. Introduce ES cells into blastocyst.
6. Transfer blastocyst into pseudo-pregnant female.
7. Breed chimeric mouse.
8. Back and self-crosses to gene homozygous knockout.

Applications in Toxicology

In toxicology, the main utility of transgenic or gene-knockout mice has been to understand the

mechanisms of toxicity of chemicals. A classic example is the elucidation of the mechanism of the bioactivation of acetaminophen. Acetaminophen is a commonly use analgesic, antipyretic drug with high potential for liver toxicity at supra-pharmacological doses. Although, it has been clear that a reactive intermediate is necessary for toxicity, the P450 isozymes involved are were not clear. Studies from Lee *et al.* unequivocally demonstrated that mice lacking CYP2E1 were remarkably resistant to acetaminophen-induced liver injury. Further mice lacking both P450s, CYP2E1 and CYP1A2 were even more resistant to acetaminophen toxicity than CYP2E1 null mice alone. Mechanisms of not only drug metabolism/bioactivation pathways but also information about critical molecular factors involved in mediating or mitigating toxicity has been uncovered using transgenic and knockout animals.

See also: Mechanisms of Toxicity.

Further Reading

Gonzalez FJ and Kimura S (1999) Role of gene knockout mice in understanding the mechanisms of chemical toxicity and carcinogenesis. *Cancer Letters* 143: 199–204.

Lee SS, Buters JT, Pineau T, Fernandez-Salguero P, and Gonzalez FJ (1996) Role of CYP2E1 in the hepatotoxicity of acetaminophen. *The Journal of Biological Chemistry* 271: 12063–12067.

Wolf CR and Henderson CJ (1998) Use of transgenic animals in understanding molecular mechanisms of toxicity. *The Journal of Pharmacy and Pharmacology* 50: 567–574.

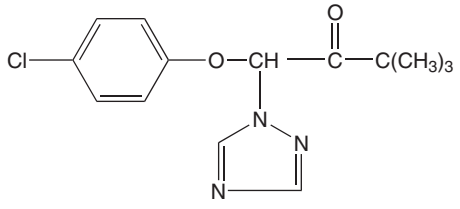
Zaher H, Buters JT, Ward JM, *et al.* (1998) Protection against acetaminophen toxicity in CYP1A2 and CYP2E1 double-null mice. *Toxicology Applied Pharmacology* 152: 193–199.

Triadimefon

Marcia D Howard

This article is a revision of the previous print edition article by Todd A Bartow, volume 3, pp. 371–372, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 43121-43-3
- Synonyms: 1,2,4-triazole; 1H-1,2,4-triazole, 1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazolyl)-2-butanone; 1-(4-Chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)-2-butanone; Acizol; Amiral; Bayleton; Bay MEB 6447
- Chemical/Pharmaceutical/Other Class: Systemic triazole fungicide
- Chemical Formula: $C_{14}H_{16}ClN_3O_2$
- Chemical Structure:



Uses

Triadimefon is a fungicide used to control powdery mildews, rust and other fungal pests on cereals, fruits, vegetables, turf, shrubs, and trees.

Exposure Routes and Pathways

Exposure to triadimefon may occur via inhalation, dermal contact and absorption, eye contact, and by ingestion of contaminated foods.

Toxicokinetics

Triadimefon is absorbed through dermal, inhalation or oral exposure routes. Triadimefon has a half-life of 2.5 h in blood plasma. Metabolism occurs in the liver resulting mostly in the formation of triadimenol and glucuronic acid conjugates. In mammals, 83–98% is excreted unchanged in the urine and feces within 2–3 days following oral administration. Metabolism of triadimefon occurs more rapidly in male rats compared to females. Radioactivity in male rats was found mainly in feces but more equally distributed between the urine and feces in females.

Mechanism of Toxicity

Triadimefon binds to hepatic cytochrome P450 and inhibits microsomal enzyme activities. It inhibits sterol demethylation and thus sterol synthesis. Fungi sensitive to triadimefon utilize ergosterol as the primary sterol, the production of which is inhibited. It is also thought that triadimefon may have actions similar to those caused by indirect-acting dopamine agonists.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute effects on the central nervous system may occur. Minimum irritation to the conjuctiva was observed in rabbits with irritation resolved within 1 day. Triadimefon is a skin sensitizer according to primary dermal sensitization studies. There was slight dermal irritation in rabbits. Acute dermal toxicity LD_{50} is $>2000\,mg\,kg^{-1}$ for rats and rabbits. However, absorption following dermal exposure occurs more rapidly in young rats compared to adults. LD_{50} values are reported to be 90–1500 $mg\,kg^{-1}$ (rats), $500\,mg\,kg^{-1}$ (rabbits and dogs), and $1000\,mg\,kg^{-1}$ (mice) following acute oral exposure. Values for LC_{50} following a 4 h exposure were similar in rats and mice ($0.48\,mg\,l^{-1}$).

Human

Mild toxicity generally results from oral or dermal exposure. Triadimefon may cause dermal sensitization and moderate irritation of the eyes.

Chronic Toxicity (or Exposure)

Animal

The no-observed-effect level (NOEL) in dogs was 100 ppm ($2.5\,mg\,kg^{-1}\,day^{-1}$) in a 2 year feeding study. Studies in rats and rabbits suggest that triadimefon has little or no teratogenic potential. In rats, the teratogenic NOEL is $50\,mg\,kg^{-1}\,day^{-1}$. Both maternal toxicity and reproductive NOELs were 300 ppm in rats three-generation reproductive toxicity studies. Responses to low or moderate doses of the toxicant in rats, mice and dogs included decreased body weight, increased cholesterol levels, and increased liver weights.

In both male and female rats, the neurobehavioral NOEL was $2\,mg\,kg^{-1}$ based on hyperactivity and increased motor activity. Triadimefon has been shown to increase motor activity and stereotyped response in a manner resembling psychomotor stimulants by potentiation of dopaminergic transmission. One study noted enhanced locomotor and stereotypic behavioral patterns in Sprague–Dawley rats treated with repeated triadimefon exposure ($100\,mg\,kg^{-1}$ every other day for 14 days). Furthermore, this study suggested that chronic exposure to triadimefon may involve effects of dopamine uptake in the striatum and nucleus accumbens.

Human

No deleterious effects or symptoms are anticipated from chronic exposure under normal usage.

In Vitro Toxicity Data

An *in vitro* study by Vinggaard and colleagues in 2000 reported that triadimefon was able to inhibit aromatase activity in human placental microsomes ($IC_{50} = 32\,\mu mol\,l^{-1}$).

Clinical Management

Eyes should be flushed with copious amounts of water for 15 min. Further medical attention should be sought if irritation persists or develops after flushing. For dermal exposure, contaminated clothing should be removed and the affected area washed with soap and water. A physician should be consulted if irritation develops or persists. In cases of poisoning by ingestion, a physician should be contacted or poison control should be called immediately. Vomiting should be induced after administering one to two glasses of water to the victim. Vomiting should not be induced, nor anything given orally if the person is unconscious. If suffering from inhalation exposure, the exposed individual should be removed to fresh air or an uncontaminated area. Artificial respiration (e.g. cardiopulmonary resuscitation) should be administered if the victim is not breathing. Medical treatment should be sought as soon as possible.

Environmental Fate

Triadimefon is reported to have a half-life in soil of a few weeks to a few months. Its primary metabolite is more persistent.

Ecotoxicology

Triadimefon toxicity in wild birds is thought to be low while acute toxicity to aquatic invertebrates is moderate. LD_{50} values for quail and ducks ranged from 2 to $4\,g\,kg^{-1}$. Triadimefon is not toxic to bees.

Exposure Standards and Guidelines

The reference dose for triadimefon is 0.04 $mg\,kg^{-1}\,day^{-1}$ while the acceptable daily intake is $0.03\,mg\,kg^{-1}\,day^{-1}$.

See also: Biocides; Neurotoxicity; Pesticides.

Further Reading

Hill D, Ikaiddi M, Mazzio E, and Soliman KFA (2000) The neurochemical basis for the behavioral effects of

triadimefon. *Annals of the New York Academy of Sciences* 914: 336–353.
Vinggaard AM, Hnida C, Breinholt V, and Larsen JC (2000) Screening of selected pesticides for inhibition of CYP19 aromatase activity *in vitro*. *Toxicology In Vitro* 14: 227–234.

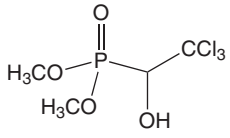
Relevant Website

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

Trichlorfon

Ramesh C Gupta

- Chemical Name: *O,O*-dimethyl-1-hydroxy-2,2,2-trichloroethylphosphonate
- Chemical Abstracts Service Registry Number: CAS 52-68-6
- Synonyms: Chlorofos; Dipterex; Dylox; Dyrex; Dyvon; Neguvon; Proxol; Tugon and Wotex. The common name for trichlorfon in Great Britain is trichlorphon, in Turkey is dipterex, and in Russia is chlorofos.
- Chemical and Physical Properties: Trichlorfon is an organophosphate compound, which has an empirical formula of $C_4H_8Cl_3O_4P$ and a molecular weight of 257.44. It is a racemic mixture of two isomers. Trichlorfon is a pale clear, white or yellow crystalline powder, melting point 75–84°C, boiling point 100°C, vapor pressure 7.8 mmHg at 20°C, and is stable at normal temperatures and pressure. At higher temperatures and pH less than 5.5, trichlorfon decomposes to form dichlorvos (*O,O*-dimethyl-*O*-(2,2-dichlorovinyl) phosphate, DDVP). It is readily soluble in chloroform and methylene chloride, and less soluble in water, benzene, and diethyl ether.
- Chemical Structure:



Uses

Trichlorfon was introduced in 1950, and has been used as an insecticide since 1952. It is available in the form of dust, granular, emulsifiable concentrate, soluble powder, injectable solution, tablets, and fly bait. Trichlorfon is a broad-spectrum insecticide that is particularly used against *Diptera*. It is used to control a variety of other insects in field, vegetable, and fruit crops, and forestry. In domestic animals, trichlorfon is used for the control of internal and external parasites. It has selective insecticidal action. Under the generic name metrifonate, trichlorfon is used as an anthelmintic in the treatment of schistosomiasis. Trichlorfon was shown in a number of clinical trials to have efficacy in treating Alzheimer's disease and was pending drug certification, but the application for this use was subsequently withdrawn due to pulmonary toxicity. But currently, it is labeled as a mutagen, carcinogen, and possibly teratogen, and therefore used as an investigational agent.

Exposure Routes and Pathways

Poisonings have occurred after oral, dermal, and inhalation exposure to trichlorfon.

Toxicokinetics

In humans and animals, the absorption, distribution, and excretion of trichlorfon is rapid. The biological half-life of trichlorfon in mammalian blood is estimated to be ~30 min. It eliminates from the blood stream within a matter of 1.5–4 h, and only low levels are detected after 8–24 h. In mice, ~70–80% of an oral dose of trichlorfon gets excreted within the first 12 h. Trichlorfon undergoes transformation via dehydrochlorination to form dichlorvos in all biological fluids and tissues. The major metabolites of trichlorfon found *in vivo* are demethyl trichlorfon, demethyl dichlorvos, dimethyl hydrogen phosphate, methyl hydrogen phosphate, phosphoric acid, and trichloroethanol. The excretion of trichlorfon and its metabolites occurs primarily via the urine.

Mechanism of Toxicity

Trichlorfon is primarily an indirect inhibitor of AChE, that is, it is converted in the body to the active chemical inhibitor dichlorvos. In fact, trichlorfon is considered to be a slow release cholinesterase inhibitor, transformed nonenzymatically to dichlorvos. This leads to irreversible AChE inhibition by phosphorylation, primarily at the synapses of the nervous system and at the neuromuscular junctions. Dichlorvos is

estimated to be at least 100 times more potent than trichlorfon as a cholinesterase inhibitor. As a result, signs and symptoms due to trichlorfon overexposure develop after a latent period and may even continue to increase after exposure has been discontinued.

Acute and Short-Term Toxicity (or Exposure)

Animal

Trichlorfon is moderately toxic for laboratory animals by ingestion or dermal absorption. The oral LD_{50} for trichlorfon in rats is 150–649 mg kg^{-1}, 300–1370 mg kg^{-1} in mice, 97 mg kg^{-1} in cats, 400 mg kg^{-1} in dogs, 420 mg kg^{-1} in guinea pigs, and 160 mg kg^{-1} in rabbits. The dermal LD_{50} of trichlorfon is 2000–5000 mg kg^{-1} in rats, and from 1500 to >2100 mg kg^{-1} in rabbits. The LC_{50} for trichlorfon in rats is 1300 mg m^{-3}. Oral acute toxicity studies conducted on rats, dogs, monkeys, rabbits, and guinea pigs revealed that trichlorfon poisoning caused the usual OP-cholinergic signs attributed to the accumulation of ACh by virtue of AChE inhibition. Exposure with very high doses of trichlorfon is known to produce neurotoxicity. Interestingly, trichlorfon may cause delayed symptoms beginning 1–4 weeks after an acute exposure, which may or may not have produced immediate symptoms. In such cases, numbness, tingling, weakness, and cramping may appear in the lower limbs and progress to incoordination and paralysis. Improvement may occur over months or years, but some residual impairment will remain.

Human

Several cases of acute trichlorfon poisoning from suicidal, accidental, or occupational exposure have occurred. Signs and symptoms of intoxication include those characteristic of AChE inhibition, such as weakness, exhaustion, excessive salivation, sweating, vomiting, chest pain, miosis, and muscle spasms. In severe cases, convulsions and unconsciousness develop, and death ensues from respiratory failure. In some cases, victims surviving because of medical interventions developed a delayed polyneuropathy with weakness of the lower limbs after a few weeks of exposure.

Chronic Toxicity (or Exposure)

Animal

Repeated or prolonged exposure to trichlorfon, like with other organophosphates, may result in the same effects as with acute exposure, including delayed symptoms. With 45 mg kg^{-1} day^{-1} trichlorfon administered to dogs for 3 months, serum cholinesterase was reduced to 60% of normal. A dietary level of ~10.5 mg kg^{-1} day^{-1} for 12 weeks produced a similar effect. During a 60 day testing period with repeated doses of trichlorfon at 100 mg kg^{-1} day^{-1}, cholinesterase activity was reduced to 50–75% of normal levels. Trichlorfon produced no pathological changes in rats that were fed 500 mg trichlorfon per kg diet for 1 year. In a 16 week study on rats, a 4 year study on dogs, and a 26 week study on monkeys, no-observed-effect levels (NOELs) of 100 mg kg^{-1} diet, 50 mg kg^{-1} diet, and 0.2 mg kg^{-1} body weight (based on plasma, erythrocyte or brain AChE activity), respectively, were observed. Inhalation exposure of rats over a 3 week period indicated a NOEL of 12.7 mg m^{-3}.

Exposure of laboratory animals (rats, mice, and hamsters) to trichlorfon at higher doses during the gestation period caused adverse effects on reproduction. An increased number of embryonic deaths, a decreased number of live fetuses and an increased number of fetal abnormalities were observed in rats given a single oral dose of 80 mg kg^{-1} body weight, p.o., on the 13th day of pregnancy. During a three-generation study conducted on rat reproduction, a dietary level of 3000 ppm trichlorfon or 150 mg kg^{-1} day^{-1} resulted in a marked decrease in the rate of pregnancy, and underdeveloped rat pups at birth, none of which survived to weanling. A dietary dose of 50 mg kg^{-1} day^{-1} reduced the number of pups per litter, as well as the weight of individual pups; however, a dietary level of 300 ppm (about 15 mg kg^{-1} day^{-1}) had no detectable effect on reproduction.

Trichlorfon and its active metabolite dichlorvos can cross the placenta and produce fetal abnormalities. Trichlorfon was found to be teratogenic when given to pregnant rats through a stomach tube, at a dose level of 480 mg kg^{-1} day^{-1}, on days 6–15 of pregnancy, but not when administered only on days 8 or 10 of pregnancy. Teratogenic effects have also been found in hamsters given 400 mg kg^{-1} day^{-1} on days 7–11 of pregnancy. In a three-generation study, rats fed dietary doses of trichlorfon as high as 150 mg kg^{-1} day^{-1} did not show any evidence of teratogenesis. It is important to note that these exposure levels are within the LD_{50} range.

Trichlorfon produced mutations in mice when it was given in the maximum tolerable single dose or repeated smaller doses. Trichlorfon has been shown to produce carcinogenic effects in rats given oral doses of 186 mg kg^{-1} or intramuscular doses of 183 mg kg^{-1} for 6 weeks. However, no evidence of carcinogenicity in rats given trichlorfon orally or intraperitoneally for 90 days.

In Vitro Toxicity Data

In vitro studies suggest that trichlorfon or its degradation products can be mutagenic in bacterial and mammalian cell assays.

Clinical Management

The diagnosis of a trichlorfon or related organophosphate intoxication should be confirmed as soon as possible by the determination of cholinesterase activity in venous blood. If dermal exposure occurs, decontamination procedures include removal of contaminated clothes and washing of the skin with soap or with a sodium bicarbonate solution. Extensive eye irrigation with water or saline should be performed. In the case of ingestion of a liquid formulation of trichlorfon, which may contain hydrocarbon solvents, avoid inducing emesis because of the risk of aspiration pneumonia. Instead, the stomach should be emptied, as soon as possible, by careful gastric lavage using 5% sodium bicarbonate (with a cuffed endotracheal tube already in place). Artificial respiration should be applied, if necessary. In a severe poisoning case, as early as possible, administer 2 mg of atropine sulfate intravenously and 1000–2000 mg of pralidoxime chloride or 250 mg of obidoxime chloride intramuscularly or intravenously. Repeated doses of 2 mg of atropine sulfate should be given until muscarinic receptor associated effects are completely subsided. For children, the doses are 0.04–0.08 mg of atropine sulfate per kg body weight, and 250 mg of pralidoxime chloride per child or 48 mg of obidoxime chloride per kg body weight. In adults, a single dose of diazepam (10 mg, i.p. or s.c) is found to be beneficial.

Environmental Fate

Trichlorfon rapidly degrades in the environment.

Ecotoxicology

Trichlorfon is highly toxic to birds, as the oral LD_{50} is $37\,mg\,kg^{-1}$ in wild birds, $36.8\,mg\,kg^{-1}$ in mallards, $22.4\,mg\,kg^{-1}$ in bobwhite quail, $59.3\,mg\,kg^{-1}$ in California quail, $95.9\,mg\,kg^{-1}$ in male pheasant, and $23\,mg\,kg^{-1}$ in rock doves. Signs of poisoning, such as regurgitation, imbalance, trembling, ataxia, and wingbeat convulsions, occur as early as within 10 min after exposure. Death usually occurs within 30 min to 3 h of exposure.

Trichlorfon is also highly toxic to both cold and warm water fish, and its acute toxicity is between 1.67 and 180 ppm. The 24 h LC_{50} for striped bass is 10.4 ppm. The 48 h LC_{50} for rainbow trout is 3.2 ppm, and the 96 h LC_{50} for fathead minnow is 180 ppm. Trichlorfon does not bioaccumulate.

Exposure Standards and Guidelines

The maximum permissible concentration of trichlorfon in air is $0.5\,mg\,m^{-3}$. The US Environmental Protection Agency (EPA) has set an acute population adjusted dose (PAD) for trichlorfon at $0.01\,mg\,kg^{-1}\,day^{-1}$. The chronic PAD is set at $0.2\,\mu g\,kg^{-1}\,day^{-1}$. Currently, none of the organizations – Occupational Safety and Health Administration, the National Institute for Occupational Safety and Health, or the American Conference of Governmental Industrial Hygienists – has established any occupational exposure limits for trichlorfon.

See also: Organochlorine Insecticides; Organophosphates; Pesticides.

Further Reading

Hayes WJ (1982) *Pesticides Studied in Man*. Baltimore, MD: Williams and Wilkins.

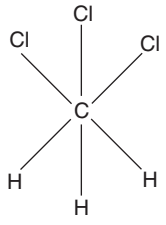
Hayes WJ and Laws ER (1990) *Handbook of Pesticide Toxicology, Vol. 3, Classes of Pesticides*. New York: Academic Press.

National Institute for Occupational Safety and Health (NIOSH) (1981–1986) Registry of Toxic Effects of Chemical Substances (RTECS). Cincinnati, OH.

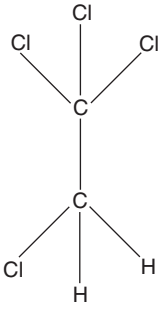
Trichloroethane

Robert Kapp

- Chemical Abstracts Service Registry Numbers: 1,1,1-Trichloroethane (CAS 71-55-6); 1,1,2-Trichloroethane (CAS 79-00-5)
- Synonyms:
  - 1,1,1-Trichloroethane: 1,1,1-TCE; Aerothene TT; Baltana; Chloroform, methyl-Chloroethene; Chlorten; Ethane, 1,1,1-trichloro-; Inhibisol; Methylchloroform; Methyltrichloromethane; Tafclean; Trichloroethane; Trichloromethylmethane; Alpha-Trichloroethane
  - 1,1,2-Trichloroethane: 1,2,2-Trichloroethane; Ethane, 1,1,2-trichloro-; Trojchloroetan(1,1,2); Vinyl trichloride; Vinyltrichloride; Beta-Trichloroethane
- Related Compounds: Tetrachloroethane (CAS 79-34-5); Carbon tetrachloride (CAS 56-23-5); 1,1-Dichloroethane (CAS 107-06-2); 1,2-Dichloroethane (CAS 75-34-3)
- Chemical/Pharmaceutical/Other Class: Chlorinated hydrocarbons
- Chemical Formula: $C_2H_3Cl_3$
- Chemical Structures:



1,1,1-Trichloroethane



1,1,2-Trichloroethane

Uses

1,1,1-Trichloroethane is a solvent used for resins, oils, waxes, tars, paints, and glues. It is widely used as a degreasing agent in the manufacture of metals and plastics, silicon chips, and other electronic parts. It is used in pesticides, textile processing, cutting fluids, aerosols, lubricants, cutting oil formulations, drain cleaners, spot cleaners, printing inks, and stain repellents.

1,1,2-Trichloroethane is used primarily as a chemical intermediate in the production of 1,2-dichloroethene and vinylidine chloride. It is also used in adhesives, lacquers, coating formulations, for chlorinated rubbers and polyesters, as a solvent for fats, oils, waxes, resins, and in the production of Teflon tubing.

Exposure Routes and Pathways

A primary pathway for trichloroethane exposure is by inhalation of contaminated air both indoors (from building materials, aerosols, cleaning products, paints, or metals degreasing agents) and outdoors near industrial sites or accidental releases. Other routes of exposure include ingesting contaminated water or food or through the skin upon dermal contact.

Toxicokinetics

Human data on both forms of trichloroethanes indicate that they are both rapidly and extensively absorbed upon inhalation, dermal, or gastrointestinal exposure. Animal studies show that 1,1,1-trichloroethane is metabolized slowly, but it is distributed by the blood to virtually all tissues and organs with a preference to fatty tissues. In humans and animals the principal pathway of elimination is by exhalation of the unchanged material via the lungs. The biological half-life is estimated to be 8.7 h. Only very limited studies on distribution and elimination were available for 1,1,2-trichloroethane; however, it is likely that these mechanisms are very similar to that of 1,1,1-trichloroethane.

Mechanism of Toxicity

1,1,1-Trichloroethane is oxidized to 2,2,2-trichloroethanol and trichloroacetic acid by the cytochrome P450 mixed-function oxidase system, which are excreted in the urine while unchanged 1,1,1-trichloroethane, carbon dioxide, and acetylene are excreted in expired air. It is estimated that less than 7% of 1,1,1-trichloroethane is absorbed and metabolized by any exposure route and the toxicokinetic behavior is qualitatively identical across all species. Less than 1% of 1,1,1-trichloroethane remains in the human body after 9 days. 1,1,2-Trichloroethane is metabolized to

chloroacetic acid, *S*-carboxymethylcysteine, and thiodiacetic acid. Thiodiacetic acid and *S*-carboxymethylcysteine are formed following glutathione conjugation while chloroacetic acid is the metabolite formed by hepatic cytochrome P450. This reaction is thought to proceed through acyl chloride. Acyl chlorides and free radicals that are formed from both 1,1,1-trichloroethane and 1,1,2-trichloroethane are believed to bind nucleic acids and proteins causing various cytoxic, mutagenic, and carcinogenic effects.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute exposure to 1,1,1-trichlororethane is considered to be of a low-order toxicity with a rat oral LD_{50} of 10.3–12.3 g kg^{-1}. High concentrations cause disturbances of the central nervous system (CNS), cardiovascular effects, and irritation of the skin, mucous membranes, and eyes.

Studies on exposure of experimental animals to 1,1,2-trichloroethane have reported effects on the liver kidney and CNS from inhalation exposure and ingestion. Acute exposure to 1,1,2-trichloroethane is considered to be moderately to highly toxic with a rat oral LD_{50} of 580 mg kg^{-1}.

Human

Exposure of humans to high concentrations of 1,1,1-trichloroethane can produce severe CNS depression, respiratory arrest, decrease blood pressure, as well as lung, kidney, and liver damage. 1,1,1-Trichloroethane has been intentionally inhaled to alter mood or consciousness. Deaths have been attributed to abuse of this solvent. It is mildly irritating to the skin and eyes of both humans and experimental animals.

Exposure of humans to high concentrations of 1,1,2-trichloroethane via the skin has caused burning sensations and transient whitening of the skin. No other acute human exposure data are available.

Chronic Toxicity (or Exposure)

Animal

Chronic exposure to 1,1,1-trichloroethane has resulted in some liver damage and neurological effects in experimental animals. US Environmental Protection Agency (EPA) has not established a reference concentration (RfC) or a reference dose (RfD) for this material.

Some liver and immune effects have been noted in chronic oral studies in experimental animals exposed to 1,1,2-trichloroethane.

Human

US EPA's IRIS gives 1,1,1-trichloroethane a class D (not classifiable as to human carcinogenicity) carcinogenic potential.

US EPA's IRIS lists 1,1,2-trichloroethane as a class C (possible) human carcinogen. This is based upon hepatocellular carcinomas and pheochromocytomas in one strain of mice. Carcinogenicity was not shown in rats.

Reproduction/Developmental Effects

There are no data in humans or in experimental animals indicating any potential for reproductive or developmental effects from exposure to either 1,1,1- or 1,1,2-trichloroethane.

Carcinogenicity

There are no epidemiology studies on both compounds regarding the potential for carcinogenic effects in humans. The carcinogenicity categories for these materials are presented in the table below:

Agency	*1,1,1-Trichloroethane classification*	*1,1,2-Trichloroethane classification*
EPA	D	C
IARC	3	3
ACGIH	A4	A3
NIOSH	NL	Ca
MAK	NL	3B

US EPA Classification C = Possible human carcinogen: limited evidence of carcinogenicity in animals in the absence of human data; US EPA Classification D = Not classifiable as to human carcinogenicity: inadequate human and animal evidence of carcinogenicity or no data available; IARC (International Agency for Research on Cancer) Classification 3 = Unclassifiable as to carcinogenicity in humans; ACGIH (American Conference of Governmental Industrial Hygienists) Classification A3 = Confirmed animal carcinogen with unknown relevance to humans; ACGIH Classification A4 = Not classifiable as a human carcinogen; NIOSH (US National Institute for Occupational Safety and Health) Classification Ca = Potential occupational carcinogen, with no further categorization; MAK (Federal Republic of Germany Maximum Concentration Values in the Workplace) Classification 3B = Substances which are suspected of being germ cell mutagens because of their genotoxic effects in mammalian somatic cells *in vivo*; in exceptional

cases, substances for which there are no *in vivo* data but which are clearly mutagenic *in vitro* and structurally related to known *in vivo* mutagens.

Clinical Management

Upon ocular exposure, eyes should be generously washed with tap water; medical attention should be sought. In case of dermal exposure, contaminated clothing should be removed and the skin should be rinsed with soap and tap water. Upon ingestion, the mouth should be rinsed and vomiting should be induced in conscious victims only; medical attention should be sought. Upon inhalation, the victim should be removed to fresh air and given artificial respiration if indicated; medical attention should be sought.

Environmental Fate

1,1,1-Trichloroethane tends to be stable in the atmosphere and is transported considerable distances. The rate of degradation is increased by the presence of chlorine radicals and nitrogen oxides. In water, its primary loss is by evaporation into the atmosphere. At a vapor pressure of 23 mmHg at 25°C, 1,1,2-trichloroethane is expected to exist almost entirely in the vapor phase in the ambient atmosphere. It will gradually degrade by reaction with photohemically produced hydroxyl radicals.

Exposure Standards and Guidelines

The ACGIH threshold limit value (TLV), time-weighted average (TWA), for 1,1,1-trichloroethane is reported to be 350 ppm or 1910 mg m^{-3}. The ACGIH TLV short-term exposure limit (STEL)/TLV ceiling (CEIL) is reported to be 450 ppm or 2460 mg m^{-3} on skin.

The ACGIH TLV, TWA, for 1,1,2-trichloroethane is reported to be 10 ppm or 55 mg m^{-3} on skin. The ACGIH TLV, STEL/CEIL, is reported to be 10 ppm or 45 mg m^{-3} on skin. The NIOSH recommended exposure limit, TWA, is also 10 ppm or 45 mg m^{-3} on skin.

See also: Pollution, Water.

Further Reading

Bruckner JV, Kyle GM, Luthra R, *et al.* (2001) Acute, short-term, and subchronic oral toxicity of 1,1,1-trichloroethane in rats. *Toxicological Sciences* 60(2): 363–372.

Wang RY (2004) Hydrocarbons In: Dart RC (ed.) *Medical Toxicology*, 3rd edn., pp. 1329–1340. Philadelphia: Lippincott Williams and Wilkins.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profiles for 1,1,1-Trichloroethane and 1,1,2-Trichloroethane.

Trichloroethylene

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 3, pp. 372–374, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 79-01-6
- Synonyms: Trichloroethene; 1,1,2-Trichloroethene; TCE; TRI; Trichlor; Acetylene trichloride; Ethylene, trichloro; Ethylene trichloride; Triclene
- Chemical/Pharmaceutical/Other Class: Chlorinated olefinic hydrocarbon
- Chemical Formula: C_2HCl_3

Uses

Manufactured by chlorination of acetylene or other two carbon hydrocarbons or by dehydrohalogenation of tetrachloroethane, trichloroethylene (TCE) may contain one of numerous additives for stabilization. This chlorinated solvent has been used extensively as a degreasing and metal cleansing agent, as a heat exchange liquid, as a diluent in paints and adhesives, in textile processing and in the manufacture of organic chemicals and pharmaceuticals, as a cleaning solvent, for dyeing of polyester textiles, as an extractant for caffeine from coffee, in typewriter correction fluid, and as a solvent for insecticides and other chemicals. It is a volatile liquid whose vapor is heavier than air. The vapor has a chloroform-like odor detectable at ~21 ppm in air.

Exposure Routes and Pathways

The two main sources of human exposure to TCE are the environment and the workplace, although home use of products containing TCE is not uncommon. Background levels of TCE can be found in the

outdoor air we breathe (30–460 ppt) and in many lakes, streams, and underground water used as sources of tap water for homes and businesses. An important source of environmental release of TCE is evaporation into the atmosphere from work performed to remove grease from metal (degreasing).

Toxicokinetics

Absorption of TCE following inhalation exposure in humans is characterized by an initial rate of trichloroethylene uptake that is quite high. Retention of inhaled TCE has been measured at 37% and 75% of the amount inhaled. Absorption of TCE following oral exposure in both humans and animals is rapid and extensive. In animal studies, absorption from the gastrointestinal tract has been measured at 91–98%, and peak TCE blood levels are attained within a matter of hours. Dermal absorption of TCE in both humans and animals is slow, but dermal absorption studies are complicated by the fact that pure liquid TCE can act to defat the skin and thereby enhance its own absorption.

TCE is extensively metabolized (40–75% of the retained dose) in humans to trichloroethanol, glucuronides, and trichloroacetic acid (TCA). Saturation of metabolism has not been demonstrated in humans up to an exposure concentration of 300 ppm. Mathematical models predict, however, that saturation of metabolism is possible at TCE concentrations previously used for anesthesia (i.e., 2000 ppm). Although the liver is the primary site of TCE metabolism in animals, there is evidence for extrahepatic metabolism of trichloroethylene in the kidneys and lungs.

The distribution of TCE in rats following exposure to 200 ppm, 6 h day^{-1} for 5 days was studied. Seventeen hours after exposure on Day 4, there were relatively high levels of TCE in the perirenal fat (0.23 nmol g^{-1}) and in the blood (0.35 nmol g^{-1}) and virtually no TCE in the other tissues. Following exposure on Day 5, tissue levels in brain, lungs, liver, fat, and blood reached a steady state within 2 or 3 h.

In humans, ~11% of TCE is eliminated through the lungs, whereas more than 50% of the absorbed dose is metabolized and excreted in the urine as TCA and other metabolites. Elimination is relatively slow in humans, with TCA being detected in the urine of exposed individuals up to 12 days postexposure suggesting a cumulative process, probably related to storage in fatty tissue. The biological half-life of urinary metabolites of TCE in humans is ~41 h.

Mechanism of Toxicity

Extended exposure (e.g., occupational exposure) to a chlorinated solvent like trichloroethylene typically results in signs of central nervous system (CNS) disturbance and hepatotoxicity. Administration of this chemical to mice induces neoplasms in the liver, as is typical of virtually all the chlorinated hydrocarbons. TCE is readily converted to TCA and other metabolites including the corresponding alcohol. TCA acts as a peroxisome proliferator, and hepatic neoplasms in mice may arise through this mechanism. Glutathione adducts of TCE are thought to be converted to the reactive metabolite in the kidneys through the action of β-lyase. These processes may account for nephrotoxicity exhibited in rats. TCE induces liver tumors in mice and the contributing metabolites are thought to include trichloroacetate, chloral hydrate, and dichloroacetate. Peroxisome proliferation is thought to be involved in the mechanism of formation of mouse liver tumors. TCE is lipophilic and easily crosses the blood–brain barrier resulting in the observed CNS effects.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute toxicity data indicate that trichloroethylene is relatively nontoxic by the inhalation and oral routes. In mice, LC_{50} values ranged from 7480 to 49 000 ppm, whereas in rats the range was 12 500–26 300 ppm. By the ingestion route, acute LD_{50} values in dogs, cats, rats, mice, and rabbits ranged from ~2000–8000 mg kg^{-1}. Inhalation and oral studies indicate that the bone marrow, CNS, liver, and kidneys are the principal targets of TCE in animals. Effects on the liver and kidney include enlargement with hepatic and biochemical and/or histological alterations. Other reported effects include indication of impaired heme biosynthesis and other hematological alterations in rats exposed by inhalation and immunosuppression in orally exposed mice.

Inhalation studies with mice and rats indicate that TCE is a developmental toxicant. Fetotoxicity is expressed mainly as skeletal ossification anomalies and other effects consistent with delayed maturation. Oral studies with rats and mice showed no trichloroethylene-related effects on fertility or other indicators of reproductive performance. No definitive teratogenic effects have been reported regarding exposure to TCE.

Autoimmune responses to TCE in susceptible mice were demonstrated including significant increases in antinuclear antibodies and total serum globulin among other parameters. Some indications of an autoimmune response have also been reported in Brown Norway rats.

Human

TCE is readily absorbed through ingestion, inhalation, and dermal exposure, the latter producing a defatting effect if contact is prolonged, resulting in erythema and vesiculation followed by exfoliation. The liquid solvent is also an eye irritant producing pain and irritation but apparently no permanent injury. Exposure to high vapor concentrations of TCE has been reported to result in irritation of the mucous membranes of the eyes, nose, and throat, conjunctivitis, rhinitis, and pharyngitis. Exposures exceeding 100 ppm in air are reported to result in restlessness, peripheral neuritis, impaired concentration, irritability, euphoria, lightheadedness, dizziness, depression, reversible trigeminal degeneration, psychic disturbances, cranial nerve deafness, alterations in electrical patterns in the brain, bronchoconstriction, fatal cardiac arrhythmias, and renal and hepatic damage. In combination with alcohol, trichloroethylene exposure produces a vasodilation which has been described as 'degreasers flush'. Optic neuritis, hallucinations, and gastrointestinal changes have been reported after ingestion of trichloroethylene. These symptoms are often accompanied by nausea and vomiting, as well as major cardiovascular effects including hypotension, conduction defects, myocardial injury, and cardiac arrhythmias. The latter has been reported to be the cause of death in some individuals who have been exposed to high levels of TCE, but usually death is preceded by coma and subsequent hepatic or renal failure. Occasional sudden deaths have been attributed to ventricular fibrillation. The estimated oral dose in humans to cause death is reported to be 3–5 ml kg^{-1} while the lowest reported concentration in air to produce unconsciousness in adult humans is 3000 ppm. Reversible trigeminal nerve degeneration and psychic disturbances have also been reported.

Chronic Toxicity (or Exposure)

Animal

Chronic inhalation exposure to TCE produced lung and liver tumors and leukemia in mice and Leydig cell tumors in rats. Chronic oral exposure to TCE produced increased incidences of hepatocellular carcinomas in mice and marginally significant increased incidences of renal adenocarcinomas in rats. TCE was neither embryotoxic nor teratogenic in Sprague–Dawley rats or Swiss Webster mice. Since there was a question about some impurities in these bioassays, the National Toxicology Program treated F344 rats and B6C3F1 mice with epichlorohydrin-free TCE producing renal tubular cell neoplasms in rats and increased incidences of hepatocellular carcinomas in male and female mice and hepatocellular adenomas in female mice.

Human

There is limited evidence in humans for the carcinogenicity of trichloroethylene in humans. There are reports of increased risks of multiple myeloma, non-Hodgkins lymphoma, and cancer of the biliary passages, but the studies are limited. There is sufficient evidence, however, in experimental animals for the carcinogenicity of TCE; therefore, TCE has been classified as being a probable human carcinogen. TCE in drinking water has been associated with leukemia in women and children and, in a study of the Tucson water supply, low birth weight was also reported.

Prolonged TCE exposure has been associated with impairment of peripheral nervous system function, persistent neuritis and temporary loss of tactile sense and paralysis of the fingers after direct solvent contact. Chromosomal effects have been reported in those involved in the use of TCE for degreasing and symptoms of systemic lupus erythematosis have been reported after chronic TCE exposure. In addition, organic dementia has been noted after occupational exposure to TCE and there have been some reports of an association between exposure and scleroderma, an autoimmune disease.

In Vitro Toxicity Data

Mutagenic responses generally occurred with metabolic activation only, suggesting the involvement of metabolites of TCE. The mutagenic potential of pure trichloroethylene is unclear; however, the limited information available suggests that TCE would be a weak mutagen. Both positive and negative findings showing frameshift and base pair mutations in *Saccharomyces cerevisiae* and reverse mutations in *Escherichia coli* K12 have been reported. Fisher rat embryo cells were transformed by TCE producing foci which, when transplanted into host animals, grew as undifferentiated fibrosarcomas at the site of inoculation. However, attempts to induce morphological transformations in the BALB/3T3 mouse cell line were not successful and an attempt to produce increased unscheduled DNA synthesis in a rat hepatocyte primary culture also failed.

Clinical Management

After oral ingestion, emesis is not recommended due to potential for CNS depression and cardiovascular instability. Gastric lavage may be appropriate shortly

after ingestion and activated carbon may also be administered. Cardiovascular monitoring should be instituted and treated as appropriate. For inhalation exposure, the patient should be moved to an uncontaminated environment and monitored for respiratory symptomatology. Bronchospasm if present may be treated with a $\beta 2$ agonist and corticosteroids. Oxygen should be administered and ventilation assist applied when appropriate. Seizures may result and should be addressed using diazepam.

Environmental Fate

In the atmosphere, TCE will be degraded by reaction with photochemically produced hydroxyl radicals. Its half-life in the atmosphere is ~ 7 h. In soils, TCE is expected to have a high mobility since its average K_{oc} is 101 but volatilization is expected to be an important fate process in both wet and dry soils. Under anaerobic conditions, TCE is slowly degraded by reductive dechlorination leading to dichloroethylene and vinyl chloride, among other decomposition products. Studies in aquifers have reported half-lives ranging from 35 days to 6 years. TCE has a bioconcentration factor ranging from 4 to 39 suggesting moderate to low bioconcentration in aquatic organisms. Estimated volatilization half-lives in a model river and lake are 3.5 h and 5 days, respectively.

Other Hazards

TCE decomposes at high temperatures forming toxic gases including hydrogen chloride, chlorine, and phosgene. Welding or smoking in a TCE-contaminated environment may result in inhalation of these toxic gases. TCE pools in low-lying areas when released to the environment. It converts to vinyl chloride monomer under anaerobic conditions in the environment.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value for TCE is 50 ppm (8 h time-weighted average) and the short term exposure limit is 100 ppm for a 15 min period.

The Occupational Safety and Health Administration's permissible exposure limit is 100 ppm with an acceptable ceiling exposure concentration of 200 ppm.

The National Institute for Occupational Safety and Health considers TCE as a potential occupational carcinogen and recommends an exposure limit of 25 ppm for a 10 h day.

The International Agency for Research on Cancer states that there is sufficient evidence for the carcinogenicity of TCE in experimental animals and classifies it as being probably carcinogenic to humans (Category 2B).

ACGIH classifies TCE as not being suspected as a human carcinogen (Category A5) but this classification was made in 1993.

TCE is designated as a hazardous substance under Section 311(b) (2) (A) of the Federal Water Pollution Control Act and is further regulated by the Clean Water Act Amendments of 1977 and 1978.

The US Environmental Protection Agency Federal Drinking Water Standard for TCE is $5\,\mu g\,l^{-1}$.

Miscellaneous

TCE is a volatile chlorinated organic solvent having excellent solvent characteristics. It is a colorless liquid having a boiling point of 87.2°C and an ethereal odor. As a liquid it has a vapor density of ~ 1.5 and is miscible in oils and other lipophilic organic solvents. Its vapor pressure is 69 mmHg at 25°C and a vapor density of 4.53 causing the vapors to 'pool' in lower elevations. TCE has an odor threshold of ~ 21 ppm in air and $\sim 10\,mg\,l^{-1}$ in water.

See also: Pollution, Water.

Further Reading

Bruening T and Bolt HM (2000) Renal toxicity and carcinogenicity of trichloroethylene. *Critical Reviews in Toxicology* 30(3): 253–285.

World Health Organization (WHO) (1985) Environmental Health Criteria 50: Trichloroethylene.

Relevant Websites

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

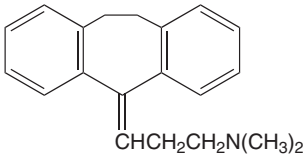
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Trichloroethylene.

Tricyclic Antidepressants

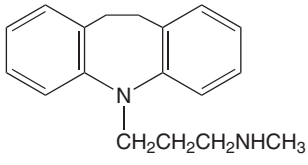
Fermin Barrueto Jr.

This article is a revision of the previous print edition article by Michael J Hodgman, volume 3, pp. 375–376, © 1998, Elsevier Inc.

- Representative Chemicals: Imipramine; Amitriptyline; Doxepin; Desipramine; Nortriptyline
- Chemical/Pharmaceutical/Other Class: The tricyclic antidepressants are a group of drugs that have a three-ring molecular core and share a similar pharmacologic effect, inhibiting the reuptake of biogenic amines at central presynaptic terminals
- Chemical Structures:



Amitriptyline



Desipramine

Uses

Tricyclic antidepressants are used to treat depression. They are also used for treatment of enuresis in children, chronic pain syndromes, neuropathic pain, the fibromyalgia syndrome, and chronic headaches.

Exposure Routes and Pathways

Ingestion is the most common route of exposure. Several tricyclic antidepressants are also available in injectable form.

Toxicokinetics

The tricyclic antidepressants are well absorbed following oral ingestion. Large ingestions will be more slowly absorbed because of the anticholinergic effects and resulting decreased gut motility. There is extensive first-pass metabolism that limits oral bioavailability.

Tricyclic antidepressants are highly lipid soluble and bind extensively to tissue and plasma proteins. The volume of distribution ranges from 10 to 50 $l\,Kg^{-1}$.

The half-life of various tricyclic antidepressants ranges from 10 to 50 h. Less than 5% of these drugs appear unchanged in the urine.

The tricyclic antidepressants are extensively metabolized by the liver and partially enterohepatically recirculated. They undergo demethylation, hydroxylation, and glucuronide conjugation. The demethylated metabolites of the tertiary amine tricyclic antidepressants are pharmacologically active. Drugs that induce hepatic microsomal enzymes speed the metabolism of tricyclic antidepressants.

Mechanism of Toxicity

There are six main properties that tricyclic antidepressants have: antihistaminic, GABA antagonism, Na^+ channel blockade, peripheral alpha antagonism, inhibition of the reuptake of biogenic amines, and anticholinergic properties. The cardiac toxicity of tricyclic antidepressants is mostly related to their quinidine-like sodium channel blockade leading to prolongation of the QRS complex. This leads to altered conduction, slowing of both depolarization and repolarization, and decreased inotropy. This can lead to ventricular dysrhythmias, bradydysrhythmias, and asystole. Decreased inotropy, peripheral alpha blockade, and dysrhythmias can all contribute to hypotension and shock. The antihistaminic properties are largely responsible for the sedation seen in overdose. The anticholinergic affects produce tachycardia, which is further exacerbated by the alpha antagonism and, together with the GABA antagonism, cause central nervous system (CNS) excitation and seizures.

Acute and Short-Term Toxicity (or Exposure)

Animal

Toxic effects are similar to those seen in humans.

Human

Early signs of tricyclic antidepressant toxicity are due to anticholinergic effects and include tachycardia, mydriasis, dry mouth, low-grade fever, diminished bowel sounds, CNS excitation, and delirium. More serious toxicity is manifested by coma, respiratory depression, seizures, and cardiovascular toxicity including conduction disturbances, hypotension, ventricular arrhythmias, and asystole. Seizures cause hyperthermia, rhabdomyolysis, and metabolic acidosis. Clinical deterioration can be rapid and catastrophic in patients with tricyclic antidepressant overdose. Death most often occurs due to dysrhythmia and circulatory collapse. The typical therapeutic dose of a tricyclic antidepressant is 2–4 $mg\,kg^{-1}\,day^{-1}$. Doses of 15–20 $mg\,kg^{-1}$ are potentially lethal. Therapeutic drug levels for most tricyclic antidepressants range from 100 to

260 ng ml^{-1}. Toxicity can be seen at levels only modestly elevated, although severe symptomatology is usually associated with levels >1000 ng ml^{-1}. However, drug levels are not useful in predicting toxicity, complications, or patient management. Electrocardiogram changes include sinus tachycardia, a rightward deviation of the terminal vector of the frontal plane QRS complex to >120°, intraventricular conduction disturbances with a prolongation of the QRS duration >100 ms, and T wave changes. A QRS duration >100 ms is associated with a 32% chance of seizure and a QRS >160 ms is associated with a 50% chance of a ventricular dysrhythmia. In distinction to the tricyclic antidepressants, newer cyclic antidepressants such as the bicyclics and dibenzoxazepines are less cardiotoxic but are associated with an increased risk of seizures.

Chronic Toxicity (or Exposure)

Animal

Pregnant rats were given imipramine 5 mg kg^{-1} day^{-1} during pregnancy. Basal body temperature was higher in female offspring compared to male offspring. Puppies given intravenous imipramine showed fewer arrhythmias and caused less of a fall in blood pressure compared to puppies who were administered intravenous amitryptilline or doxepin.

Human

Tricyclic antidepressants have a wide range of uses in humans and seem to be generally well tolerated. Patients taking therapeutic doses chronically have rarely developed blood dyscrasias (e.g., agranulocytosis, thrombocytopenia). Common adverse effects include CNS effects (CNS depression, confusion, aggression, nightmares, tremor, seizures, etc), anticholinergic effects (e.g., dry mouth, urinary retension), and gastrointestinal effects (e.g., nausea, vomiting, weight gain).

In Vitro Toxicity Data

Some studies have implied that several tricyclic antidepressants may be able to stimulate tumor growth in animals or humans with existing tumors. However, recent research using human and murine *in vitro* models have not been able to support these findings.

Clinical Management

If the patient is seen early postingestion (e.g., within 60 min), gastric decontamination by lavage may be considered. Because of the risk of rapid CNS depression, ipecac should be avoided and airway protection by endotracheal intubation should be aggressively considered. Activated charcoal should also be given. Flumazenil and physostigmine should be avoided. Sodium bicarbonate administration is beneficial in treating cardiac toxicity and hypotension though it is not clear if the effects are a consequence of sodium administration or alkalinization of the serum. In case of signs of impaired conduction (QRS >100 ms), ventricular arrhythmias, or hypotension, alkalinize serum to pH 7.45–7.55. Sodium bicarbonate should be used by bolus injection (1–2 mEq kg^{-1} of $NaHCO_3$) followed by continuous infusion to maintain target pH. Ventricular dysrhythmias unresponsive to alkalinization should be treated by standard ACLS methods, avoiding the class Ia antidysrhythmics (quinidine, procainamide, and disopyramide). Hypotension is multifactorial and treatment should include volume resuscitation if not contraindicated, serum alkalinization, and pressor support if needed. Norepinephrine may be the more effective pressor as the hypotension is caused by both negative inotropy and vasodilation. Central hemodynamic monitoring should be useful in this setting. Seizures should be treated using benzodiazepines. For uncontrolled seizures, paralysis is indicated as the associated acidosis and hyperthermia will aggravate cardiac toxicity. Hemodialysis and hemoperfusion are not effective treatment modalities. Only patients free of any signs of toxicity during the first 6 h, with the exception of a resolved tachycardia, can be considered medically clear at that time.

Further Reading

Boehnert MT and Lovejoy FH Jr. (1985) Value of the QRS duration versus serum drug level in predicting seizures and ventricular arrhythmias after an acute overdose of tricyclic antidepressants. *New England Journal of Medicine* 313: 474–479.

McCabe JL, Cobaugh DJ, Menegazzi JJ, and Fata J (1998) Experimental tricyclic antidepressant toxicity: A randomized, controlled comparison of hypertonic saline solution, sodium bicarbonate and hyperventilation. *Annals of Emergency Medicine* 32: 329–333.

Nattel S and Mittleman M (1984) Treatment of ventricular tachyarrhythmias resulting from amitriptyline toxicity in dogs. *Journal of Pharmacology and Experimental Therapeutics* 231: 430–435.

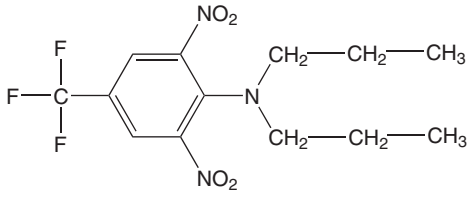
Niemann JT, Bessen HA, Rothstein RJ, and Laks MM (1986) Electocardiographic criteria for tricyclic antidepressant cardiotoxicity. *American Journal of Cardiology* 57: 1154–1159.

Weld FW and Bigger JT (1980) Electrophysiological effects of imipramine on ovine cardiac purkinje fibers and ventricular muscle fibers. *Circulation Research* 46: 167–175.

Trifluralin

David R Wallace

- Chemical Abstracts Service Registry Number: CAS 1582-09-8
- Synonyms: Agreflan; Elancolan; Nitran; Olitref; Synfloran; Trefancocide; Treflan; Trifloran; Trifluraline; Trikepin; Tristar
- Chemical/Pharmaceutical/Other Class: Herbicide
- Chemical Formula: $C_{13}H_{16}F_3N_3O_4$
- Chemical Structure:



Uses

Trifluralin is a herbicide, first approved in 1963, for control of annual grasses and broadleaf weeds on a variety of crops. Trifluralin is registered for nonfood uses, including residential use. Trifluralin comes in a variety of formulations and is applied as a soil-incorporated treatment.

Exposure Routes and Pathways

Trifluralin can enter the body by inhalation of contaminated air or absorption through the skin or by diet if contaminated plants or animals are consumed.

Toxicokinetics

The predominant metabolic pathway appears to be hydroxylation of alkyl groups or *N*-dealkylation. To a lesser extent, a cyclized compound, benzimidazole, and the reduction products of nitro groups or amines, are also possible metabolic products.

Mechanism of Toxicity

Technical trifluralin has low acute toxicity, whereas solvents often used for the emulsification of trifluralin have been shown to be irritating to the eyes and skin. An active component of trifluralin toxicity is the volatile nitrosamine, *N*-nitroso-di-*n*-propylamine, which may be the active compound in trifluralin toxicity.

Acute and Short-Term Toxicity (or Exposure)

Trifluralin poses the greatest risk following acute exposure. It can be a strong irritant following inhalation, oral, or dermal routes of administration. As noted above, however, this irritation may be primarily due to the solvents used for emulsification. Generally, trifluralin is well tolerated and relatively safe.

Animal

Animal studies have shown that trifluralin is moderately toxic to rats exposed by inhalation, oral ingestion, or dermal contact. Dogs appear to be more sensitive to the actions of trifluralin and exhibited weight loss, hematological changes, and increase liver weights.

Human

Trifluralin is classified as a Toxicity Category IV (practically nontoxic) agent for acute oral toxicity and dermal irritation; and Toxicity Category III (slightly toxic) for acute dermal toxicity, acute inhalation toxicity, and eye irritation. Ocular irritation is characterized by increased lacrimation, photophobia, and redness. Conjunctivitis can continue for 5–7 days.

Chronic Toxicity (or Exposure)

Trifluralin is irritating to the eyes and produces mild skin irritation after prolonged exposure. Central nervous system and respiratory depression are observed following lethal doses of trifluralin.

Animal

There have been reports of increased incidence of urinary tract tumors and thyroid tumors in rats exposed chronically to trifluralin. Trifluralin is structurally similar to ethalfluralin, which is a known carcinogen in rats, and formulations contain *N*-nitroso-di-*n*-propylamine, an omnipresent contaminant, also a known carcinogen. Trifluralin has been classified as a group C (possible carcinogen) due to evidence of increased combined malignant and benign urinary bladder tumors in female rats and renal pelvis carcinomas in male rats. There is also evidence for an increased incidence of thyroid and follicular cell tumors in male rats.

Human

The risk of carcinogenicity to the general population is low. The primary risk group consists of individuals

who will come directly into contact with trifluralin on a long-term, daily basis.

In Vitro Toxicity Data

Trifluralin is strongly mutagenic in plants, producing a 3 to 4 times increase in spontaneous mitosis and chromosomal aberrations. A commercial trifluralin formulation induced chromosomal aberrations in bone marrow, embryonic cells, and male germ cell line in mice. Aneuploidy was induced in several lower eukaryotes.

Clinical Management

Emesis should not be induced due to the potential for central nervous system and respiratory depression. Instead, trifluralin should either be diluted with water or milk, or activated charcoal should be administered. In severe cases gastric lavage should be initiated.

Environmental Fate

Trifluralin is relatively nonmobile and is persistent in soil. Due to the lack of mobility of trifluralin and the fact that annual average surface water concentrations are not likely to exceed the lifetime health advisory level, the threat from drinking water is minimal. There may be some risk of run-off contamination.

Ecotoxicology

Effects of trifluralin on birds and mammals in their natural habitat on an acute basis are very low. Acute risk to endangered species is a potential concern. For aquatic animals, trifluralin is considered moderately to highly toxic and poses acute risk to endangered species.

Exposure Standards and Guidelines

The Environmental Protection Agency (EPA) has estimated that an exposure of $0.0075\,\mathrm{mg\,kg^{-1}\,day^{-1}}$ or less over a lifetime would not result in noncancer endpoints of toxicity. The EPA uses mathematical modeling to estimate the probability of a person developing cancer from drinking water containing specified amounts of trifluralin. Based on these estimates, if a person drank water containing $5\,\mu\mathrm{g\,l^{-1}}$ over their lifetime, that person would have no more than a 1:1 000 000 chance of developing cancer as a result of this exposure.

See also: Nitrosamines.

Further Reading

Environmental Protection Agency (EPA) R.E.D. FACTS: Trifluralin. EPA-738-F-95-035, Washington, DC, April 1996.

Relevant Websites

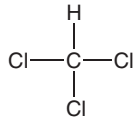
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Trifluralin.
http://www.epa.gov – Environmental Protection Agency, Technology Transfer Network Air Toxics Website: Trifluralin.
http://www.weblakes.com – Lakes Environmental Software. Air Toxic Index: Trifluralin Factsheet.

Trihalomethanes

Shayne C Gad

This article is a revision of the previous print edition article by Shayne C Gad and Jayne E Ash, volume 3, pp. 376–377, © 1998, Elsevier Inc.

- Representative Compounds: Bromoform; Dichlorobromomethane; Dibromochloromethane
- Chemical Abstracts Service Registry Number: CAS 67-66-3 (Chloroform; representative compound)
- Synonyms: Carbonyl chloride; Chloroformyl chloride; Trichloromethane; Freon-20 or HCF C-22; THM
- Chemical/Pharmaceutical/Other Class: Halogenated hydrocarbons
- Chemical Formula: $CH(halogen)_3$
- Chemical Structure:



Trichloromethane

Background Information

Trihalomethanes are by-products of the chlorination process. Almost all of the chloroform used in the

United States has been for chlorofluorocarbon refrigerant production, although these uses are being discontinued due to concerns about ozone depletion. Chloroform was once used as an anesthetic.

Exposure Routes and Pathways

Ingestion, inhalation, and dermal contact are possible routes of exposure.

Toxicokinetics

Trihalomethanes are absorbed readily through the skin, breathing, or ingestion, and then distributed primarily to stomach, liver, and kidneys. Elimination of the parent compounds is primarily by the lungs. Chloroform undergoes more conjugation than other trihalomethanes. The metabolism of chloroform is well understood. Approximately 50% of an oral dose of 0.5 g was metabolized to carbon dioxide in humans. Metabolism was dose dependent, decreasing with higher exposure. Approximately 38% of the dose was converted in the liver and $<17\%$ was exhaled unchanged from the lungs before reaching the systemic circulation. Metabolism studies indicated that chloroform was, in part, exhaled from the lungs or was converted by oxidative dehydrochlorination of its carbon–hydrogen bond to form phosgene. This reaction was mediated by cytochrome P450 and was observed in the liver and kidneys. Covalent binding of chloroform to lipids can occur under anaerobic and aerobic conditions; binding to protein occurs only under aerobic conditions. Chloroform can induce lipid peroxidation and inactivation of cytochrome P450 in rat liver microsomes under anaerobic conditions. Evidence that chloroform is metabolized at its carbon–hydrogen bond is provided by experiments using the deuterated derivative.

Mechanism of Toxicity

Chloroform inhibits the function of kidney tubules. It increases nitrogen in blood urea, renal concentrating ability, and glomerular filtration rate. It also increases the metallothionein concentration and reduces the level of cytochrome P450. P450 oxidation also contributes to metabolic release of carbon monoxide. Glutathione is a cofactor of this process. Toxic intermediates such as phosgene, a conjugate by-product of chloroform, may bind covalently with proteins and lipids, contributing to toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

Rat oral $LD_{50} = 444–2000\,mg\,kg^{-1}$ (limited research finds similar toxicity levels for all trihalomethanes).

Human

Chloroform is an irritant to skin and mucous membranes. The fatal human dose is estimated to be 10 ml. Central nervous system depression (narcosis) is a prominent response to high levels of chloroform.

Chronic Toxicity (or Exposure)

Animal

Chloroform is a proven carcinogenic in the liver, kidneys, and/or intestines of rodents. It inhibits kidney function and increases chromosomal aberrations. Chloroform is also a developmental toxicant.

Human

Chloroform produces liver and kidney damage and central nervous system depression. Carcinogenic properties are suspected but not confirmed.

Clinical Management

Respiratory therapy should be administered. If ingested, emesis should be induced. Blood pressure and normal urinary output should be maintained. A high carbohydrate diet can assist in restoring normal liver function. Epinephrine should not be used.

Environmental Fate

Chloroform exists almost entirely as a vapor in the atmosphere. Chloroform is effectively eliminated by wet deposition but can reenter the atmosphere following subsequent volatilization. Long-range transport within the atmosphere is possible. Chloroform is eliminated from surface waters primarily by volatilization. Chloroform is not expected to substantially adsorb to organic matter in surface water. Chloroform does not appreciably bioconcentrate in higher aquatic organisms. Chloroform has a moderate potential to concentrate in some aquatic plants.

In surface soil, chloroform is volatilized. Remaining chloroform travels through the soil, as confirmed by detection of chloroform in groundwater. In air, chloroform is degraded through reactions with free radicals. In water and soil, chloroform is degraded under both aerobic and anaerobic conditions. Hydrolysis and direct photolysis are not significant.

Ecotoxicology

The 96 h LC_{50} in channel catfish and rainbow trout is 75 and 44 ppm, respectively. In fathead minnows and bluegills, 96 h (static conditions) LC_{50} values were 129 and 100.0 mg l^{-1}, respectively. A 96 h (static conditions) LC_{50} in *Daphnia* was 29 mg l^{-1}.

Exposure Standards and Guidelines

The threshold limit value – time-weighted average for chloroform is 10 ppm. The permissible exposure limit is 50 ppm. No reference concentration has been set.

See also: Metallothionein; Neurotoxicity; Phosgene; Pollution, Water.

Further Reading

Bingham E, Cohrssen B, and Powell CH (2001) *Patty's Toxicology*, 5th edn., vol. 5, pp 50–61. New York: Wiley.
Komulainen H (2004) Experimental cancer studies of chlorinated by-products. *Toxicology* 198: 239–248.

Relevant Website

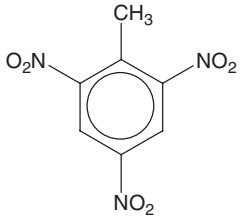
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Trihalomethanes.

Trinitrotoluene

Shayne C Gad

This article is a revision of the previous print edition article by Shayne C Gad and Jayne E Ash, volume 3, p. 377, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 118-96-7
- Synonyms: TNT; 2,4,6-Trinitrotoluene; Methyltrinitrobenzene
- Chemical/Pharmaceutical/Other Class: Aromatic nitro compound
- Chemical Formula: $C_7H_5N_3O_6$
- Chemical Structure:



Uses

Trinitrotoluene (TNT) is used as a high explosive. It is also an intermediate in the production of dyes and photographic chemicals.

Exposure Routes and Pathways

Ingestion, inhalation, and dermal contact are possible routes of exposure.

Toxicokinetics

TNT is readily absorbed through skin, especially when skin is moist. It is excreted in urine more than in feces; some is found in bile. The major biotransformation reaction is nitroreduction and, to a lesser extent, oxidation. The main metabolite formed by nitroreduction seems to be 4-amino-2,6-dinitrotoluene (4-ADNT). Other metabolites include 2-amino-4,6-dinitrotoluene (2-ADNT), 2,4-diamino-6-nitrotoluene, and 2,6-diamino-4-nitrotoluene. The metabolites are excreted in the urine as glucuronide conjugates and in the free form. Ring oxidation products of TNT such as trinitrobenzylalcohol, trinitrobenzoic acid, and simultaneous oxidation and reduction metabolites such as 2,6-dinitro-4-amino-benzylalcohol and 2,6-dinitro-4-amino-*m*-cresol are of less importance. Untransformed TNT is also excreted in the urine. ADNT and TNT concentrations were found in workers in explosives factories. 4-ADNT excretion was reported to be complete within 3–4 days after exposure. However, another study reported detectable urine concentration of ADNT in explosives workers even after 17 days away from the workplace.

Mechanism of Toxicity

TNT increases UDPglucuronsyltransferase in the liver and kidneys. It increases renal epoxide hydrolase activity. Animal studies have suggested covalent binding between TNT and macromolecular proteins including serum albumin, hemoglobin (Hb), hepatic and renal proteins, and possibly lens protein. The Hb adduct was dose dependent. Macromolecular binding is likely to be correlated with toxic effects;

however, it is unclear if a cause and effect relationship can be established. Formation of organic nitro radicals was also hypothesized based on hemolysis in glucose 6 phosphate dehydrogenase (G6PD)-deficient TNT workers. G6PD is a limiting factor in the maintenance of cellular glutathione, which protects against oxidative damage. TNT was also found to be oxidized oxyhemoglobin, resulting in methemoglobin formation.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} values are $795\,mg\,kg^{-1}$ in rats and $660\,mg\,kg^{-1}$ in mice.

Human

TNT stains the skin orange and yellow. It can cause dermatitis, irritation of eyes, nose, throat, and skin. High exposures may cause weakness, anemia, headaches, liver, or central nervous system damage.

Chronic Toxicity (or Exposure)

Animal

TNT can cause anemia, methemoglobinemia, splenomegaly (dogs, 6 month oral study) in animals. Also, note that TNT is a mutagen in animals.

Human

Chronic exposure may cause cataracts, cyanosis, jaundice, methemoglobin aplastic anemia or hepatitis. TNT is a possible human carcinogen.

Clinical Management

Methylene blue should be administered with oxygen therapy. Contaminated skin or eye should be irrigated with large amounts of water.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value is $0.5\,mg\,m^{-3}$ of air, the Occupational Safety and Health Administration permissible exposure limit is $63\,mg\,m^{-3}$, and the National Institute for Occupational Safety and Health recommended exposure limit is $0.5\,mg\,m^{-3}$.

See also: Pollution, Water.

Further Reading

Bingham E, Cohrssen B, and Powell CH (eds.) (2001) *Patty's Toxicology*, vol. 4. New York: Wiley.
Dart RC (2004) *Medical Toxicology*, 3rd edn. Philadelphia: Lippincott.
IARC (1996) 2,4,6-Trinitrotoluene. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans 65: 449–475.

Tungsten

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 7440-33-7
- Synonym: Wolfram
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formula: W^{6+}

Uses

Tungsten compounds (e.g., tungsten oxides and sulfides) are used as catalysts, and to increase hardness, toughness, elasticity, and tensile strength of steel. Tungsten metal and alloys are also used in filaments, for example, incandescent lamps. Tungsten (e.g., organic tungsten) is used in dyes and pigments. Tungsten carbides are used in cutting and forming tools, for example, in rock drills and machine tools.

Background Information

Tungsten was discovered in 1781. The average tungsten concentration in the earth's crust is $\sim$0.006%. Tungsten occurs naturally as tungstate, mainly in compounds such as wolframaites and scheelites.

Exposure Routes and Pathways

Occupational exposure to tungsten compounds may occur through inhalation of dust and dermal contact. The production and use of tungsten compounds as catalysts, and in cutting and forming tools, filaments, and dyes and pigments may result in the release of tungsten to the environment through various waste

streams; however, only small concentrations of tungsten have been released into the atmosphere, primarily by industrial emissions and nuclear fall-out. If released to air, most tungsten compounds have low vapor pressures and are expected to exist solely in the particulate phase in the ambient atmosphere.

Toxicokinetics

Amount ($\sim$70%) of inhaled tungsten is cleared within 4 h. Absorbed portions are distributed primarily to the liver and kidneys, skeleton and skeletal muscles.

Mechanism of Toxicity

Reported inhalation effects are probably due to cobalt in exposures, a competitive inhibitor of molybdenum utilization.

Acute and Short-Term Toxicity (or Exposure)

Animal

The LD_{50} of tungsten metal powder in rats is $5\,g\,kg^{-1}$ body weight. Fifty milligrams of tungsten dust introduced into the trachea of rats resulted in proliferation of the intraalveolar septa. Chamber exposures of animal to tungsten dust produced only minor changes.

Human

Eye, skin, and respiratory irritations.

Chronic Toxicity (or Exposure)

Animal

Intratrachael instillation or inhalation of dusts (of tungsten or tungsten carbide) into rodents causes only minor lung damage. When tungsten coils were implanted into the subclavian artery of rabbits, the mean tungsten levels rose from $0.48\,\mu g\,l^{-1}$ prior to the implantation to $12.4\,\mu g\,l^{-1}$ 4 months postimplantation. The study concluded that tungsten coils corrode and lead to a steady increase in serum tungsten levels starting as early as 15 min after implantation; however, despite the markedly elevated serum tungsten levels 4 months after implantation, the degradation of tungsten coils was not associated with local or systemic toxicity.

Human

Diffuse pulmonary fibrosis ('hard metal disease'), loss of appetite, nausea, cough, blood changes. No known mutagenicity, carcinogenicity, or developmental/reproductive toxicity.

Clinical Management

Dimercaprol may be useful as a chelating agent.

Ecotoxicology

Tungsten inhibits molybdenum utilization, which is essential for the induction of nitrate reductase.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) has an 8 h time-weighted average (TWA) of $5.0\,mg\,m^{-3}$ and a 15 min short-term exposure limit (STEL) of $10.0\,mg\,m^{-3}$ for tungsten metal and insoluble tungsten compounds. ACGIH has a TWA of $1\,mg\,m^{-3}$ and an STEL of $3\,mg\,m^{-3}$ for soluble tungsten compounds. In addition, the US National Institute for Occupational Safety and Health recommends a 15 min STEL of $3.0\,mg\,m^{-3}$ for tungsten and insoluble compounds, and a TWA of $5\,mg\,m^{-3}$ for tungsten and a TWA of $1\,mg\,m^{-3}$ for soluble tungsten compounds.

See also: Metals; Toxicity Testing, Inhalation.

Further Reading

Bingham E, Cohrssen B, and Powell CH (eds.) (2001) *Patty's Toxicology*, 5th edn., vol. 3, pp. 106–121. New York: Wiley.

Goyer RA, Klaassen CD, and Waalkes MP (1995) *Metal Toxicology*. San Diego, CA: Academic Press.

Kraus T, Schramel P, Schaller KH, *et al.* (2001) Exposure assessment in the hard metal manufacturing industry with special regard to tungsten and its compounds. *Occupational and Environmental Medicine* 58: 631–634.

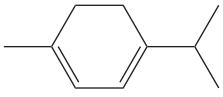
Lagarde F and Leroy M (2002) Metabolism and toxicity of tungsten in humans and animals. *Metal Ions in Biological Systems* 39: 741–759.

Peuster M, Fink C, Wohlsein P, *et al.* (2003) Degradation of tungsten coils implanted into the subclavian artery of New Zealand white rabbits is not associated with local or systemic toxicity. *Biomaterials* 24: 393–399.

Turpentine

Vijay M Vulava

- Representative Chemicals: Turpentine is primarily composed of $C_{10}H_{16}$ terpene hydrocarbons such as α-pinene, β-pinene, limonene, 3-carene, and camphene. It may also contain other acyclic, monocyclic, or bicyclic terpenes, oxygenated terpenes, and anethole
- Chemical Abstracts Service Registry Number: CAS 8006-64-2
- Synonyms: Gum spirits; Turps; Gum thus; D.D. turpentine; Wood turpentine; Oil of turpentine; Rectified turpentine oil; Spirits of turpentine; Sulfate wood turpentine; Sulfate turpentine; Gum turpentine; Steam-distilled turpentine; Turpentine oil; G 4134
- Chemical/Pharmaceutical/Other Class: Terpene
- Chemical Formula: $C_{10}H_{16}$ (approximate formula)
- Chemical Structure (Generalized Structure):



Uses

Turpentine is commonly used in several food and chemical products. Some of the flavoring agents (e.g., menthol) used in candy, baked goods, and chewing gum have the same chemicals that make up turpentine. Turpentine is also used in perfumery, sprays, deodorizers, and stimulating ointments. It is commonly used in the manufacture of synthetic pine oil, insecticides, beta-pinene resins, disinfectants, and human and veterinary medicines. It is also used in the preparation of shoe, stove, furniture, and other polishes; manufacture of synthetic camphor and menthol, cleaning materials, inks, putty, mastics, cutting and grinding fluids, paint thinners, degreasing agents, and paints. Turpentine is often used as a solvent thinner for paint, varnishes, waxes, resins, fats, oils, lacquers, and rubber. It is a starting component in the production of a variety of volatile bases.

Exposure Routes and Pathways

Turpentine can affect humans and animals by multiple routes – inhalation, ingestion, or dermal contact. Exposure to turpentine vapors has been reported to cause irritation of eyes, skin, and upper respiratory tract in addition to headache, dizziness, and nausea. Other effects include depression of central nervous system (CNS), kidney problems, and bladder irritation. Inhalation exposure to turpentine can also result in asthmatic symptoms such as cough, hoarseness, rhinorrhea, wheezing, and conjunctivitis. Exposure to turpentine can be fatal to small children.

All terpenes, a key component of turpentine, are local irritants. Ingestion of terpenes usually produces gastrointestinal signs and symptoms while aspiration causes pulmonary toxicity. Terpene absorption, which may begin in oral cavity, is associated with alteration in mental status, ranging from coma to seizures. Renal toxicity has also been reported. Following ingestion, pine oil (a popular terpene product) may be concentrated in the lungs, resulting in chemical pneumonitis without evidence of aspiration.

Toxicokinetics

Terpenes are oxidized by cytochrome P450, conjugated principally with glucuronic acid in the liver, and are excreted by the kidney. Part of inhaled turpentine is eliminated unchanged in expired air and in urine, but most is metabolized and excreted in urine conjugated with glucuronic acid. The excretory product of turpentine has a characteristic odor of violets.

Mechanism of Toxicity

Turpentine is readily absorbed through the gastrointestinal and respiratory tracts and skin. Turpentine, as a lipophilic substance, accumulates in fatty tissues. In animal studies, the highest concentrations of turpentine following inhalation by rats were found in the spleen, kidneys, brain, and peripheral and perinephric fat. Liver microsomal epoxide hydrase and uridine diphosphoglucuronosyl transferase activities were elevated during chronic turpentine exposures. While some turpentine may be eliminated unchanged through the lungs, most turpentine and its metabolites are eliminated through the urinary tract as glucuronides.

Acute and Short-Term Toxicity (or Exposure)

The signs and symptoms of acute inhalation exposure to turpentine may include irritation of the skin, eyes, mucous membranes, and upper respiratory

tract; salivation, cough, chest pain, and shortness of breath; confusion, headache, dizziness, nausea, anxiety, painful urination, bloody urination, or decreased urine output. The signs and symptoms of turpentine ingestion include a burning sensation in the mouth and throat; nausea, vomiting, diarrhea, and abdominal pain; excitement, confusion, ataxia, stupor and seizures; fever; and increased heart rate.

Animal

Turpentine is an eye, mucous membrane, and skin irritant and a CNS depressant in animals. The oral LD_{50} in rats is 5760 mg kg^{-1} and the LC_{50} in the same species is 12 g m^{-3} for 6 h. Cats exposed to a 540–720 ppm concentration of turpentine exhibited signs of immediate eye and mucous membrane irritation and had mild convulsions; at a concentration of 1440 ppm, they developed paralysis within 150–180 min. No adverse effects were noted in dogs exposed to 180 ppm for 3.5 h day^{-1} for 8 days; however, raising the concentration to 818 ppm and exposing the dogs for 3.5–4.5 h caused nausea, lack of coordination, mild paralysis, and weakness. Exposure of rats to a 12–20 mg l^{-1} (2150–3600 ppm) concentration for 1–6 h and of mice to a 29 mg l^{-1} (5200 ppm) concentration for 2 h produced seizures and apnea; at autopsy, however, no pulmonary lesions were noted in these animals. Injection of turpentine into rabbits' eyes produced shrinkage of the orbit and corneal opacification. In one study, dermal application of turpentine produced skin tumors in rabbits but not in mice; in another experiment, however, painting the skin of mice with 240 g kg^{-1} turpentine did cause tumors.

Human

Turpentine is a skin, eye, mucous membrane, and upper respiratory tract irritant in humans. It may also cause skin sensitization and adverse effects to CNS, gastrointestinal, and urinary tract. The lowest estimated oral dose reported to be lethal in humans is 441 mg kg^{-1}. Exposure to a 75 ppm concentration for 3–5 min irritates the nose and throat, and exposure to a 175 ppm concentration irritates the eyes and may be considered intolerable by human volunteers. Ingestion of turpentine causes a burning pain in the mouth and throat, nausea, vomiting, diarrhea, abdominal pain, excitement, ataxia, confusion, stupor, seizures, fever, and tachycardia and may cause death due to respiratory failure. Toxic glomerulonephritis and bladder irritation, with hematuria, albuminuria, oliguria, and dysuria, have been associated with overexposure to the vapor of turpentine in the past; however, the more purified form of turpentine now in use appears to have decreased the incidence of or eliminated turpentine-induced nephritis. Splashes of the liquid in the eye produce severe pain and blepharospasm; conjunctival redness and temporary corneal erosion may also occur, but these effects are reversible.

Chronic Toxicity (or Exposure)

Chronic effects associated with occupational exposures to turpentine include cerebral atrophy, behavioral changes, anemia, bone marrow damage, glomerulonephritis, and dermatitis. Urinary disturbances, albuminuria, and urinary casts were observed in workers exposed to paints and varnishes. However, renal damage associated with occupational exposures to turpentine was transient and reversible.

A number of epidemiology studies have been completed that were associated with the pulp and paper industry. Cancers considered included lung, lymphoproliferative diseases (Hodgkin's disease, multiple myeloma, leukemia, lymphosarcomas), and cancers of the digestive organs. These studies were confounded by other possible chemical exposures. Without job-exposure matrices, it is difficult to pinpoint exposures to specific chemicals and corresponding risks of developing cancers. Workers exposed to terpenes (a principal component of turpentine) for longer than 5 years may also be at greater risk of developing lung cancer.

Chronic skin exposure to turpentine may produce a hypersensitivity reaction, with bullous dermatitis and/or eczema. A case–control study of workers in particle-board, plywood, sawmills, and formaldehyde glue factories demonstrated a statistically significant association between chronic exposure (longer than 5 years) to terpenes (the principal component of turpentine) and the development of respiratory tract cancers.

Clinical Management

Degree of exposure should be considered when determining initial treatment. If eyes are exposed to turpentine or a solution containing turpentine, the eyes should be flushed with large amounts of water for a minimum of 15 min, lifting the lower and upper lids occasionally. Medical attention will be required as soon as possible. Upon skin exposure, the contaminated skin should be washed with soap and water. If irritation persists or a large skin area was affected, medical attention will be required.

A victim of turpentine vapor inhalation should be moved quickly to fresh air and provided medical

care. If the victim is not breathing, cardiopulmonary resuscitation should be performed; if breathing is difficult, supplemental oxygen should be provided. Careful attention to the airway, including an attempt to prevent emesis, is important. It is best to administer nothing by mouth. All precautions should be taken to minimize the victim's risk of vomiting and further aspiration.

If turpentine or a solution containing turpentine is ingested, the victim should be given copious amounts of water to dilute stomach contents. Emesis induction is contraindicated because of the initial risk of aspiration and the risk of CNS depression or seizure development before ipecac syrup can produce vomiting. If gastric decontamination is considered, the airway must be stabilized to minimize the risk of aspiration secondary to the victim's vomiting. Because a major complication of hydrocarbon ingestion is aspiration, the use of gastric decontamination should be reserved for only cases of large intentional ingestions or those involving an increased risk of systemic toxicity. Absorption of some toxic terpenes, such as camphor, is so rapid as to make any attempt at gastric emptying ineffective. Seizures may be managed with benzodiazepines. In all cases, professional medical help will be required. The victim should be kept warm and quiet until medical help arrives.

Environmental Fate

Turpentine is a natural product and is completely biodegradable. It is immiscible in water and miscible in organic solvents such as alcohol, ether, chloroform, and glacial acetic acid. Below the solubility limits, turpentine does not represent a hazard to biological wastewater-treatment plants. However, the biological and chemical oxygen demand for turpentine is exceptionally high and therefore effluent discharges are regulated. Environmental releases of turpentine may occur at production facilities where faulty equipment or spills occur. Facilities are required to use best management practices to reduce the amount of turpentine released to the air during turpentine production processes. Turpentine released into the environment either evaporates rapidly or is completely degraded by natural processes within a few days. The rate of degradation depends on the concentration of turpentine, temperature, availability of air, and presence of bacteria. Turpentine has been ranked as having zero potential as an ozone depleting substance or for global warming. Turpentine has a specific gravity of $0.86\,g\,cm^{-3}$ and hence floats on water. However, there is strong potential for it to seep deep into soil and subsurface environment when discharged in large quantities at the surface. This leads to eventual contamination of groundwater sources.

Ecotoxicology

Several animal studies involving a variety of exposure pathways to turpentine indicate extreme toxicity to small mammals. Inhalation of turpentine vapors resulted in acute toxicity in rats with symptoms including salivation, weakness, incoordination, bloody nasal discharge, paraplegia, ataxia, tremor, convulsions, tachypnea, decreased tidal volume, coma, and death due to sudden apnea. High-level exposures cause irritation of the skin, nose, and mucosal membranes. CNS depression is accompanied by an increased respiration rate and decreased tidal volume. Systemic effects include damage to the kidney and liver. Hyperplasia was demonstrated within 48 h of a single cheek painting in the hamster cheek pouch model. Thirty percent turpentine in acetone elicits a moderate degree of skin irritation free from ulcer formation.

Turpentine is also toxic to aquatic organisms and may cause long-term adverse effects in the aquatic environment. Coating action of resins can destroy water birds, plankton, algae, and fishes. Penaeid shrimp given abdominal injections were highly sensitive to turpentine. Turpentine-induced cellular inflammatory response was fibrous scar tissue in all tissues. Early gill and hepatopancreas tissue destruction, and extensive heart and abdominal tissue destruction was also observed.

Other Hazards

Turpentine should be stored in a cool, dry, well-ventilated area in tightly sealed containers. Turpentine can undergo auto-oxidation in contact with air and can generate heat that may spontaneously ignite in a confined space. Containers of turpentine should be protected from physical damage and should be stored separately from strong oxidizers (especially chlorine), heat, sparks, and open flame. Only nonsparking tools may be used to handle turpentine. To prevent static sparks, containers should be grounded and bonded for transfers. Because containers that formerly contained turpentine may still hold product residues, they should be handled appropriately.

Toxic gases and vapors (such as carbon monoxide and the partial oxidation products of terpenes) may be released in a fire involving turpentine. Turpentine attacks some coatings and some forms of plastic and rubber.

The odor threshold for turpentine is 200 ppm of air. Because this value is above the US Occupational Safety and Health Administration (OSHA) current permissible exposure limit (PEL) of 100 ppm, turpentine is considered to have inadequate warning properties. The eye irritation threshold for turpentine is 175 ppm.

Exposure Standards and Guidelines

- The current OSHA PEL for turpentine is 100 ppm ($560\,mg\,m^{-3}$) as an 8 h time-weighted average (TWA) concentration.
- The US National Institute for Occupational Safety and Health (NIOSH) has not issued a recommended exposure limit (REL) for turpentine; however, NIOSH concurs with the PEL established for this substance by OSHA. NIOSH estimates that turpentine concentration of 800 ppm ($4457\,mg\,m^{-3}$) is immediately dangerous to life or health (IDLH).
- The American Conference of Governmental Industrial Hygienists (ACGIH) has assigned turpentine a threshold limit value (TLV) of 100 ppm ($560\,mg\,m^{-3}$) as a TWA for a normal 8 h workday and a 40 h workweek. The OSHA and ACGIH limits are based on the risk of irritation associated with exposure to turpentine.
- The value of the maximum contact with skin in countries such as Australia, Austria, Belgium, France, Germany, New Zealand, Singapore, The Netherlands, The Philippines, Turkey, and Vietnam is a TWA of 100 ppm ($560\,mg\,m^{-3}$).
- In UK and Finland, the following values are used: long-term exposure limit 8 h TWA of 100 ppm ($560\,mg\,m^{-3}$) and short-term exposure limit of 15 min at 150 ppm ($840\,mg\,m^{-3}$).

See also: Camphor; Food Additives; Fragrances and Perfumes.

Further Reading

Haneke KE and Masten S (2002) *Turpentine (Turpentine Oil, Wood Turpentine, Sulfate Turpentine, Sulfite Turpentine) (8006-64-2) Review of Toxicological Literature*. Research Triangle Park, NC: National Institute of Environmental Health Sciences.

Irwin RJ, Van Mouwerik M, Stevens L, Seese MD, and Basham W (1997) *Environmental Contaminants Encyclopedia*. Fort Collins, CO: National Park Service, Water Resources Division.

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UN *See* Food and Agriculture Organization of the United Nations.

Uncertainty Analysis

Virginia Lau

Uncertainty analysis provides an evaluation of the key parameters that contribute to the uncertainty (e.g., variability and imprecision) involved in performing a risk assessment. Also known as probabilistic risk assessment, it provides information that enables decision makers to better understand the strengths, weaknesses, and assumptions inherent in the assessment and to evaluate the conclusions of the risk assessment accordingly. The result of the uncertainty analysis is a distribution of risks that a population may be potentially exposed to and thus, may be used by the risk manager to better understand the implication of the conclusions derived from the risk assessment and to support scientifically based and economically feasible hazardous waste management decisions.

Uncertainty analysis has become a popular method for assessing the uncertainty associated with risk estimates calculated for specific receptors under designated routes of exposures. Two types of uncertainty analysis are typically performed in risk assessment: qualitative and quantitative. Qualitative uncertainty analysis literally describes and lists the parameters or assumptions likely to produce the highest uncertainties and quantifies whether the risks have been over- or underpredicted. Although the qualitative analysis is simple to perform, it generally addresses the overall uncertainty of the assessment in vague and general terms and yields very imprecise results. Quantitative uncertainty analysis, on the other hand, is the mathematical investigation of the uncertainty in the risk output performed by varying the input parameters such that the relative contribution of each parameter is determined. This method has numerous advantages over the qualitative analysis since (1) it provides precise results that are for the most part reproducible and consistent; (2) with the advent of many commercially available software, the analysis is straightforward and easy to perform; and (3) it does not require mathematics beyond that commonly used in risk assessments.

In most risk assessments, calculations are based on deterministic values (i.e., all of the variables are treated as known constants). Many of these values are actually estimates of either an average, high-end, or conservative worst-case condition used in place of a range of values that would better characterize a population or condition. For example, one value may be used to represent the body weight of all the individuals in a population that is composed of people of different ages, sexes, and sizes. Clearly, the deterministic values commonly used in risk assessments represent only a portion of the overall available data.

The current practice in risk assessment of using multiple point estimates that are either high end or worst case to calculate risk often results in a compounding of conservatism intended to significantly overestimate risk. The uncertainty associated with each parameter commonly used to calculate risk usually has not been fully characterized. Major sources of uncertainty associated with risk assessment include:

- Incomplete information on the potential adverse health effects that can be caused by a chemical in the species, at the dose, and over the anticipated length of time the exposure might occur.
- Natural variability (e.g., uncertainty about the range of sensitivity in the population of interest).
- Measurement and sampling errors (i.e., errors resulting from direct measurement due to instrument and observation variations).
- Bias (i.e., difference between the estimated value and true value).

- Judgmental error (i.e., estimated value based on professional opinion).
- Randomness.
- Unpredictability (i.e., systems that exhibit extreme sensitivity).
- Disagreement (i.e., differences in opinion based on expert opinion).
- Approximations (i.e., simplification of the real world).

The uncertainty associated with certain exposure parameters may decrease once probability distributions are used that describe the parameter of interest (e.g., body weight) in place of point estimates as inputs into the calculations. This methodology is described as stochastic modeling and utilizes the full range of data available by selecting random variables from a defined probability distribution. There are three modeling methods used to propagate uncertainty: analytical methods using mathematical statistics, the delta method, and Monte Carlo analysis. The analytical and delta methods are only used for analysis with limited complexity and thus are not discussed further. It should be noted that although increasing the complexity of the uncertainty model may initially improve its accuracy (i.e., consistent and reproducible results), uncertainty within the analysis increases with complexity as less characterized parameters are included in the model.

The Monte Carlo method is a well-established approach used in characterizing uncertainty that can be used to incorporate ranges of data (distributions) into calculations. 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 parameters of interest. Monte Carlo analysis can be selectively used to generate input parameters and mixed with point estimates, as appropriate, to calculate risk.

The use of this method has become increasingly popular as the availability of commercial software that allows the probability distribution functions to be input directly into a computer spreadsheet have become available. Once the probability distribution is incorporated into a spreadsheet cell, each time the spreadsheet is recalculated, a new value for the random variable is selected from the distribution and used in the calculations. The key to appropriately using this method is to run the entire simulation (choosing random samples from each distribution) hundreds to thousands of times. After each selection, a new representative parameter is generated and can be used as the basis for an estimate of exposure or risk. When the method is used to calculate risk or exposure, the results of all risk estimates are summarized in a histogram which provides risk assessors a full possible distribution of risk based on probability.

The Monte Carlo analysis is performed using the following steps:

- Standard spreadsheet calculation results are entered for all chemicals and pathways to be modeled following the methods used for deterministic calculations. For each of the random variables, discrete or continuous probability density functions are placed in the appropriate cells.
- Any correlations among the exposure parameters must be identified. For example, body weight and skin surface area would be positively correlated such that when a high body weight is selected a corresponding high skin surface area should likewise be used. It is important to identify these correlations so that individual simulations avoid selecting values of two different random variables that are not representative of an individual (i.e., high body weight and low skin surface area). In addition, variability in some exposure assumptions should likewise be accounted for in the toxicity metric. All other variables are assumed to be independent and are not correlated with any other parameter selected.
- The simulation should be run thousands of times to fully sample from each distribution. The summaries of the risk estimates can include statistical tables and histograms of resulting risks and intermediate calculations.

The most difficult aspect of performing a Monte Carlo analysis is estimating the probability distributions underlying many of the variables used. Because it is not immediately obvious what distributions best characterize the exposure parameters for a particular population, the risk assessor must carefully evaluate the available data and choose the appropriate distributions based on the level of information known. A sensitivity analysis may provide additional insight into any distribution selected by indicating the significance of the parameter in affecting the conclusions. There are several general rules used in uncertainty analysis in determining the most unbiased distribution for a specific parameter. The data should initially be tested using a robust goodness-of-fit test (i.e., chi-square test, Shapiro–Wilk test, or Kolmogorov–Smirnov test) to determine if the data are normally or lognormally distributed. If only a range of values is known for a variable, a uniform distribution is the least biased assumption. If the range and mode of values are known, a triangular distribution could be used although it may result in

values being selected more from the extremes than would be expected. A beta distribution can be selected when estimates of the mean, lower bound, and upper bound are available. If the data cannot be adequately described by a standard distribution, the empirical data may be 'bootstrapped' into the simulation in which the model randomly selects individual data points from the data provided. It should be noted that the use of full distribution functions (e.g., lognormal or normal) is not entirely accurate since it is impossible to have mass values beyond physical plausibility within the extreme tails of these distributions although these values may be selected during the analysis (i.e., body weights that are negative, zero, or infinite). Users of packaged software have the flexibility of describing the exposure distributions in terms of parametric and nonparametric functions including cumulative percentiles, bootstrapped values, and moments to limit the values selected from a distribution to within the physical realm.

Uncertainty analysis should account for and characterize the variability inherent in most data sets. In certain cases (e.g., use of Monte Carlo analysis), it is used to more accurately represent a parameter (e.g., body weight) that influences the calculation of risk. Quantitative analysis provides information that enhances understanding and implications of the risk assessment. In the case of human health risk assessment, the risk assessor attempts to quantify the likelihood an individual in a population will develop cancer or other adverse effect due to contact with a chemical at a particular dose level over a specified exposure period. Uncertainty analysis allows the risk assessor to more accurately account for the differences in the population being evaluated (e.g., body weight, exposure duration, ingestion rates, and other exposure parameters) that potentially impact the overall estimate of risk and the conclusions that can be made based on the assessment. It does not, however, address the uncertainty or validity of the methodologies used to develop the parameter distributions or test the underlying uncertainty model itself.

See also: Hazard Identification; Risk Assessment, Ecological; Risk Assessment, Human Health; Risk Characterization; Risk Communication; Risk Management; Sensitivity Analysis.

Uncertainty Factors

Michael Dourson

Health organizations throughout the world utilize a 'safe' dose concept in the dose–response assessment of noncancer toxicity. This safe dose has often been referred to by different names, such as acceptable daily intake (ADI), tolerable daily intake (TDI) or tolerable concentration (TC), minimal risk level (MRL), reference dose (RfD), and reference concentration (RfC). The approaches used by various health organizations share many of the same underlying assumptions, judgments on critical effect, and choices of uncertainty (or safety) factors.

There is enormous variability in the extent and nature of different databases for risk assessment. For example, in some cases, the evaluation must be based on limited data in experimental animals; in other cases detailed information on the mechanism of toxicity and/or toxicokinetics may be available. In some cases the risk evaluation can be based on effects data in exposed human populations; however, few chemicals have been adequately studied in humans to accurately identify a safe dose directly. Therefore, scientists typically rely on existing human epidemiologic and animal laboratory data to estimate safe doses for humans. In estimating a safe dose for a given chemical, scientists first review all toxicity data, judge what constitutes an adverse effect, and determine the critical effect. The critical effect is the first adverse effect that occurs as dose or concentration increases. Not all effects are adverse effects, and the judgment of what constitutes an adverse effect is sometimes difficult.

Scientists then determine the appropriate uncertainty (or safety) factors to apply to the no-observed-adverse-effect level (NOAEL) or lowest-observed-adverse-effect level (LOAEL) for the critical effect, based on considerations of the available toxicity, toxicodynamic, and toxicokinetic data. Uncertainty factors (UFs) used in the estimation of safe doses are necessary reductions to account for the lack of data and inherent uncertainty in these extrapolations. Other areas of uncertainty include extrapolations of subchronic-to-chronic exposure, LOAEL to NOAEL, and use of an incomplete database. The major assumptions underlying each of these UFs are described in **Table 1**.

The various major areas of uncertainty are described briefly below. These areas represent most of the considerations of estimating safe doses from differing toxicity databases. However, this list may

Table 1 Major assumptions for individual uncertainty factors[a]

Factor	*Assumption*
Interhuman variability	Assumes that there is variability in response from one human to the next and that this variability may not have been detected in the study, usually due to small sample size; may also assume that subpopulations of humans exist that are more sensitive to the toxicity of the chemical than the average population
Animal to human	Assumes that results seen in experimental animals are relevant to humans and that humans are more sensitive than animals at a given $mg\,kg^{-1}\,day^{-1}$ dose or $mg\,m^{-3}$ concentration; this UF may also account for assumptions about specific toxicokinetic and toxicodynamic properties
Less than chronic to chronic	Assumes that an effect seen at subchronic exposures will be seen at lower doses after chronic exposures; may also assume that effects may only be seen after an experimental group is exposed chronically
LOAEL to NOAEL	Assumes that the chosen LOAEL is reasonably close to the projected NOAEL in an experiment, and that the use of this uncertainty factor will drop the LOAEL into the range of the expected NOAEL
Incomplete data	Assumes that the critical effect can be discovered in a reasonably small selection of toxicity studies

[a] This list of assumptions is not exhaustive.

not be exhaustive. **Table 2** shows these areas of uncertainty and how different expert bodies use them.

Interhuman Variability

Whenever possible, data on humans is used to conduct noncancer risk assessment, thereby avoiding the problems inherent with interspecies extrapolation. If sufficient data on sensitive individuals exist, the safe dose can be estimated directly, that is, without the need of an UF. If adequate data on sensitive humans do not exist, an uncertainty is encountered that must be addressed, most often with a 10-fold factor. This UF assumes that variability in response from one human to the next occurs and that this variability may not have been detected in the study, usually due to small sample size. This factor may also assume that subpopulations of humans exist that are more sensitive to the toxicity of the chemical than the average population.

Some groups use data on differences in dynamics and kinetics among humans for this UF. This concept is based on the work of Renwick and is described more fully below.

Animal to Human

If adequate toxicity data on humans do not exist, then experimental animal data are used as the basis of the assessment, and an UF of 10 is routinely applied to the NOAEL. The basic assumptions for this UF are that the results seen in experimental animals are relevant to humans, that toxicokinetic and toxicodynamic differences exist among species, and that humans are more sensitive than animals at a given milligram per kilogram per day dose or milligram per meter cube concentration. Researchers have tried to quantify this area of uncertainty by investigating the ratios between animals and humans. Also ongoing is physiologically based pharmacokinetic modeling as applied to this area of uncertainty.

Some groups use data on differences in dynamics and kinetics between humans and common laboratory animals, such as rats, mice, and dogs, for this UF. This concept is based on the work of Renwick, and is also described in detail below.

Less-than-Chronic Studies to Chronic

The subchronic-to-chronic UF is based on the assumption that an effect seen at shorter durations will also be seen after a lifetime of exposure, but at lower doses. This factor also assumes that effects may only be seen after an experimental group is exposed chronically. In fact, several investigators have examined subchronic-to-chronic ratios of NOAELs and LOAELs, and the average differences between subchronic and chronic values are only 2–3, while some small percentage of chemicals has ratios that exceed 10-fold. Data suggest that the routine use of a 10-fold default factor for this area of uncertainty should be examined closely. For example, short-term (2 weeks) and subchronic (90 days) NOAELs are often available for comparison, which can give an indication of the possible differences in the subchronic NOAEL and the expected chronic NOAEL. When such data are not available, a 10-fold UF may not be unreasonable, but it should be considered as a loose upper-bound estimate to the overall uncertainty.

LOAEL to NOAEL

If an LOAEL exists on which to base the estimation of a safe dose, the uncertainty in the NOAEL must be addressed. Analysis of several sets of data suggests

Table 2 Description of typical uncertainty and modifying factors in the development of 'safe' doses for several groups[a]

		Agency				
Uncertainty factors (UFs)[b]	Guidelines[c] from Health Canada, the International Programme on Chemical Safety (IPCS), the Netherlands National Institute for Public Health and the Environment (RIVM), the US Agency for Toxic Substances and Disease Registry (ATSDR), and the US Environmental Protection Agency (EPA)	Health Canada	IPCS	RIVM	ATSDR	EPA
Interhuman variability	Generally use when extrapolating from valid results from studies of prolonged exposure to average healthy humans. This factor is intended to account for the variation in sensitivity among humans and is thought to be composed of toxicokinetic and toxicodynamic uncertainties	1–10	10 (3.16×3.16)	10	10	10
Animal to human	Generally use when extrapolating from valid results of long-term studies on experimental animals when results of studies of human exposure are not available or are inadequate. This factor is intended to account for the uncertainty in extrapolating animal data to humans, and is also thought to be composed of toxicokinetic and toxicodynamic uncertainties	1–10	10 (2.5×4)	10	10	10
Less than chronic to chronic	Generally use when extrapolating from less than chronic results on experimental animals or humans. This factor is intended to account for the uncertainty in extrapolating from less than chronic NOAELs or LOAELs to chronic NOAELs or LOAELs			10	Not available/ not used (NA)[d]	$\leqslant 10$
LOAEL to NOAEL	Generally use when extrapolating an LOAEL to an NOAEL. This factor is intended to account for the experimental uncertainty in developing a safe dose from an LOAEL, rather than an NOAEL			10	10	$\leqslant 10$
Incomplete data	Generally use when extrapolating from valid results in experimental animals when the data are 'incomplete'. This factor is intended to account for the inability of any single study to adequately address all possible adverse outcomes	1–100	1–100	NA	NA	$\leqslant 10$
Modifying factor	Generally use upon a professional assessment of scientific uncertainties of the study and database not explicitly treated above (e.g., the number of animals tested)	1–10	1–10	NA	NA	0 to $\leqslant 10$

[a] *Sources*: Dourson (1994), Jarabek (1995), IPCS (1994), Meek *et al.* (1994), Pohl and Abdin (1995), and Rademaker and Linders (1994).

[b] The maximum uncertainty factor used with the minimum confidence database is generally 10 000.

[c] Professional judgment is required to determine the appropriate value to use for any given UF. The values listed in this table are nominal values that are frequently used by these agencies.

[d] ATSDR develops MRLS for specified durations of exposure, and generally does not extrapolate among durations. Therefore, an uncertainty factor for extrapolation between subchronic and chronic exposures is not used.

that a factor of 10 or lower is adequate and that use of data does support a lower factor with certain chemicals. Such a result is not surprising, since experiments are seldom designed with doses in excess of 10-fold apart, leading to the common belief that these ratios depend more on dose spacing than inherent toxicity. The choice of dose spacing, however, often reflects the judgment on the likely steepness of the dose–response slope, with steeper slopes resulting in tighter dose spacing.

Incomplete Data

If data are only available from one chronic study on which to base the estimation of a safe dose, the question may be asked whether data from chronic studies in other species or data from different types of bioassays (such as reproductive or developmental toxicity) would yield lower NOAELs. If so, an uncertainty exists that must be addressed. The default approach to address this uncertainty is by dividing by a 3- or 10-fold UF, based on the assumption that the critical effect can be discovered in a reasonably small selection of toxicity studies. This area of uncertainty has been investigated through the comparisons of NOAELs of different types of studies.

Data-Derived or Compound Specific Adjustment Factors (CSAF)

The science supporting the use of UFs has evolved considerably over the past years. Increased knowledge of inter- and intraspecies sensitivity, mechanism of action, and detailed evaluation of databases has led to improvements that allow for the incorporation of more scientific data into the dose–response assessment of noncancer toxicity, and permit the use of factors other than the standard default values.

Renwick examined the nature of the UFs generally applied for intraspecies and interspecies extrapolations. He proposed the division of each of these UFs into subfactors to allow for separate evaluations of differences in toxicokinetics and toxicodynamics. The toxicokinetic considerations include absorption, distribution, metabolism, and excretion of a toxic compound, and therefore address differences in the amount of the parent compound or active metabolite available to the target organ(s). The toxicodynamic considerations are based on variations in the inherent sensitivity of a species or individual to chemical-induced toxicity, and may result from differences in host factors that influence the toxic response of a target organ to a specified dose. The advantage to such a subdivision is that components of these UFs can be addressed where data are available (e.g., if data exist to show similar toxicokinetic handling of a given chemical between laboratory animals and humans, then the interspecies extrapolation factor would need to account only for differences in toxicodynamics).

Renwick examined in detail the relative magnitude of toxicokinetic and toxicodynamic variations between and within species. Results suggested that toxicokinetic differences were generally greater than toxicodynamic differences. Thus, he proposed that the 10-fold overall UF be subdivided into factors of 4 for kinetics and 2.5 for dynamics. The International Programme on Chemical Safety (IPCS) has adopted the principles set forth by Renwick, but has suggested that while the UF for interspecies extrapolation be subdivided unequally into four-fold (toxicokinetics) and 2.5-fold (toxicodynamics), the UF for intraspecies extrapolation should be split evenly (3.16-fold for both kinetics and dynamics) (see **Figure 1**).

This equal subdivision of the human variability factor was supported by a subsequent, more extensive analysis of appropriate kinetic parameters for 60 compounds in humans and concentration–effect data for 49 compound-related effects.

The quantitative toxicokinetic and toxicodynamic data used to inform interspecies and interindividual extrapolations in dose/concentration–response assessment was initially referred to as 'data-derived UFs'. But the new nomenclature of 'chemical-specific

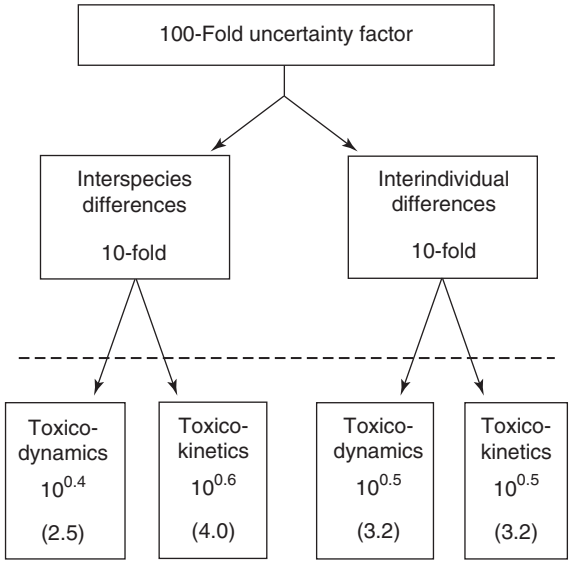

Figure 1 Subdivision of the 100-fold uncertainty factor showing the relationship between the use of uncertainty factors (above the dashed line) and proposed subdivisions based on toxicokinetics and toxicodynamics (IPCS, 1994, based on Renwick, 1993). Actual data should be used to replace the default values if available. (Reproduced with permission from IPCS.)

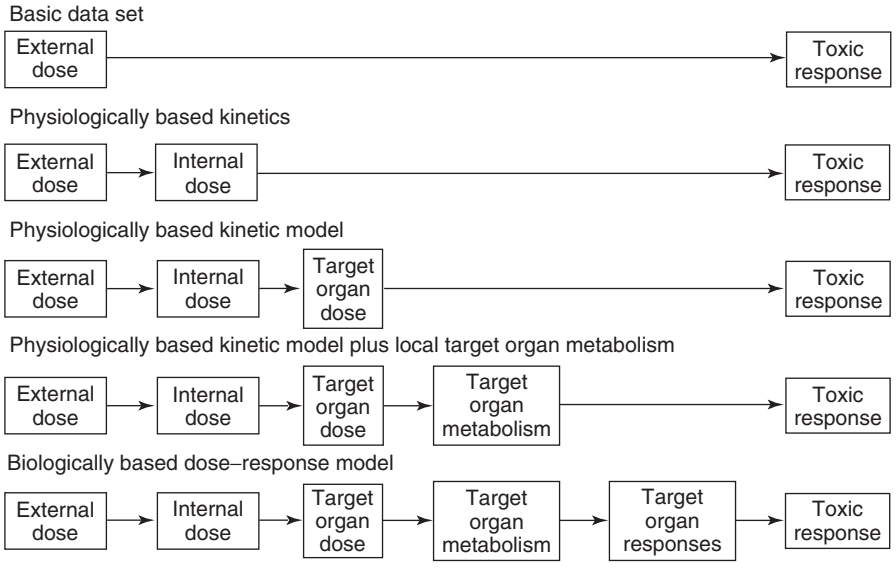

Figure 2 The relationship between external dose and toxic response for specific compounds. (Reproduced from Renwick AG, Dorne JLCM, and Walton K (2001) Pathway-related factors: The potential for human data to improve the scientific basis of risk assessment. *Human and Ecological Risk Assessment* 7: 165–180, with permission.)

adjustment factors' (CSAFs) has been adopted because it more accurately describes the nature of the refinement to the usual default approach. Also, it avoids confusion with factors that are based on an analysis of data for a group of chemicals sharing a common characteristic, that is, 'categorical' default factors such as those based on common physical/chemical characteristics or pathways of metabolism, which are sometimes referred to as data-derived factors, and which are not chemical-specific.

It is acknowledged that for many substances there are few data to serve as a basis for development of CSAFs. Indeed, currently, relevant data for consideration are often restricted to the component of uncertainty related to interspecies differences in toxicokinetics. While there are commonly fewer appropriate, relevant data at the present time to address the three other components considered here, namely interspecies (animal to human) differences in toxicodynamics, interindividual (human) variability in toxicokinetics, and interindividual (human) variability in toxicodynamics, it is anticipated that the availability of such information will increase with a better common understanding of its appropriate nature. Application of the approach even in the absence of data is considered to be informative, therefore, since it focuses attention on gaps in the available information that, if filled, would permit development of more appropriate measures of dose/concentration–response.

It should be recognized that CSAFs represent part of a broader continuum of increasingly data-informed approaches to account for interspecies differences and human variability, which range from default ('presumed protective') to more 'biologically based predictive' (**Figure 2**). The approach along this continuum adopted for any single substance is necessarily determined principally by the availability of relevant data. The extent of data available is, in turn, often a function of the economic importance of the substance.

The development of CSAFs may not always be possible or even necessary. For example, if the margin between the no- or lowest-effect level or bench mark concentration/bench mark dose (BMC/BMD) and anticipated human exposure is very wide, the generation of the more sophisticated data necessary to replace part of a default UF would not warrant the necessary experimentation in animals and humans and the associated resource expenditure. However, where this margin is small, development of additional chemical-specific quantitative data may be justified to refine the dose–response analyses and scientific credibility of the outputs, such as ADIs, TDIs, margins of exposure, or margins of safety.

Summary

As risk assessment scientists continue to accumulate and develop knowledge of toxicokinetics, toxicodynamics, mechanisms of toxicity, and temporal effects of critical effects for various chemicals, evaluations become increasingly more accurate and detailed. Moreover, the science behind the use of UFs has progressed considerably. Increased understanding of

inter- and intraspecies sensitivity, mechanisms of action, and detailed evaluation of databases can support the use of data-derived or CSAFs, which ultimately results in a risk assessment with greater confidence.

See also: Benchmark Dose; Chemical-Specific Adjustment Factor (CSAF); Environmental Protection Agency, US; International Programme on Chemical Safety; Risk Assessment, Human Health; Uncertainty Analysis.

Further Reading

Dourson ML (1994) Methods for establishing oral reference doses (RfDs). In: Mertz W, Abernathy CO, and Olin SS (eds.) *Risk Assessment of Essential Elements*, pp. 51–61. Washington, DC: ILSI Press.

Dourson ML, Felter SP, and Robinson D (1996) Evolution of science-based uncertainty factors in noncancer risk assessment. *Regulatory Toxicology and Pharmacology* 24: 108–120.

Dourson ML and Stara JF (1983) Regulatory history and experimental support of uncertainty (safety) factors. *Regulatory Toxicology and Pharmacology* 3: 224–238.

Jarabek A (1995) Interspecies extrapolation based on mechanistic determinants of chemical disposition. *Human and Ecological Risk Assessment* 1: 641–662.

Jarabek A (1995) The application of dosimetry models to identify key processes and parameters for default dose–response assessment approaches. *Toxicology Letters* 79: 171–184.

Kalberlah F and Schneider K (1998) Quantification of extrapolation factors. Final report of the research Project No. 116 06 113. Germany: Federal Environmental Agency.

Kroes R, Munro I, and Poulsen E (1993) Scientific evaluation of the safety factor for the acceptable daily intake. *Food Additives and Contaminants* 10: 269–373.

Meek ME, Newhool R, Liteplo RG, and Armstrong VC (1994) Approach to assessment of risk to human health for priority substances under the Canadian Environmental Protection Act. *Environmental Carcinogenesis & Ecotoxicology Reviews* C12: 105–134.

Meek M, Renwick A, Ohanian E, *et al.* (2001) Guidelines for application of compound specific adjustment factors (CSAF) in dose/concentration response assessment. *Comments in Toxicology* 7: 575–590.

Pohl HR and Abadin HG (1995) Utilizing uncertainty factors in minimal risk levels derivation. *Regulatory Toxicology & Pharmacology* 22: 180–188.

Rademaker BC and Linders JBHJ (1994) *Estimated-Concentrations-of-no-Concern of Polluting Agents in Drinking Water and Air for Humans*. Bilthoven, The Netherlands: Public Health and Environment.

Renwick AG (1993) Data-derived safety factors for the evaluation of food additives and environmental contaminants. *Food Additives and Contaminants* 10: 275–305.

Renwick AG and Lazarus NR (1998) Human variability and noncancer risk assessment – An analysis of the default uncertainty factor. *Regulatory Toxicology & Pharmacology* 27: 3–20.

Relevant Websites

http://www.inchem.org – International Program for Chemical Safety (IPCS) Principles for the Assessment of Risks to Human Health from Exposure to Chemicals. Environmental Health Criteria 210 (1999). Principles for Evaluating Health Risks to Reproduction Associated with Exposure to Chemicals. Environmental Health Criteria 225 (2001).

http://www.epa.gov – US Environmental Protection Agency (EPA) (1994) Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry. EPA/600/8-90/066F. Washington, DC: Office of Research and Development.

http://www.who.int – International Programme on Chemical Safety, World Health Organization.

Uranium

Fletcher F Hahn and Raymond A Guilmette

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- Chemical Abstracts Service Registry Number: CAS 7440-61-1
- Selected Compounds: Ammonium diuranate, $(NH_4)_2U_2O_7$ (CAS 7783-22-4); Uranium dioxide, UO_2 (CAS 1344-57-6); Uranium octaoxide, U_3O_8 (CAS 1344-59-8); Uranium tetrafluoride, UF_4 (CAS 10049-14-6); Depleted uranium, U (CAS 7440-61-1)
- Chemical/Pharmaceutical/Other Class: Metals

Uses

The primary use for uranium is in nuclear power reactors and in weapons. Low-enriched metal or ceramic UO_2 fuel pellets (enriched in fissile U-235) are produced for commercial power reactors. Smaller quantities of high-enriched fuel are produced for shipboard power reactors and weapons manufacture. Depleted uranium, a by-product of the enrichment process, is used for armor-piercing ammunition for the military, for counter balances and weights, and for radiation shielding. A small amount of uranium is used in specialty chemicals and catalysts.

Background Information

The chemical toxicity of uranium compounds is well known when compared to the toxicity of most other compounds. In 1824, a treatise described uranium salts as 'feeble poisons' when given by mouth to animals. In the late 1800s, uranium salts were used as homeopathic therapeutic agents in humans, primarily for treatment of diabetes. In the early 1900s, the renal toxicity of uranium became apparent in humans, and its use as a therapeutic agent ceased.

Toxicity studies of uranium compounds were initiated during World War II as nuclear weapons were developed. Initially the occupational exposure limits for lead were used for uranium. From studies in animals, however, it became clear that the amount of soluble uranium salts depositing and remaining in the lung or bone would never constitute a sufficient radiation hazard to override the chemical toxicity to the kidney. Based on these studies in animals a threshold concentration of $3\,\mu g\,U\,g^{-1}$ kidney and a limiting air concentration of $50\,\mu g\,m^{-3}$ were recommended for soluble uranium. Subsequently, all exposure standards for uranium have been based on the renal concentration of $3\,\mu g\,U\,g^{-1}$ kidney. Numerous calls for a reduction in this standard have been made based on more recent studies in animals and epidemiologic studies of individuals living in areas with high concentrations of uranium in the drinking water.

Exposure Routes and Pathways

Inhalation and ingestion are the primary ways for uranium to enter the body. Uranium is ubiquitous in the environment and is present in all soils and rocks in the form of a variety of minerals. Trace amounts of uranium are found in all foods. The intake of uranium in food in the United States ranges from 1 to $4\,\mu g\,day^{-1}$. Drinking water is also a source of uranium and may contribute more than food to the human intake. The quantity of uranium in drinking water varies widely. People in the United States ingest between 0.2 and $2\,\mu g\,U\,day^{-1}$ from water. Air concentrations of uranium also vary widely, but typically are low, ranging from 0.01 to $0.2\,ng\,m^{-3}$, resulting in estimated intakes of $0.002–0.020\,\mu g\,U\,day^{-1}$. Industrial processes, such as mining or milling of uranium ore and nuclear manufacturing facilities, can increase the uranium concentrations present in the air, resulting in potential occupational exposures to uranium.

Toxicokinetics

Three isotopes of uranium, all radioactive, occur together in natural uranium: U-238, U-234, and U-235. The chemistry of these isotopes, which is identical, determines the reactions of the isotopes within the environment as well as their transport and reactions within the body. All the isotopes of uranium emit primarily α particles. The U-238 isotope is the longest lived with a half-life of 4.5 billon years and constitutes more than 99% of the mass of natural uranium and half of its radioactivity. Uranium-234, a decay product of U-238, is responsible for nearly all the remainder of the radioactivity of natural uranium. A small amount (0.7% by weight) of fissionable U-235 is present in natural uranium.

Depleted uranium (DU) is a by-product of the enrichment process in which about 70% of the U-235 in natural uranium is separated from U-238. The remaining uranium, DU, contains about 0.2% U-235 by weight and emits about 60% of the radioactivity of natural uranium. US military specifications designate that DU contain less than 0.3% U-235.

Mixtures of uranium oxides ('yellow cake') are produced in the processing of uranium ores and can result in occupational exposures in uranium mill workers. Exposure to uranium tetrafluoride and hexafluoride is a potential for workers in the uranium enrichment industry.

After deposition in the lung or in wound sites, the movement or translocation of uranium depends primarily on the solubility of the uranium compound. Relatively insoluble compounds may be retained in the lung or a wound with a half-life of years. After absorption to blood from the lungs, a wound site or intestines, uranium is deposited systemically or is excreted by the kidneys. A substantial fraction of the metal ions filtered by the kidneys is retained in the renal tubules before it is passed into the urinary bladder. Over 90% of the uranium remaining in systemic tissues at one day is excreted with half-life ranging from 2 to 6 days and the remainder with half-lives ranging from 30 to 340 days. After a few days, most of the remaining uranium in the body is found in the kidneys, skeleton and, in the case of insoluble compounds, the site of entry (lung or wound).

Mechanism of Toxicity

The kidneys are considered to be the target organ for uranium chemical toxicity. Upon entering the bloodstream, $\sim$40% of the uranium in plasma is complexed with transferrin. The remaining 60% is in stable low molecular weight complexes with carbonate or bicarbonate, that are filtered though the renal glomeruli. As the glomerular filtrate passes through the proximal tubules, the complexed

uranium dissociates with decreasing pH. This dissociation liberates the reactive uranyl ion, which can interact with other complexing species in the filtrate or with components of the proximal tubular membrane. Some of the uranium that remains complexed in the lumen, and a portion of the freed uranyl ions, may traverse the length of the tubules and enter the bladder, thus resulting in a high rate of urinary excretion of uranium soon after exposure. Heavy metals, including uranium, have a great affinity for ionic sites of the brush border membrane of the proximal tubule. The suggested mechanism for renal damage is that the binding of uranium to these cells may alter cellular permeability to sodium, which, in turn, interferes with the transport of glucose, amino acids, and phosphates, resulting in the increased release of these compounds into the urine.

Acute and Short-Term Toxicity (or Exposure)

Renal Effects in Humans

Health effects related to immediate kidney injury are of primary concern following inhalation or ingestion of large quantities of soluble uranium compounds. Acute nephrotoxicity, manifest by acute renal failure with severe oliguria, proteinuria, and increased nonprotein nitrogen has been described in occupationally exposed workers. Transient biochemical effects from lower-level exposures include proteinuria, albuminuria, enzymuria, and the appearance of casts in the urine. Because these biochemical changes typically resolve within a few days, it is possible that individuals exposed to uranium resulting in low kidney burdens will not have detectable renal effects if they are not assessed within a few days of the exposure. Protracted biochemical changes noted are similar to the transient changes, the principal difference being the duration of the change. These changes were not necessarily irreversible. Following recovery from acute effects, individuals typically have no persistent effects.

Pulmonary Effects in Humans

The acute pulmonary toxicity of inhaled uranium is dependent on the chemical form of the uranium. Uranium hexafluoride is the only uranium compound that has been associated with acute effects after inhalation. Two accidents involving uranium hexafluoride have resulted in the deaths of three workers in the US uranium processing industry. However, the lethal effects were due to liberated hydrogen fluoride rather than the uranium.

Chronic Toxicity (or Exposure)

Renal Effects in Humans

Two situations are of primary concern for chronic toxicity of uranium, inhalation of yellowcake or ore concentrate by uranium process workers, and ingestion of drinking water by the general population. These exposures are chronic, lasting for years. Uranium millers, exposed over a period of years to aerosols of yellowcake, had calculated kidney concentrations up to $\sim 1\,\mu g\,U\,g^{-1}$. Biochemical indicators of renal effects, but not clinical symptoms, were noted in some of these workers. The lowest estimated kidney concentrations of uranium reported to result in renal effects were related to high concentrations ($\sim 80\,\mu g\,l^{-1}$) of uranium in the drinking water seen in two studies. In both of these studies, biochemical indicators of subtle renal effects of undetermined significance were seen. The length of time of the exposure, however, may be an important factor. The groups with the longer exposure times have the greatest effects.

Pulmonary Effects in Humans

Based on epidemiologic studies of workers in the uranium processing industry, the chronic exposure to aerosols of uranium compounds has not been related to chronic pulmonary health effects, such as chronic obstructive pulmonary disorder or lung cancer.

In Vitro Toxicity Data

Soluble compounds of uranium are genotoxic in cultured cells. They have caused micronuclei, chromosomal aberrations and sister chromatid exchanges in Chinese hamster ovary cells and chromosomal aberrations in human peripheral lymphocytes.

Clinical Management

For treatment when acute nephrotoxicity is possible, alkalinization of the urine is important to increase urinary excretion of uranium. Systemic chelating agents, such as calcium or zinc salts of diethylenetriaminepentaacetic acid (Ca-DTPA or Zn-DTPA), although recommended in some publications, have not been shown to be useful in increasing the excretion of uranium.

Environmental Fate

Uranium is naturally occurring in the environment with an average abundance in the earth's crust of $\sim 2\,mg\,kg^{-1}$ (range $0.1–20\,mg\,kg^{-1}$). It is more

abundant than silver or gold. Concentrations of uranium in water, food, and soil are variable (typically 0.1–5 $\mu g\,l^{-1}$ in water; 0.1–2 $mg\,kg^{-1}$ in soil and 0.01–2 μg in food) and depend largely on the presence of uranium in soil or rocks or proximity of industries that may introduce uranium into the environment. Extreme concentrations (up to a factor of 100 times the typical ranges noted) may be found in certain geologic environments or where uranium has been concentrated, such as mine tailings. Uranium concentrations in surface and ground waters, as well as many bottled mineral waters, commonly exceed current drinking water standards.

Exposure Standards and Guidelines

- Water: Environmental Protection Agency National Primary Drinking Water Standard – 30 $pCi\,l^{-1}$ (20 $\mu g\,l^{-1}$) proposed.
- Air: Occupational Safety and Health Administration permissible exposure level time-weighted average (corrected rule) – soluble 0.05 $mg\,m^{-3}$; insoluble 0.25 $mg\,m^{-3}$.

Further Reading

Agency for Toxic Substances and Disease Registry (ATSDR) (1999) *Toxicological Profile for Uranium*, Report TP 90-29. Atlanta, GA: ATSDR.

BEIR IV (1988) Uranium. In: *Health Risks of Radon and Other Internally Deposited Alpha-Emitters*, chap. 6. Committee on the Biological Effects of Ionizing Radiations, National Research Council. Washington, DC: National Academy Press.

Fulco CE, Liverman CT, Sox HC (eds.) (2000) Depleted uranium. In: *Gulf War and Health*, Vol. 1, Depleted Uranium, Sarin, Pyridostigmine Bromide, Vaccines, chap. 6, pp. 88–167. Washington, DC: National Academy Press.

Hodge HC, Stannard JN, Hursh JB (eds.) (1973). Uranium, plutonium, transplutonium elements. In: *Handbook of Experimental Pharmacology*, vol. 36. New York: Springer.

Leggett RW (1989) The behavior and chemical toxicity of U in the kidney: A reassessment. *Health Physics* 57: 365–383.

Stannard JN (2003) Internal emitter research and standard setting. *Health Physics* 85: 275–279.

Voegtlin C and Hodge HC (eds.) (1953) (1949) *The Pharmacology and Toxicology of Uranium Compounds National Nuclear Energy Series*. New York: McGraw-Hill.

World Health Organization (WHO) (2001) *Depleted Uranium: Sources, Exposure and Health Effects*. Geneva: Department of the Protection of the Human Environment, World Health Organization.

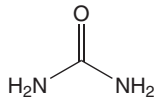
Relevant Websites

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

Urea

Midhun C Korrapati and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 57-13-6
- Synonyms: Aqua Care; Carbamide; Keratinamin
- Chemical/Pharmaceutical/Other Class: Diuretic, osmotic agent, and dermatological agent
- Chemical Formula: N_2H_4CO
- Chemical Structure:



Uses

Urea is used as an osmotic to treat problems like high pressure in the eye ball (glaucoma). It is also used as a diuretic and as a topical dermatological agent in treating psoriasis, and other dry, scaly conditions.

Exposure Routes and Pathways

Exposure to urea may occur through inhalation and dermal contact with this compound at workplaces where urea is produced or used, especially to workers applying urea fertilizers. General population may be exposed to urea via ingestion of food, drinking water, and dermal contact with products containing urea.

Toxicokinetics

Urea is a nonionizable compound that readily traverses mammalian membranes, probably along with water, through the pores.

Mechanism of Toxicity

The primary mechanism of toxicity appears to be inhibition of the citric acid cycle. It leads to blockade of electron transport and a decrease in energy

production and cellular respiration, which leads to convulsions.

Acute and Short-Term Toxicity (or Exposure)

Animal

Intravitreal injection of 0.2 ml of 10 M solution into vitreous humor of rabbits has caused inflammation, chorioretinitis, and degeneration of retina. Urea mixed with soy meal is particularly dangerous, as urease in the latter leads to formation of ammonia. Poisoning of cattle may also be caused by urea as fertilizer and spread unevenly on pasture lambs given $2\,g\,kg^{-1}$ of urea died in 90–200 min. Adult sheep given same dose exhibited almost continuous convulsions after 165 min.

Human

Adverse reactions include headache, nausea, vomiting, disorientation, and transient confusion. Urea causes redness and irritation of skin and eyes.

Chronic Toxicity (or Exposure)

Animal

Urea was tested for mutagenicity in the Salmonella/microsome preincubation assay using the standard protocol approved by the National Toxicology Program. Urea was tested at doses of 0.10, 0.33, 1.0, 3.3, and 10 mg per plate in as many as five *Salmonella typhimurium* strains (TA1535, TA1537, TA97, TA98, and TA100) in the presence and absence of rat or hamster liver S-9. Urea was negative in these tests and the highest ineffective dose tested in any *Salmonella typhimurium* strain was 10 mg per plate.

Human

Urea is found to be mutagenic in humans.

Clinical Management

Urea is effectively eliminated by the kidney. If normal renal function exists, diuresis will ensue. Patients should have adequate hydration. When diuresis is extensive electrolytes should be monitored. Patients should be moved to fresh air when exposed through inhalation. Respiratory distress should also be monitored. If cough or difficulty in breathing develops, patient should be evaluated for respiratory tract irritation, bronchitis, or pneumonitis. Administer oxygen and assist ventilation as required. Bronchospasm should be treated with inhaled $\beta 2$ agonist and oral or parenteral corticosteroids. When dermally exposed, contaminated clothing should be removed and exposed area should be washed thoroughly with soap and water. A physician should examine the area if irritation or pain persists.

Environmental Fate

Terrestrial Fate

Urea is expected to have very high mobility in soil. Urea is not expected to volatilize from dry soil surfaces based upon its vapor pressure. Various field and laboratory studies have demonstrated that urea degrades rapidly in most soils. Urea is rapidly hydrolyzed to ammonium ions through soil urease activity, which produces volatile gases, that is, ammonia and carbon dioxide. However, the rate of hydrolysis can be much slower depending upon the soil type, moisture content, and urea formulation.

Aquatic Fate

Urea is not expected to adsorb to suspended solids and sediment. Volatilization from water surfaces is not expected. Urea is rapidly hydrolyzed to ammonia and carbon dioxide in environmental systems by the extracellular enzyme, urease, which originates from microorganisms and plant roots.

Atmospheric Fate

According to a model of gas/particle partitioning of semivolatile organic compounds in the atmosphere, urea, which has a vapor pressure of 1.2×10^{-5} mmHg at 25°C, will exist in both the vapor and particulate phases in the ambient atmosphere. Vapor-phase urea is degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 9.6 days.

Ecotoxicology

The toxicity threshold for *Scenedesmus quadricauda* (green algae) is $>10\,000\,mg\,l^{-1}$. Toxic effect is multiplication inhibition of cell division. Toxicity threshold for *Entosiphon sulcatum* (protozoa) is $>29\,mg\,l^{-1}$ and the toxic effect is inhibition of cell multiplication.

Exposure Standards and Guidelines

Residues of urea are exempted from the requirement of a tolerance when used as a stabilizer, inhibitor in accordance with good agricultural practices as inert

(or occasionally active) ingredient in pesticide formulations applied to growing crops or to raw agricultural commodities after harvest. This action promulgates standards of performance for equipment leaks of volatile organic compounds (VOCs) in the Synthetic Organic Chemical Manufacturing Industry (SOCMI). The intended effect of these standards is to require all newly constructed, modified, and reconstructed SOCMI process units to use the best demonstrated system of continuous emission reduction for equipment leaks of VOC, considering costs, nonair quality health and environmental impact and energy requirements. Food and Drug Administration requirements: substance added directly to human food affirmed as generally recognized as safe.

Workplace environmental exposure level: 8 h time-weighted average 10 mg m^{-3}.

See also: Volatile Organic Compounds (VOC).

Further Reading

Environment Canada (1985) Technological Information for Problem Spills: Urea.

Haliburton JC and Morgan SE (1989) Nonprotein nitrogen-induced ammonia toxicosis and ammoniated feed toxicity syndrome. *The Veterinary Clinics of North America – Food Animal Practice* 5: 237–249.

WHO/IPCS (1993) Toxicological Evaluation of Certain Food Additives and Contaminants. WHO Food Additives Series 32.

Urethane

Heriberto Robles

- Chemical Abstracts Service Registry Number: CAS 51-79-6
- Synonyms: Ethyl carbamate; Ethylurethane; Urethan
- Chemical/Pharmaceutical/Other Class: Carbamates
- Chemical Formula: $C_3H_7NO_2$

Uses

Urethane is used as a solvent for various organic materials, pesticides, and fumigants. It is also used as a chemical intermediate in the production of cross-linking agents in textiles and pharmaceutical industry. Urethane was once used as an anesthetic in veterinary medicine. Its veterinary use was discontinued in 1948 when its carcinogenic properties were revealed.

Exposure Routes and Pathways

Occupational exposure to urethane may occur through inhalation of dust particles and dermal contact with this compound at workplaces where urethane is produced or used. In addition to industrial worker exposure, urethane is unintentionally formed during the manufacture of certain consumer beverages. Urethane has been found predominantly in bourbons, sherries, fruit brandies, whiskeys, and wines. The general population may thus be exposed to urethane via ingestion of fermented foods and alcoholic beverages.

Toxicokinetics

Urethane can be excreted in the urine unchanged (0.5–1.7% of dose). Urethane can also be metabolized by the liver cytochrome P450 system. The urinary metabolites of urethane include *N*-hydroxy urethane, acetyl-*N*-hydroxyurethane, ethyl mercapturic acid, and *N*-acetyl-*S*-ethoxycarbonyl cysteine.

Mechanism of Toxicity

Urethane is activated in the liver into a carcinogenic metabolite. The activation of urethane by cytochrome P450 involves two sequential reactions. First, urethane is dehydrogenated to vinyl carbamate followed by epoxidation to form vinyl carbamate epoxide. The former is believed to be the ultimate carcinogenic metabolite of urethane.

Acute and Short-Term Toxicity (or Exposure)

Animal

Developmental defects have been produced in offspring of rats and hamsters treated *in utero* with urethane. Some of the malformations noted included eye, skeletal, neuronal tube defects, and cardiac malformations.

Human

Signs and symptoms of overexposure in humans include vomiting, anorexia, drowsiness, nausea,

vomiting, and dizziness. Exposure to high concentrations has been reported to produce hemorrhages, kidney and liver injury and, in severe cases, coma.

Chronic Toxicity (or Exposure)

Animal

Chronic administration of urethane through the oral, inhalation, subcutaneous, and intraperitoneal routes has produced cancer in mice, rats, and hamsters. Urethane exposure in laboratory animals has produced an increased incidence of spontaneous lung adenomas in susceptible mice strains.

Human

Chronic overexposure in humans has been reported to produce damage to the blood and bone marrow as well as to the liver. No data exist that link human exposure to urethane and cancer. Nonetheless, given its carcinogenic effects in animals, the International Agency for Research on Cancer has labeled urethane as a possible human carcinogen.

Clinical Management

There is no specific treatment for urethane toxicity. Supportive and symptomatic treatment is recommended.

Environmental Fate

Urethane may be released to the environment in various waste streams from its production and use in the preparation and modification of amino resins, as a solubilizer and cosolvent for pesticides and fumigants, as an intermediate in the production of pharmaceuticals, as an antineoplastic agent, and as a reagent in biochemical research. If released to the atmosphere, urethane is expected to exist solely as a vapor in the ambient atmosphere. Vapor-phase urethane will be degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals with an estimated half-life of 2.2 days. If released to soil, urethane is expected to have very high mobility. Volatilization from moist soil surfaces is not expected to occur. Biodegradation of urethane in soil may be important. If released into water, urethane is not expected to adsorb to suspended solids and sediment in the water column. Volatilization from water surfaces is not expected. The potential for bioconcentration in aquatic organisms is low based on an estimated bioconcentration factor (BCF) of 0.45. Purely aliphatic carbamates are expected to be resistant to hydrolysis under environmental conditions; hydrolysis half-lives of 3300 and 330 years at pHs 7 and 8, respectively, were estimated for urethane. Urethane was judged easy to biodegrade in river die-away tests. Other biodegradation studies using activated sludge indicate urethane may biodegrade slowly.

Other Hazards

Urethane is combustible. When heated it emits toxic nitrogen oxide fumes.

Exposure Standards and Guidelines

Urethane is classified as a Group 2B carcinogen (probable human carcinogen) and hazardous air pollutant. Special precautions must be taken when working with urethane. Personnel handling urethane must follow industrial hygiene and health protection requirements for handling potentially carcinogenic substances. At a minimum, urethane exposure should be minimized through the use of engineering controls, work practices and personal protective equipments, including impervious and disposable gowns and gloves as well as eye and respiratory protection. In addition, working areas and working instruments must be especially designed for handling potentially harmful substances.

See also: Carcinogenesis; Dithiocarbamates.

Further Reading

Ellenhorn MJ and Barceloux DG (eds.) (1988) *Medical Toxicology, Diagnosis and Treatment of Human Poisoning*. New York: Elsevier.

Klaassen CD (ed.) (2001) *Casarett & Doull's Toxicology, The Basic Science of Poisons*, 6th edn. New York: McGraw-Hill.

Rossoff IS (2002) *Encyclopedia of Clinical Toxicology*. Boca Raton, FL: The Parthenon Publishing Group.

Relevant Website

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

V

Validation of Toxicity Testing *See* Toxicity Testing, Validation.

Valium *See* Diazepam.

Valley of the Drums

Pertti J Hakkinen

A site in Brooks, Kentucky, USA, is called the Valley of the Drums. It was one of the earliest and most serious hazardous waste sites in the United States. The Valley of the Drums involved a vast quantity of illegally disposed material, and discovery of this site helped lead the US Congress to create the Superfund law.

The property was owned by Arthur Taylor until 1977, and the 13 acres of the Valley of Drums were used as a refuse dump, drum recycling center, and chemical dump from 1967 to 1977. Another 10 acres of the tract owned by Mr. Taylor was not part of the Valley of the Drums. The paints and coatings industries of the Louisville, Kentucky area were the primary waste generators using the Valley of the Drums.

In 1967, the Kentucky Department of Natural Resources and Environmental Protection (KDNREP) identified the Valley of the Drums site as an uncontrolled dump location for hazardous waste.

Some of the drums were emptied into open pits and trenches, cleaned, and recycled. Other drums were buried on site and, during the later years of operation, many drums were stored on the surface. Mr. Taylor was eventually stopped from burning the chemical waste, and soil from nearby hillsides was used to cover the pits and trenches. Thousands of drums were also stored on the surface of the site, and investigation found four or five major cells of buried wastes containing chemical liquids, sludges, and crushed drums.

The KDNREP first documented releases of hazardous chemicals from the Valley of the Drums in 1975, and they pursued legal actions against Mr. Taylor until his death in late 1977. Throughout the history of his operating the site from 1967 to 1977, Mr. Taylor never applied for the required state permits. A KDNREP investigation of the property revealed that over 100 000 drums of waste were delivered to the site. When it rained, the deteriorating drums leaked, and drainage overflowed into Wilson Creek, a tributary of the Ohio River. In 1979, large amounts of chemicals were carried into Wilson Creek by the spring snow melts. In January of 1979, at the request of KDNREP, the US Environmental Protection Agency (EPA) responded to the releases under the authority of Section 311 of the Clean Water Act. The open pits which had been used for burning solvents had been covered over before EPA's involvement.

The initial drum inventory conducted in 1979 found 17 051 drums on the surface, including 11 628 empty ones. The EPA analyzed the property and creek and found $\sim$140 chemical substances. The chemicals found most often and in the highest concentrations were xylene, methyl ethyl ketone, methylene chloride, acetone, phthalates, anthracene, toluene, fluoranthene, alkyl benzene, vinyl chloride, dichloroethylene, and aliphatic acids. Polychlorobiphenyls were detected in low concentrations and several metals including barium, zinc, copper, strontium, magnesium, and chromium were detected in concentrations exceeding background levels.

In 1980, KDNREP contacted six Responsible Parties who identified and removed some of the waste remaining on the surface of the site. Through these response activities and voluntary removal of wastes by the known generators, a majority of the drums on

the surface were removed. Actions by EPA intended to prevent further releases of chemicals into the creek included the construction of interceptor trenches and a temporary water treatment system, securing leaking drums, and segregating and organizing drums of the site.

In 1981, US EPA again inspected the site and discovered deteriorating and leaking drums and discharges of chemicals into Wilson Creek occurring again. EPA responded by upgrading the treatment system and removed the remaining several thousand drums on the surface of the site for recycling or disposal; however, some waste remained buried on the site. The area became Kentucky's first federal Superfund site in 1983.

In 1986 and 1987, the US EPA took additional remedial action to contain the site from any further impact to the surrounding environment. Overall, over $2.5 million has been spent to clean up the Valley of the Drums. The Valley of the Drums undergoes periodic scheduled reviews by US EPA and the Army Corp of Engineers to determine, if the cleanup measures that have been taken are still judged to be adequate. The reviews have found that the remedies put into place in 1987, including covering the site with a containment cap, were effective, and that a remarkable cleanup job has been accomplished. In addition to 5 year reviews, the site is also monitored regularly to make sure that tree roots or other foreign objects are not damaging the cap, and to sample water in several wells that were installed near the cap to check whether contaminated water is leaving the site.

See also: Environmental Protection Agency, US; Hazardous Waste.

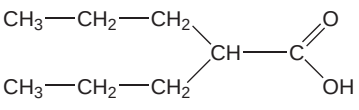
Relevant Website

http://www.epa.gov – US EPA Record of Decision System (RODS) Website on EPA/ROD/R04-86/009. 1986. EPA Superfund Record of Decision: A.L. Taylor (Valley of Drums) EPA ID: KYD980500961 OU 01, Brooks, KY, 06/18/1986. The US EPA website also has information on the history of the Valley of the Drums.

Valproic Acid

Dennis J Naas

- Chemical Abstracts Service Registry Numbers: CAS 99-66-1; CAS 76584-70-8 (Sodium valproate)
- Synonyms:
  - Valproic acid: 2-Propylpentanoic acid; Dipropylacetic acid (n-DPA); 2-Propylvaleric acid; Di-n-propylacetic acid; Depakene
  - (Semi)sodium valproate: Divalproex sodium; Sodium hydrogen bis(2-propylpentanoate); Depakote; Mylproin
  - Other proprietary names: Epilim; Convulex; Depakin; Depakine; Deprakine
- Chemical/Pharmaceutical/Other Class: Anticonvulsant
- Chemical Structure:



Uses

Valproic acid is used therapeutically as an anticonvulsant. It is a synthesized, simple, branched-chain carboxylic acid that is chemically unrelated to other anticonvulsants. Valproic acid and valproate are used in a variety of absence and generalized seizure disorders.

Exposure Routes and Pathways

Toxicity results from acute or chronic ingestion of tablets, capsules, or elixir.

Toxicokinetics

Peak plasma levels occur 1–4 h for capsules and syrup and 3–4 h for delayed-release capsules and tablets. Absorption is delayed but not diminished in the presence of food. Bioavailability appears to be complete. The majority of a dose undergoes hepatic glucuronidation or oxidation. At least two metabolites, 2-propyl-2-pentenoic acid and 2-propyl-4-pentenoic acid, have anticonvulsant activity. Biotransformation can be enhanced by enzyme-inducing drugs (e.g., primadone, carbamazine, phenobarbital, and

phenytoin), but there is no apparent autoinduction. The apparent volume of distribution is 0.2 or $0.3\,l\,kg^{-1}$ (but $\sim 1\,l\,kg^{-1}$ for the free, unbound portion), with high concentrations found in areas containing gamma-aminobutyric acid (GABA). Plasma protein binding is 90–95% at therapeutic concentrations but decreases as plasma levels increase. The therapeutic level in plasma is $50–150\,\mu g\,ml^{-1}$.

Less than 3% of a dose is excreted unchanged in the urine or through the feces. The elimination half-life from plasma is 10–15 h when valproic acid is used alone, but interaction with other anticonvulsant drugs can reduce the half-life to 4–10 h. It may be much longer in hepatic-impaired individuals, the elderly, and young children.

Mechanism of Toxicity

The anticonvulsant properties of valproic acid (and/or its metabolites) are likely attributable to enhancement (decreased metabolism or decreased re-uptake in brain tissues) of GABA activity. Valproic acid may also inhibit platelet aggregation.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} in rats is $675\,mg\,kg^{-1}$.

Human

In most cases, overdoses with valproic acid are relatively well tolerated. Most patients will have nausea and/or vomiting, and a mild degree of lethargy. Miosis and confusion can also occur. Rare cases of seizures, coma, cerebral edema, hypotension, and cardiorespiratory arrest have been reported. The incidence of these effects is unknown, but they are likely dose related. Significant depression of consciousness has been associated with ingestions exceeding $200\,mg\,kg^{-1}$. Recovery has occurred following ingestion of 25 g. A fatality was reported in a 15-year-old with a plasma concentration of $1914\,\mu g\,ml^{-1}$ and cardiorespiratory arrest occurred 20 h postingestion. Transient elevation of hepatic transaminases, acute pancreatitis, and hyperammonemia has been noted after acute overdose.

Chronic Toxicity (or Exposure)

Animal

Developmental toxicity, manifest as skeletal abnormalities and neural tube defects, have been observed in rodents treated during gestation.

Human

The first confirmed report of an infant with congenital defects after valproic acid exposure during pregnancy appeared in 1980. The mother took 100 mg of valproic acid daily throughout gestation, and delivered a growth-retarded infant with facial dysmorphism and heart and limb defects. The infant expired at age of 19 days. Since this initial report, several studies and case reports have described newborns with malformations after *in utero* exposure to either valproic acid monotherapy or combination therapy. The most serious abnormalities observed with valproic acid (or sodium valproate) exposure are defects in neural tube closure. The absolute risk of this defect is $\sim$1–2%, about the same risk for a familial occurrence of the anomaly. No cases of anencephaly have been associated with valproic acid. Exposure to valproic acid between the 17th and 30th days after fertilization must occur before the drug can be considered a cause of neural tube defects. Other predominant defects involve the heart, face, and limbs. A characteristic pattern of minor facial abnormalities has been attributed to valproic acid. Cardiac anomalies and cleft lip/palate occur with most anticonvulsants, and a causal relationship with valproic acid has not been established.

Hepatotoxicity is a concern. During the first few months of therapy, transient elevation of hepatic transaminases occurs in an average 11% (up to 40%) of patients. Fulminant hepatic failure will develop in 1 in 5000–10 000 patients. In these cases there is hepatic necrosis, steatosis, and a Reye's syndrome-like illness. Fatal hepatic injury is most likely in children less than 2 years old and in those patients on multiple-drug therapy.

In Vitro Toxicity Data

Valproic acid is an *in vitro* developmental toxicant (rodent whole embryo culture system).

Clinical Management

The majority of patients with acute overdose have a benign course and needs supportive care alone. The gut should be decontaminated with oral doses of activated charcoal. Gastric lavage can be considered after ingestion of life-threatening quantities if it can be done soon after ingestion (generally within 1 h). Airway management should be provided after severe overdose. Ipecac is not recommended due to its potential for central nervous system depression. If hypotension develops, isotonic fluids should

be infused. Measures to enhance elimination are not justified despite testimonials from case reports. Patients requiring treatment in the emergency department should be tested for valproic acid plasma concentration, complete blood count, liver function, and perhaps for the presence of other anticonvulsant drugs. Valproic acid therapy should be discontinued in patients with elevated hepatic enzymes or serum ammonia. There is no known antidote; however, one case report describes a positive response to naloxone in a child with a serum level of 185 $\mu g\,ml^{-1}$.

See also: Benzodiazepines; Carbamazepine; Developmental Toxicology; Phenytoin.

Further Reading

Briggs GG, Freeman RK, and Yaffe SJ (1994) *A Reference Guide to Fetal and Neonatal Risk. Drugs in Pregnancy and Lactation*, 4th edn., p. 869. Baltimore, MD: Williams and Wilkins.

Sztajnkrycer MD (2002) Valproic acid toxicity: Overview and Management. *Journal of Toxicology. Clinical Toxicology* 40(6): 789–801. (Erratum in: (2003) *Journal of Toxicology. Clinical Toxicology* 41(4): 215.)

Vanadium

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 7440-62-2
- Chemical/Pharmaceutical/Other Class: Transition metals
- Chemical Formulas: V^{2+}; V^{3+}; V^{4+}; V^{5+}

Uses

Vanadium is used as an alloying addition to steel, iron, titanium, copper, and aluminum, with the primary use in the steel industry. Vanadium is also used as a target material for X-rays, as a catalyst for the production of synthetic rubbers, plastics, and chemicals, and in ceramics. Vanadium is an element of pharmacological and nutritional significance; for example, it has increasing therapeutic use in diabetes, and is emerging as a potent anticarcinogenic agent.

Background Information

Vanadium was discovered in 1830. It is present at 0.01% in earth's crust. Vanadium is released naturally into the air through the formation of continental dust, marine aerosols, and volcanic emissions. The natural release of vanadium into water and soils occurs primarily as a result of weathering of rocks and soil erosion. Anthropogenic sources include the combustion of fossil fuels, particularly residual fuel oils, which constitute the single largest overall release of vanadium to the atmosphere. Deposition of atmospheric vanadium is also an important source both near and far from industrial plants burning residual fuel oils rich in vanadium. Other anthropogenic sources include leachates from mining tailings, vanadium-enriched slag heaps, municipal sewage sludge, and certain fertilizers. Natural releases to water and soil are far greater overall than anthropogenic releases to the atmosphere.

Exposure Routes and Pathways

The general population is exposed to background levels of vanadium primarily through ingestion of food. Workers in industries processing or using vanadium compounds are commonly exposed to higher than background levels via the inhalation pathway. A 1980 estimate by the National Institute for Occupational Safety and Health indicates that in 1980 about 5319 people were exposed to vanadium pentoxide in their workplace. Exposure through inhalation may also be of importance in urban areas where large amounts of residual fuel oil are burned. Other populations possibly exposed to higher than background levels include those ingesting foodstuffs contaminated by vanadium-enriched soil, fertilizers, or sludge. Populations in the vicinity of vanadium-containing hazardous waste sites may be exposed under these circumstances.

Toxicokinetics

In humans, 0.1–1% of orally administered vanadium is absorbed through the gut. Lung and gut absorption increases with the solubility of the vanadium compound. Vanadium pentoxide is ~100% absorbed by inhalation. Vanadium is not absorbed through the skin. When absorbed, 60% of the vanadium is excreted by the kidneys within 24 h of administration. Vanadium can pass through the blood–brain barrier.

Mechanism of Toxicity

In the consolidated form, vanadium metal and its alloys may pose no particular health or safety hazard; however, the toxicity of vanadium alloys may be a function of other components of the alloy. Vanadium compounds have been proven to be associated with the pathogenesis of some human diseases and also in maintaining normal body functions. Salts of vanadium interfere with many enzyme systems, for example, ATPases, protein kinases, ribonucleases, and phosphatases. Vanadium may also be an essential trace element, contributing to glucose balance; however, the importance of this element as a micronutrient is yet to be unequivocally accepted. Vanadium deficiency has been associated with disturbances in physiological functions, for instance, thyroid, glucose, and lipid metabolism. Vanadate (VO_3^-) mimics the action of insulin in target tissues and is a potential inhibitor of the sodium pump. Vanadium toxicity is enhanced by dietary zinc. Several genes are regulated by this element or by its compounds, including those for tumor necrosis factor-alpha, interleukin-8, activator protein-1, ras, c-raf-1, mitogen activated protein kinase, p53, and nuclear factors-kappaB.

When inhaled, vanadium is toxic to alveolar macrophages and therefore may impair pulmonary resistance to infection and clearance of particulate matter. An increase in inflammatory cells of the nasal mucosa has been observed in workers exposed to vanadium.

Acute and Short-Term Toxicity (or Exposure)

Animal

Inhalation of vanadium in animals results in lung irritation, coughing, wheezing, chest pain, atrophic rhinitis, and conjunctivitis. Pulmonary edema has been observed in animals after exposure to some vanadium compounds. The acute oral toxicity of vanadium is low: In mice, $1000\,mg\,kg^{-1}$ causes catarrhal gastritis. Acute oral exposure in rats results in distress, hemorrhagic exudates from the nose, diarrhea, hind limb paralysis, labored respiration, convulsions, organ congestion, fatty degeneration of the liver and kidney, focal hemorrhage of the lung and adrenal cortex, and death. Rat (oral) $LD_{Lo} = 225\,mg\,kg^{-1}$ over 5 days.

Human

In general, vanadium has a very low oral and dermal toxicity and a moderately low toxicity by the inhalation route. The toxicity of vanadium increases with its valence state, with vanadium pentoxide being the most toxic of the vanadium compounds. Vanadium fumes are more toxic than vanadium dust. Acute inhalation exposure has resulted in lung irritation, coughing, wheezing, chest pain, nosebleeds, atrophic rhinitis, pharyngitis, epistaxis, tracheitis, asthma-like diseases, irritation of the eyes, and a metallic taste in the mouth. Symptoms generally disappear within 2 weeks of exposure. A quantity of $2–10^4\,mg\,m^{-3}$ has resulted in mild to moderate respiratory effects and no systemic effects in humans.

Acute oral exposure results in abdominal cramping, diarrhea, black stools, and a greenish-black coating on the tongue. Skin exposure may result in dermatitis, allergic skin lesions, and a green discoloration of the skin. A fatal dose may result in central nervous system depression with tremors, headache, and tinnitus.

Chronic Toxicity (or Exposure)

Animal

Chronic inhalation and oral exposure to vanadium in laboratory animals has resulted in kidney and liver changes, decreased erythrocyte count and hemoglobin levels, and increased reticulocyte count in peripheral blood. Chronic oral exposure to vanadium has caused an increase in minor birth defects and fetal death in pregnant rats. Vanadium has not been found to cause mutagenic, carcinogenic, teratogenic, or reproductive effects in short-term studies. Among laboratory animals, rabbits and guinea pigs are particularly susceptible.

Human

Systemic symptoms of exposure to vanadium are extremely rare but could include peripheral vasoconstriction of the lungs, spleen, kidneys, and intestines. Chronic exposure to vanadium may result in arrhythmias and bradycardia.

Clinical Management

Irrigate exposed skin and eyes with copious amounts of tepid water (with soap for exposed skin). After inhalation exposures, move to fresh air and monitor for respiratory distress. Administer 100% humidified supplemental oxygen with assisted ventilation as required. If coughing or breathing difficulties are noted, the patient should be evaluated for irritation or bronchitis, including chest X-rays and determination of blood gases. For ingestion exposures, emesis may be indicated for recent, substantial ingestion.

Activated charcoal may be considered, depending on the form of vanadium ingested. Chelation is not usually indicated since systemic effects are rare.

Ecotoxicology

It is unlikely that there is bioaccumulation or biotransformation.

Miscellaneous

Vanadium can react violently with bromine trifluoride, chlorine, lithium, and oxidants; for example, powdered vanadium can explode in contact with chlorine.

See also: Metals; Zinc.

Further Reading

Dart RC (ed.) (2004) *Medical Toxicology*, 3rd edn. Baltimore, MD: Williams and Wilkins.
Goyer RA, Klaassen CD, and Waalkes MP (1995) *Metal Toxicology*. San Diego, CA: Academic Press.
Mukherjee B, Patra B, Mahapatra S, *et al.* (2004) Vanadium – An element of atypical biological significance. *Toxicology Letters* 150: 135–143.
Rydzynski K (2001) Vanadium, niobium, and tantalum. In: Bingham E, Cohrssen B, and Powell CH (eds.) *Patty's Toxicology*, 5th edn., vol. 3, pp. 1–74. New York: Wiley.

Relevant Website

http://www.who.int – Vanadium Pentoxide and Other Inorganic Vanadium Compounds (Concise International Chemical Assessment Document 29 from the International Programme on Chemical Safety).

Vanillin

Lu Yu

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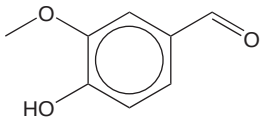
- Chemical Abstracts Service Registry Number: CAS 121-33-5
- Synonyms: *m*-Anisaldehyde; Vanillic aldehyde; Lioxin; 3-Methoxy-4-hydroxybenzaldehyde; *p*-Hydroxy-*m*-methoxybenzaldehyde; Lioxin; Vanillaldehyde; Vanillic aldehyde; 2-Methoxy-4-formylphenol; 3-Methoxy-4-hydroxybenzaldehyde; Vanilla; Protocatechualdehyde, methyl-; Zimco; *p*-Vanillin; Methylprotcatechuic aldehyde; Methylprotocatechuic aldehyde
- Chemical/Pharmaceutical/Other Class: Essential oil
- Chemical Formula: $C_8H_8O_3$
- Chemical Structure:



Uses

Vanillin is used in flavorings for food, perfumes, and pharmaceuticals (flavor, antidepressant drugs); it is a source of L-dopa; reagent in analytical chemistry; and formulating insect attractants.

Exposure Routes and Pathways

Occupational exposure to vanillin may occur through inhalation and dermal contact at workplaces where vanillin is produced or used. The general population may be exposed to ethyl vanillin via dermal contact with perfumes and ingestion of food products that contain vanillin as a flavor additive.

Toxicokinetics

In rats fed with vanillin at 100 mg kg^{-1}, most of the metabolites were excreted in the urine within 24 h. The majority of administrated vanillin will be excreted as vanillic acid. Glucurovanillin and some other forms of conjugates of vanillin were also excreted.

Mechanism of Toxicity

Vanillin in solution is an acid. It has an irritating action on eyes, gastrointestinal tract, and mucous membranes of the respiratory tract.

Pharmacologically, vanillin can accelerate bile secretion. Vanillin is capable of effectively minimizing methotrexate-induced chromosomal damage. Vanillin is an anticlastogenic agent; it has also been demonstrated to inhibit gene mutations in both bacterial and mammalian cells. Vanillin enhances or suppresses chemical-induced cytotoxicity, mutations, and chromosome aberrations.

Acute and Short-Term Toxicity (or Exposure)

Animal

Vanillin is a weak dermal sensitizer in guinea pigs and mice. Treatment with vanillin caused eye irritation in rabbits. Rat LD_{50} values are 1580 mg kg^{-1} (oral), 1500 mg kg^{-1} (subcutaneous), and 1160 mg kg^{-1} (intraperitoneal). The mouse LD_{50} is 475 mg kg^{-1} (intraperitoneal). Guinea pig LD_{50} values are 1400 mg kg^{-1} (oral) and 1190 mg kg^{-1} (intraperitoneal). Oral ingestion at high doses causes hyperpnea, muscular weakness, dyspnea, collapse, and circulatory failure in rats.

Human

Vanillin is a weak human sensitizer. It has induced skin sensitization in humans, and it was also reported to have highly irritating action on the eyes and mucous membranes of the respiratory tract. Ingestion of vanilla has provoked intolerance reaction; it is pharmacologically active and may cause depressed blood pressure, increased respiratory rate, and even death due to cardiovascular collapse. Probable oral lethal dose to human is 500 mg kg^{-1} for a 70 kg person.

Chronic Toxicity (or Exposure)

Animal

Repeated administration in rats may induce tissue effects at various sites. Growth depression, enlargement of liver, kidney, and spleen were reported in rats after administration of vanillin at 50 000 ppm for 91 days. Administration of vanillin at 64 mg kg^{-1} day^{-1} for 10 weeks caused growth depression, damage to myocardium, liver, kidney, lung, spleen. Vanillin has not been shown to cause cancer in animals. No excess of lung tumor was observed in mice given an intraperitoneal dose of 3.6–18 g kg^{-1} over 24 weeks.

In Vitro Toxicity Data

Vanillin was found to directly suppress the *in vitro* antisheep RBC antibody response at a noncytotoxic dose (200 µg per culture). Vanillin induced chromosomal damage in human cells treated in culture, but showed no genotoxic activity in mice treated orally or in hamster cells in culture. There was also no evidence of mutagenic activity in bacterial (including Ames test) or in yeast.

Clinical Management

The patient should be moved from the source of exposure. If there is respiratory distress, an airway should be established. Patients should be closely observed for esophageal or gastrointestinal tract irritation, or signs of respiratory insufficiency. Eyes should be gently flushed with water immediately after exposure. Activated charcoal should be administered if vanillin is ingested. Early removal of ingested vanillin by cautious gastric lavage should be considered if there is significant gastrointestinal tract irritation or if life-threatening amount of vanillin has been ingested. Skin burns should be covered with dry sterile dressing after decontamination. Further treatment is needed for patients who develop a dermal hypersensitivity reaction.

Environmental Fate

Vanillin's production and use as a flavoring agent in foods and in perfumery may result in its release to the environment through the waste stream. It is also a naturally occurring compound in vanilla beans and may be released to the environment through decay of plant material. If released into the air, vanillin will exist as a vapor and may be degraded by reaction with photochemically produced hydroxyl radicals with a half-life of 14 h. In the soil, vanillin is expected to be highly mobile; volatilization from soil surface is estimated to be less and it degrades rapidly. When vanillin is released into water, it exists in the ionized form at environmental pH, and is not expected to adsorb to suspended solids and sediments in water. Volatilization from the water surface is also expected to be low. Vanillin has a low potential to bioaccumulate in aquatic organisms.

Other Hazards

Some synthetic fragrant substances and intermediate products are flammable. Violent reactions occur when a small amount of vanillin was added to thallium trinitrate trihydrate (up to 50%) in 90% formic acid. When heated to decomposition it emits acrid smoke and irritating fumes.

Exposure Standards and Guidelines

There are exposure standards for vanillin. US Environmental Protection Agency (EPA) promulgated a model Health and Safety Data Reporting Rule, which requires manufacturers, importers, and processors of listed chemical substances and mixtures to submit to US EPA copies and lists of unpublished

health and safety studies. Vanillin is included in this list.

See also: Consumer Products; Limonene.

Further Reading

Clark GS (1994) Menthone. *Perfumes and Flavors* 19: 41–45.

Clayton GD and Clayton FE (eds.) (1993–1994) *Patty's Industrial Hygiene and Toxicology, vols. 2A, 2B, 2C, 2D, 2E, 2F: Toxicology*, 4th edn. New York: Wiley.

Relevant Website

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

Vein *See* Blood.

Venoms and Poisons from Animals *See* Animals, Poisonous and Venomous.

Vesicants *See* Blister Agents/Vesicants.

Veterinary Toxicology

Wilson K Rumbeiha and Frederick W Oehme

This entry focuses on understanding and managing chemically induced disorders in domestic animals. Approximately 10% of veterinary practice is devoted to the diagnosis and treatment of poisonings. The species treated range from small domestic animals (i.e., cats and dogs) to food-producing animals (i.e., dairy cattle, beef cattle, and swine), to horses, pet birds, zoo animals, and, occasionally, wild game (i.e., rabbits and fish).

Species Differences

Small animals react to chemicals more or less the same way as do humans because they are all monogastrics. Ruminants (i.e., cattle and sheep), however, react differently than the monogastrics; they have evolved a unique digestive tract structure and microbial flora, which play a major role in the fermentation of the forage ingesta. The ruminant's microflora are usually capable of metabolizing toxic chemicals. For example, cattle are more susceptible to nitrate poisoning than are horses, whereas dogs and cats are very resistant to nitrate poisoning. Cattle are very susceptible to nitrate poisoning because their digestive tract microbes will convert nitrates to the proximate toxic metabolite, nitrite.

Dogs, because of their relatively small gastrointestinal microbial population, are resistant to nitrate poisoning. The horse may succumb to nitrate poisoning because of the presence of microorganisms in the cecum in its posterior digestive tract. However, by the time nitrate reaches the cecum, more than 70% will have been absorbed; little will be available for biotransformation into the toxic nitrite ion. Horses, therefore, require threefold higher nitrate concentrations to be poisoned than do cattle.

Physiological differences among species will markedly alter the susceptibility to toxicants. Birds are more sensitive to toxic vapors and gases than mammals. Canaries have been used in mines to test for the presence of poisonous gases because their elaborate respiratory system causes them to succumb to lower concentrations of toxic gases than would endanger humans.

Biochemical differences also contribute to differential susceptibility between and within species. Cats are more susceptible to acetaminophen poisoning than other domestic animals. The cat's glucuronyl

transferase activity for conjugating acetaminophen is much lower than that of other domestic species, and feline hemoglobin is more susceptible to oxidation than that of other animals. Therefore, cats given what would be considered a therapeutic dose of acetaminophen in humans die of methemoglobinemia. Biochemical differences are also found within the same species. For example, the Bedlington terrier is much more susceptible to copper poisoning than other species of dogs. Most biochemical differences are of genetic origin.

Adequate comprehension of the variance in toxicity from chemicals in the domesticated species requires an understanding of the anatomy, physiology, and biochemistry of the affected animals. Other general factors that affect the toxicity of chemicals must also be considered when dealing with clinical toxicities in domestic animals. These factors include the animal's age, sex, health, nutritional status, environment, and concurrent exposure to other chemicals.

The effects of these and other factors in modifying the outcome of poisoning can be of vital significance in determining its outcome and also point to appropriate management options. There is a vast amount of literature in this area.

Common Toxicoses in Food-Producing Animals

Food-producing species are cattle, swine, and small ruminants. Swine differ from other animals in this category in that they have a simple stomach (monogastric), whereas the other animals have a compound stomach. Most of the toxicants affecting other animals also affect food-producing animals, but some toxicants are peculiar to or predominantly seen in food-producing animals. Toxicoses frequently encountered in ruminants include nonprotein nitrogen toxicoses; copper, lead, or arsenic poisoning; mycotoxicoses; nitrite poisoning; plant poisoning; and blue-green algae poisoning. In swine, salt poisoning, mycotoxicoses, organic arsenicals, plant poisoning, and gases generated in swine confinement operations are often is involved in toxic episodes.

Nonprotein Nitrogen Compounds

Nonprotein nitrogenous sources include urea, biuret, and ammoniated feeds. These compounds are cheap sources of the nitrogen required by the animals for protein synthesis. Nonprotein nitrogen poisoning is a common problem and is often seen in animals not gradually introduced to diets containing these compounds. It is an acute fatal condition characterized by bloating, intense abdominal pain, ammonia breath, frequent urination, and frenzy. Often several animals are affected.

In ruminant animals, the rumen microflora normally convert urea to ammonia, and the ammonia is rapidly utilized by the liver for protein synthesis. However, in cases of excess ammonia production, the blood ammonia concentration builds up to toxic levels very fast and induces central nervous system (CNS) derangement. Therefore, in addition to the gastrointestinal signs, the animals will show fulminating CNS signs. Treatment of the condition involves giving a weak acid such as vinegar and plenty of cold water orally. The rationale for giving cold water and acetic acid is to slow down the action of urease, the enzyme responsible for converting urea to ammonia, which requires body core temperature and pH for optimal function. The cold water lowers the temperature and the acetic acid lowers the pH. Infusions of calcium and magnesium solutions are administered to alleviate tetany.

Another source of nonprotein nitrogen (urea) poisoning in ruminants is the accidental ingestion of nitrogen-based fertilizers such as ammonium phosphate. Occasionally, cattle break into drums or bags of fertilizers containing these nitrogen-based compounds. The prognosis is grave in most cases if several animals are affected. If only a few valuable animals are affected, a rumenotomy can be performed. Although small ruminants (e.g., sheep and goats) have the same anatomical predisposition to suffer from nonprotein nitrogen poisoning, they are rarely involved, probably because they are not usually fed rations containing these compounds.

Nitrate–Nitrite

Excessive exposure of ruminant animals to nitrates causes nitrite toxicity, an acute rapidly fatal disease. The most common source of nitrates in ruminants is through consumption of forage that was grown on heavily fertilized fields and has accumulated high levels of nitrates. All common animal feeds, such as sorghum, alfalfa, and milo, can accumulate excessive amounts of nitrates. Another common source of dietary nitrates is contaminated drinking water. Nitrates are highly water-soluble and underground water can become contaminated from heavily fertilized fields. Runoff from fertilized fields is another source of contamination in surface pools and ponds. Nitrate from different sources is additive.

Nitrates are reduced to nitrites by rumen microflora. In normal circumstances the nitrite ion is rapidly utilized for ammonia synthesis, but in cases of excessive acute intake of nitrate, the rapidly formed nitrite ion is absorbed into the bloodstream. In blood

the nitrite ion reacts with hemoglobin to form methemoglobin. Methemoglobin is incapable of oxygen transport, and the animal compensates for the anoxia by increasing its respiratory rate. Therefore, affected animals will be hyperventilating, have brownish mucous membranes, and be weak. Chronic intake of nitrates has been reported to cause reproductive problems such as abortion, but experimental results regarding this claim are currently inconclusive. Besides reacting with methemoglobin, the nitrite ion also replaces iodine in the thyroid gland, thereby interfering with the function of the thyroid hormone.

Treatment of nitrate/nitrite poisoning involves intravenous infusion of 1% methylene blue at a rate of $1.5\,mg\,kg^{-1}$ body weight and withdrawal of the offending feed.

Copper–Molybdenum

Sheep are more susceptible to copper poisoning than are cattle, but cattle are more sensitive to molybdenum poisoning than are sheep. The *in vivo* relationship between copper and molybdenum is well understood. Excess copper induces molybdenum deficiency and vice versa. The most frequent cause of copper poisoning in sheep is by uninformed farmers feeding cattle feed to sheep. Copper from different sources is additive. Copper is an essential element for cattle and is usually added to their feeds; however, molybdenum is not considered essential and is therefore not added. Cattle feeds therefore have high copper concentrations and no molybdenum; feeding this ration to sheep upsets the normal 6:1 copper:molybdenum ratio *in vivo*.

Copper toxicity in sheep is an acute condition that develops after a chronic copper intake. During the chronic phase copper is stored in the liver until a critical concentration is reached. Stressful conditions, such as transportation or insufficient feed or water intake, will trigger a massive hepatic release of copper and cause a hemolytic crisis. Affected sheep have hemoglobinuria, are weak, and die acutely. The massive release of hemoglobin can block the renal tubules, inducing renal failure. The prognosis is poor for animals already showing clinical signs. Chelation therapy using D-penicillamine is recommended for the exposed animals not showing clinical signs.

In cattle, molybdenosis is characterized by a foamy diarrhea which may be bloody. Affected cattle also have depigmented hair. Molybdenosis is a subacute to chronic condition and occurs when the copper:molybdenum ratio is 2:1 or less. The condition shows geographical distribution and occurs in areas deficient in copper or having an excess of molybdenum (e.g., parts of California, Oregon, Nevada, and Florida). Treatment of this condition involves copper supplementation in the feed.

Lead

Despite awareness regarding the dangers of lead poisoning in humans and domestic species, it is surprising that lead poisoning is the most frequently encountered toxicity in food-producing animals. Lead poisoning is more commonly seen in cattle than in other food-producing species. Young animals are mostly affected because of their curiosity and because they are indiscriminate in their feeding habits. There are several sources of lead in cattle. Discarded junk automobile batteries, paint, and leaded water pipes are the most common sources. Quite often uninformed owners will discard or store old batteries in farm environments and cattle will chew on them. Discarded leaded pipes, especially those used around oil wells, are a common source of lead poisoning.

Lead interferes with heme synthesis and causes renal and CNS lesions in food-producing animals. Affected animals are initially anorectic. They may then become belligerent, blind, and have periodic seizures at the terminal stages of the poisoning. Once the CNS signs have set in, the prognosis is grave but treatment with chelating agents may be of value. Chelating agents include calcium disodium-EDTA (calcium disodium salt of ethylenediaminetetraacetic acid) and 2,3-dimercapto-1-propanesulfonic acid.

Arsenic

Arsenic poisoning is second to lead as the most frequently reported heavy metal toxicant in food-producing animals. Arsenic is present in the environment in two forms: inorganic and organic arsenicals. Inorganic arsenic is often incorporated into pesticides, which are the most common sources of arsenic poisoning in cattle. Inorganic arsenicals are also used as herbicides and cattle sometimes are exposed by eating grass clippings from recently sprayed forage.

Inorganic arsenic poisoning is a rapidly developing and fatal disease. Affected animals show severe gastrointestinal irritation without CNS involvement. They have severe abdominal pain and hemorrhagic diarrhea and are depressed. Usually, these signs appear 24–36 h after exposure to the inorganic arsenic.

Phenylarsonic arsenicals are less toxic to mammals than the inorganic arsenicals. Phenylarsonic compounds are usually incorporated in swine (and poultry) feed for disease control and to improve weight gain. Examples of these compounds include arsenilic acid, 3-nitroarsenilic acid, and 4-nitroarsenilic acid. Organic arsenicals are also available as trivalent and

pentavalent compounds, and the trivalent forms are more toxic than the pentavalent compounds.

These phenylarsonic compounds are peripheral nervous system toxicants. They cause demyelination of peripheral nerve fibers leading to ataxia and paralysis of hindquarters. The condition occurs frequently in swine kept on feed containing 1000 ppm arsenic for at least 3–10 days or 250 ppm arsenic for 20–40 days. Therefore, unlike inorganic arsenic poisoning, which is an acute form of the disease, poisoning by phenylarsonic compounds is an insidious condition. In addition, organic arsenic is commonly involved in swine toxicities because of its incorporation in swine feeds, whereas inorganic arsenic poisoning is more commonly seen in cattle.

Treatment of inorganic arsenic poisoning involves decontamination procedures and use of the antidote BAL (British anti-lewisite compound; 2,3-dimercaptopropanol). Use of demulcent to coat the gastrointestinal tract and the use of antibiotics is also recommended. Organic arsenic poisoning treatment involves only withdrawal of the feed involved, with recovery occurring in 3–5 days. Severely affected pigs should be culled.

Selenium

Selenium poisoning is a regional problem occurring in areas where the selenium content in soil is high. Selenium is then absorbed and concentrated by selenium-accumulating plants such as the *Astragalus* species. Cattle, sheep, goats, and swine are exposed by consuming these plants.

Acute selenium poisoning occurs when animals consume plants containing more than 10 000 ppm. This is characterized by sudden death or labored breathing, abnormal movement and posture, frequent urination, diarrhea, and death. Because plants containing high selenium concentrations are unpalatable, they are rarely consumed by animals. Therefore, acute selenium poisoning is rare. However, chronic selenium poisoning is relatively common. Chronic consumption of plants containing as low as 50 ppm can cause chronic selenium poisoning. Affected animals are anorexic, have impaired vision, wander, salivate excessively, are emaciated and lame, and lose hair.

Removal of animals from pastures whose forages contain high selenium is the recommended cure but may be unsuccessful if the condition has persisted for several days or more.

Mycotoxins

Some of the mycotoxins of veterinary interest are aflatoxins, deoxynivalenol (DON), diacetoxyscirpenol (DAS), T-2, zearalenone, ochratoxins, and fumonisin B_1. Mycotoxins are especially a common problem in warm climates where high temperatures and relative humidity support fungal growth and favor mycotoxin production. All food-producing animals are susceptible and clinical signs will depend on the mycotoxins is involved. Usually only one mycotoxin is involved because several species of fungi (e.g., *Fusarium, Penicillium,* and *Aspergillus*) coexist and often produce more than one type of mycotoxin.

The common sources of aflatoxins for food-producing animals include corn and oats. When aflatoxins are ingested in parts-per-million quantities, acute death can occur. The affected animals show severe gastrointestinal pain and hemorrhage. Aflatoxins are severe hepatotoxicants; therefore, hepatomegaly and jaundice may be observed in severe subacute cases. Quite often, however, aflatoxin poisoning is an insidious condition due to the chronic intake of parts-per-billion aflatoxin concentrations over a prolonged period of time. Clinical signs include poor weight gain, decreased milk production, and poor reproductive performance, including abortions. Virtually every organ function is affected by aflatoxins. The immune system of the affected animals is also impaired, and animals may more easily succumb to infectious diseases.

Toxicity due to T-2 has been reported in North America and other parts of the world, including Germany, Hungary, France, and South Africa. It is less common than aflatoxin toxicity. T-2 mycotoxins act by interfering with the blood clotting mechanism. Affected animals have gastrointestinal bleeding and will pass bloodstained feces. The animals will perform poorly (i.e., have low weight gain, decreased milk production, and decreased food intake). T-2 is also an immunosuppressant. All food-producing animals are susceptible to T-2 mycotoxicosis.

Zearalenone is an estrogenic mycotoxin that usually causes toxicity in swine that consume contaminated corn. Prepubertal swine are mostly affected. Affected females show swelling of the vulva and excessive straining, which may cause vaginal prolapses. In male animals, zearalenone will cause decreased libido. There is no effective treatment apart from withdrawing the feed containing the mycotoxin.

Other mycotoxins, including DAS, DON, and ochratoxin, are not of major economic importance although they can be toxic to food-producing animals. DAS causes necrosis and erosion of the oral mucous membranes. Consequently, affected animals exhibit feed refusal and have impaired growth. DON (also called 'vomitoxin') induces vomiting and feed

refusal in swine. Ochratoxins cause renal problems, including hydronephrosis, especially in swine.

Ergot poisoning is occasionally encountered in livestock fed grain screenings contaminated with *Claviceps purpurea*. The active constituents are ergotoxin and ergotamine, which are vasoactive compounds. These compounds cause vasoconstriction of the peripheral vessels, especially those of extremities, causing necrosis and gangrene of hooves, ears, and tails. Abortions and agalactia have been reported in cattle fed ergot-contaminated feed. Therapy consists of discontinuation of the source of the toxicant and antibiotic therapy to prevent secondary bacterial infections in the necrotic tissues.

Fumonisin B_1 is produced by *Fusarium moniliforme*, a fungus that predominantly grows worldwide on corn. Fumonisin B_1 causes pulmonary edema and respiratory distress in swine. Numerous deaths have been reported in swine fed fumonisin-contaminated corn screenings.

The most practical treatment for mycotoxicoses consists of withdrawal of the contaminated feed from the herd and supportive care for the affected animals.

Blue-Green Algae

Blue-green algae poisoning occurs in late summer and early fall when the algae forms a scum on top of ponds or other stagnant waters. Because of husbandry practices, cattle are most frequently involved. Blue-green algae poisoning has been reported in North America, South Africa, and Britain. Algae of genus *Anabaena* are most frequently involved.

There are two distinct syndromes in blue-green algae poisoning: the neurotoxic effects and the hepatotoxic syndrome. The neurotoxic disorder is peracute, and cattle drinking water containing the neurotoxic principle Anatoxin A can die within a few minutes and usually are found quite close to the pond or water (algae) source. On the other hand, the hepatotoxic principle causes an acute type of poisoning characterized by lethargy and jaundice. Death may occur 2 or 3 days after drinking contaminated water.

Because of the peracute nature of the blue-green algae-induced neurological syndrome, there is hardly time for treatment and the prognosis is universally grave. Treatment of animals affected with the liver syndrome of blue-green algae poisoning involves appropriate supportive therapy.

Toxic Gases

Toxic gases are of primary concern in closed animal housing, especially in swine operations. Because of the intensive swine confinement operations with buildings designed to save on energy, toxic gases can accumulate in swine houses and result in serious health consequences to animals and caretakers in cases of ventilation failure. These toxic gases are generated from the decomposition of urine and feces, respiratory excretion, and the operation of fuel-burning heaters.

The most important gases are ammonia, hydrogen sulfide, carbon monoxide, and methane. A number of vapors, which represent the odors of manure decomposition, such as organic acids, amines, amides, alcohols, carbonyls, and sulfides, are also produced. Respirable particles, which may be loaded with endotoxins, are also a major health problem in swine confinement operations.

Ammonia is highly soluble in water and will react with the mucous membranes of the eyes and respiratory passages. At 100 ppm or greater ammonia concentrations, toxicosis will produce excessive tearing, shallow breathing, and clear or purulent nasal discharge. The irritation of the respiratory tract epithelium leads to bronchoconstriction and shallow breathing.

Hydrogen sulfide poisoning is responsible for more animal deaths than any other gas. At concentrations of 250 ppm and above, hydrogen sulfide causes irritation of the eyes and respiratory tract and pulmonary edema. Hydrogen sulfide concentrations above 500 ppm cause strong nervous system stimulation and acute death. In order to prevent hydrogen sulfide poisoning, manure pits should not be agitated when pigs are on the premises, and proper ventilation should be in place.

Carbon monoxide is produced by incomplete combustion of hydrocarbon fuels. Poisoning by carbon monoxide is caused by operating improperly vented space heaters or furnaces in poorly ventilated buildings. Carbon monoxide binds to hemoglobin forming carboxyhemoglobin, thereby reducing hemoglobin's oxygen carrying capacity and subsequently causing hypoxia. Concentrations of carbon monoxide $\geqslant$250 ppm cause hyperventilation, respiratory distress, and stillbirths.

Methane is a flammable and colorless gas produced from organic wastes through bacterial action. It serves to displace oxygen in respirable air, thus producing oxygen starvation if present in high concentrations.

Nitrogen dioxide is a very poisonous gas that is responsible for causing silo fillers disease in humans. The gas is also toxic to animals. Nitrogen dioxide is produced during the first few weeks after silage has been cut and put into the silo. The highest nitrogen dioxide concentrations are reached during the first 48 h after filling the silo. Nitrogen dioxide dissolves in water to form nitric acid, which is very corrosive

to the respiratory tract epithelium and the lungs. Nitrogen dioxide concentrations as low as 4 or 5 ppm can cause respiratory system disturbances.

Exposure to sulfur dioxide concentrations of 5 ppm or greater causes irritation and salivation in swine. The gas is soluble in water, forming the more toxic sulfuric acid. It is the sulfuric acid that causes eye and nasal irritation, and in severe cases it produces hemorrhage and emphysema of the lungs.

The effect of these toxicants singly or in combination is to produce a hypofunctional respiratory system. Affected animals are also predisposed to respiratory tract infections. The end result is significantly retarded performance and productivity decreases in the affected animals. It is therefore important to ensure that proper animal housing is provided with adequate ventilation in all seasons of the year to provide animals with a healthy breathing and a highly productive environment.

Toxic Plants

Plant poisoning is very common in areas where open grazing is practiced, such as in the Great Plains of the United States, where plant poisoning is widely reported during spring and fall grazing seasons. The wide range of toxic plants and their variations in growth environments produce risks that can affect almost all body systems, depending on the plant consumed, its level of maturity, and the soil and environmental characteristics in which it is growing.

Body systems and organs most prominently affected by plants include the digestive tract, the liver, kidneys, and nervous system, the heart and blood, the skin, and the reproductive tract and its functioning. It is important to realize, however, that toxic plants rarely affect only one body system or organ and thus may generate a complex pattern of effects in any one poisoned animal. Toxicity of a given plant can vary widely depending on the prevailing natural conditions. It is therefore not surprising that a given toxic plant may be toxic under certain conditions (e.g., during stressful drought conditions) but safe during other times.

Sodium Chloride (Salt)

Salt poisoning/water deprivation is frequently encountered in swine operations but can also occur in feedlot cattle. The causes of this condition are twofold. Most commonly, the pigs will be on a ration containing a recommended concentration of sodium chloride, but management failures or changes can favor conditions that cause salt poisoning to occur. These poor management conditions include the sudden absence of water, which can be caused by frozen water in winter or the breakdown of water supplies. The other possibility is the accidental addition of excessive amounts of salt or sodium-containing materials to the ration.

Salt poisoning has also been reported in swine operations even when the management situation is appropriate; the only change was that the animals had been moved into a new housing facility, as occurs with weaning. In those situations, the animals are not used to the watering facilities in the new buildings and they do not know how to obtain the water; thus, they go without water while continuing to feed on the normal salt-containing ration.

Clinically, salt poisoning is a neurological disorder, and the syndrome is rather acute. Affected pigs will spin on their hindquarters and fall down convulsing. The pigs will also show a characteristic rhythmic pattern of seizures which occurs cyclically every 3–5 min. Many pigs are usually affected at the same time. The condition is corrected by the provision of adequate but restricted amounts of water made available gradually.

Common Toxicoses of Poultry

Even chickens, ducks, and turkeys are affected by poisonings. There is also much concern and interest in the toxicities seen in wild birds, especially those kept in zoos, as well those kept as pet birds in households. This discussion will emphasize the toxicoses encountered in poultry.

Drugs and Medications

Sulfonamides have been used as coccidiostats in poultry for several decades. Although sulfonamides possess inhibitory action against coccidiosis and other pathogenic agents, they can be toxic and have particularly been shown to be so to poultry. In poultry, sulfonamide toxicity is characterized by blood dyscrasia and renal and liver dysfunctions. Feeding chickens a mash containing as low as 0.2% sulfonamides for 2 weeks is toxic.

Clinically affected birds have ruffled feathers; are depressed, pale, and icteric; and have poor weight gain and a prolonged bleeding time. In laying birds, sulfonamides cause a marked decrease in egg production, thin rough shells, and depigmentation of brown eggs. The temperature of affected birds is often elevated. At postmortem, hemorrhages are found in the skin, muscles (especially those of thighs and breast), and in the internal organs. Once these effects are noticed, the concentration of sulfonamides in the ration should be evaluated and the feed involved withdrawn.

Other chemotherapeutic agents sometimes involved in poisoning poultry are the other coccidiostats, such as nicarbazine, zaolene, and nitrophenide, and the ionophore monensin. As little as 0.006% nicarbazine in the diet causes mottled yolks, and at 0.02% there is depressed rate of growth and reduced feed efficiency. Feeding 0.025% nicarbazine to day-old chicks for 1 week resulted in the chicks becoming dull, listless, weak, and ataxic.

Feeding zaolene at twice the recommended level of 0.025% will cause nervous signs and depress growth and feed efficiency. The nervous signs include stiff neck, staggering, and falling over when the birds are excited.

Nitrophenide possesses marked electrostatic properties and, therefore, sticks to the walls of a feed mixer. The last bits of feed in the feed mixer will normally contain a high concentration of nitrophenide and that elevated concentration has caused disturbances in posture and locomotion, retarded growth, and increased mortality in chickens. Postural disturbances include a tilted position of the head, tremor of the neck, and difficulty in maintaining the righting reflex.

In general poultry are more resistant to monensin toxicity than other species, but there have been reports of monensin toxicity in turkeys accidentally fed rations containing 250 ppm monensin. There is a big difference in susceptibility to monensin poisoning among various species of poultry. Chickens and turkeys less than 2 weeks old are more resistant than older birds, but keets (young guinea fowl) seem more susceptible than their adults and the young of other species. For example, diets of 200 ppm monensin were not toxic for poults, whereas 100 ppm was toxic for keets.

Cresol

Cresol was a commonly used disinfectant in poultry houses but has been gradually withdrawn and replaced by less toxic disinfectants. Nevertheless, in some regions and countries cresol is still being used. Cresol poisoning in the chicken usually occurs at 3–6 weeks of age. Affected chicks are depressed and have a tendency to huddle. There are respiratory problems such as rales, gasping, and wheezing. With prolonged cresol exposure some chicks will develop edema of the abdomen.

Sodium Chloride (Salt)

All poultry and pigeons are susceptible to salt poisoning. Young birds are more susceptible than adults. Although both acute and chronic forms of salt poisoning can occur, the chronic form is more commonly encountered and is due to prolonged ingestion of feed containing high salt content. Levels of 0.5% and above in drinking water or 5–10% in feed cause death in baby chicks.

Signs of salt poisoning in poultry include anorexia, thirst, dyspnea, opisthotonos, convulsions, and ataxia. Increased water consumption may be the most significant early indicator of hazardous exposure to salt in poultry.

Insecticides

Chlorinated hydrocarbon insecticides and organophosphate compounds are used regularly around poultry houses to control external parasites. Commonly used organochlorine insecticides include chlordane, dieldrin, DDT, heptachlor, and lindane. Occasionally, birds get exposed by gaining access to sprayed grounds such as golf courses.

Chlordane causes chicks to chirp nervously, rest on their hocks, and lie on their sides. The birds then become hyperexcitable as the condition progresses. In adult birds there is reduced food consumption, decreased body weight, and a fall in egg production.

Consumption of seeds dressed with dieldrin has been a source of exposure in wild birds. Affected birds are listless and have coordination problems while lighting; severely poisoned cases have nervous signs characterized by lateral movements of the head and tremors of the head and neck. Birds die in violent convulsions.

DDT toxicity in chickens is characterized by hyperexcitability and fine tremors in severe cases. Moderate cases are characterized by loss of weight, molting, and reduced egg production.

Lindane in the form of a dust is frequently used around chickens. Adult chickens poisoned by lindane stop eating, manifest opisthotonos and flapping of wings, have clonic muscle spasms, and die in a coma.

The organophosphate compounds commonly involved include diazinon, malathion, and parathion. Diazinon is applied to chicken premises, but this compound is very toxic to ducklings. When used at rates recommended for chickens, 100% mortality has resulted from use of this compound on 1- or 2-week-old ducklings. Experimental studies suggest that goslings are three times more sensitive than ducks, chickens, and turkeys. Poisoned birds are unable to stand, salivate profusely, and manifest tremors of the head and neck. Brain cholinesterase levels in birds that die of organophosphate poisoning are on the average 69% less than those of controls.

Other organophosphate compounds commonly used on chicken premises include dichlorvos, malathion, and parathion. Birds poisoned by these

compounds manifest signs similar to those seen in diazinon poisoning. Other effects that may be seen include depression, ataxia, and reluctance to move; paralysis and lacrimation; gasping for breath; and development of diarrhea, crop stasis, and dyspnea.

In general ducks are more sensitive to organophosphate poisoning than are chickens, and care should be exercised when using these products on premises holding ducks.

The carbamate insecticide sevin is a widely used poultry insecticide. This compound is relatively safe, but deaths have been reported in turkey poults kept on premises where the product has been excessively applied at 10 times the recommended rate. The clinical signs are similar to those caused by organophosphate insecticides.

Heavy Metals

Lead poisoning is not as common in domestic poultry as in wild birds, but it is the most common toxicity reported in the avian species. Lead shot has caused losses in waterfowl populations throughout North America. All birds are susceptible to lead poisoning, but most losses are reported in waterfowl because their feeding habits predispose them to the ingestion of lead pellets from shotguns and other sources.

Characteristic signs of lead poisoning are related to CNS derangement, such as ataxia, depression, paralysis of the wings, and convulsions. In some cases the birds presented are anemic, emaciated, regurgitating, and weak. Green diarrhea has often been reported in affected birds.

Yellow phosphorus is a highly toxic element that is still used as a rodenticide. Poultry and wild birds can be intoxicated by consumption of bait intended for rodents. Firework fragments also are a common source of poisoning in free-ranging birds. Affected birds are depressed and anorectic, have increased water consumption, and manifest diarrhea, ataxia, paralysis, coma, and death.

Rodenticides

In addition to the metal yellow phosphorus being used as a rodenticide, other rodenticides are potentially toxic to poultry and other birds. The clinical signs caused by these rodenticides in birds are similar to those observed in other animals.

Birds occasionally consume baits containing anti coagulant rodenticides. The more potent second-generation rodenticide-containing baits, such as brodifacoum, are especially dangerous to birds. These coumarin anticoagulants act by interfering with vitamin K utilization, causing bleeding because of depletion of vitamin K-dependent clotting factors. Poisoned birds bleed from their nares and subcutaneously and have oral petechiations. Quite often the birds are also weak and depressed from the resulting anemia or may be found dead due to stress superimposed on the anemic condition.

Of special interest are secondary intoxications due to free-ranging birds consuming carrions of animals that died of rodenticide poisoning. Strychnine and sodium monofluoroacetate are other rodent control compounds that are involved because they cause acute death in the primary victims and are thus present in high concentrations in carrions.

Strychnine-poisoned birds show clinical effects within 2 h of ingesting the product. The birds become apprehensive and nervous and have violent tetanic convulsions, which cause them to become exhausted and to die of hypoxia. Sodium monofluoroacetate causes overstimulation of the CNS and myocardial depression. Cardiac failure is the cause of death and occurs within 1 h of consuming the product or contaminated carcass.

Mycotoxins

Mycotoxicoses are common problems for the poultry industry in warm moist climates and in developing countries in the tropics. Aflatoxins are the most commonly involved mycotoxins. Poultry are normally exposed by consumption of contaminated feed, especially corn. Some developing countries lack the resources to adequately screen contaminated corn. In other instances poultry feed is made from the poor-quality (and contaminated) corn that has been rejected for human consumption.

Aflatoxicosis in poultry can be either acute or chronic in nature, depending on the exposure dose. Ducklings are more susceptible to aflatoxin than are turkeys, pheasant, or chickens. In acute cases, affected birds become lethargic, their wings droop, and they manifest nervous signs such as opisthotonos; they die with their legs rigidly extended backward. Chronic dietary consumption of 2.5 ppm aflatoxin causes a significant drop in weight gain and egg production.

Perhaps more important is the increased susceptibility of the affected flock to infection because chronic consumption of aflatoxin-containing feed lowers the immunity of the birds. Aflatoxicosis is therefore a disease of serious economic consequences to the poultry industry in developing countries both through lowered productivity and because of death of affected birds.

Ergot poisoning has been reported in areas where rye is commonly used as poultry feed. In acute

ergot poisoning the birds' combs are cold, wilted, and cyanotic. The animals are weak, thirsty, and have diarrhea. In severe cases the birds go into convulsions, become paralyzed, and die. Ochratoxins have been reported to cause renal toxicity in poultry.

Common Toxicoses in Dogs and Cats

Dogs and cats are commonly poisoned by pesticides, herbicides, household products such as antifreeze, and drugs such as acetaminophen applied by humans to their pets. By far the most common toxicities in these small animals involve various insecticides and the overzealous use of these products by owners attempting to control fleas and ticks on their pets.

Insecticides

The insecticides most commonly involved in poisoning dogs and cats are the organophosphates and carbamates, pyrethroids, chlorinated hydrocarbons, and diethyltoluamide (DEET). The organophosphate and carbamate insecticides have a common mode of action, which is the inhibition of acetylcholinesterase. Acetylcholinesterase is the enzyme that breaks down acetylcholine, a neurotransmitter in autonomic ganglia and at cholinergic nerve endings. The inhibition of acetylcholinesterase by organophosphate and carbamate compounds causes acetylcholine to accumulate at nerve synapses and to produce persistent firing of cholinergic nerve fibers. Affected animals are overexcited and have increased respiratory rates, excessive salivation, and muscle tremors.

Treatment of animals poisoned by organophosphate compounds involves the administration of atropine and prolidoxime. Cases involving carbamates may be treated with only atropine because of the rapid biological detoxification of carbamates. The organophosphate and carbamate compounds have a relatively high acute toxicity compared to chlorinated hydrocarbons but have a lower residual activity. As such, organophosphate compounds have largely replaced the chlorinated hydrocarbons for insecticide use because of environmental concerns.

The chlorinated hydrocarbons were among the first synthetic insecticide compounds to be used but have fallen into disfavor because of their persistence in the environment. Typical examples of chlorinated hydrocarbon insecticides are DDT, lindane, and toxaphene. The toxicity of these compounds in small animals is characterized by severe CNS effects, including ataxia and convulsions. Small animals usually get poisoned by being accidentally sprayed or by drinking chlorinated hydrocarbon insecticide concentrates intended for spraying on crops. Although most of these insecticides are banned or their use highly restricted in Western countries, they are still widely applied in developing countries. Thus, chlorinated hydrocarbon insecticide poisonings still occur in the developing countries.

Another group of insecticides commonly involved in small animal poisonings are plant product derivatives – pyrethrins and their synthetic congeners, the pyrethroids. These products are currently enjoying a resurgence because of their selective insecticidal properties and absence of environmental persistence. These compounds are mainly metabolized in the body by liver glucuronidation. The cat is the most sensitive domesticated animal to pyrethrin toxicity because of the low activity of the glucuronide conjugating system in this species. Young cats, less than 6 weeks of age, are the most sensitive.

Pyrethroid compounds formulated with the insect repellant DEET were responsible for numerous deaths in cats and dogs in the past decade. Pyrethroids interfere with sodium channels in nerves causing them to fire repetitively. Clinical signs of pyrethroid poisoning in small animals include ataxia, excitement, and muscle fasciculations and tremors. There is no antidote for pyrethrin poisoning, but symptomatic treatment, such as decontamination procedures and sedation, usually results in full recovery.

Rodenticides

Rodenticide poisoning is commonly seen in all small animals. Rodenticides are widely used around farmhouses to control rodents, such as rats and mice, which destroy property and farm produce. Several classes of rodenticides are currently in use, including the anticoagulant rodenticides (warfarin and its second-generation cousin brodifacoum), zinc phosphide, strychnine, compound 1080, and arsenicals. Small animals get poisoned by either consuming baits directly or through consumption of the carrion of animals that have died of rodenticide poisoning. The clinical signs seen will vary with the compound involved and, in the majority of cases, dogs (because of their indiscriminate eating habits) are involved.

Strychnine and anticoagulant rodenticides are the most frequently reported offenders. Strychnine poisoning in dogs is a rapidly developing syndrome characterized by tonic–clonic seizures. These signs result from strychnine competitively blocking the inhibitory neurons in the nervous system. The animals start showing clinical effects within 20 min to 1 h of ingesting strychnine and, if the animal has ingested a

sufficient amount, death from anoxia occurs acutely. Anoxia results from paralysis of the respiratory muscles. Treatment of strychnine poisoning is symptomatic and involves general decontamination procedures, use of sedatives such as phenobarbital and diazepam, maintenance of adequate urine output, and respiratory support. The sedatives control the seizures and allow the vital muscles to relax and maintain their lifesaving functioning.

The anticoagulant rodenticides have been in use for many decades. Because of the long time required for them to take effect, some strains of rats have become genetically resistant to the so-called first-generation anticoagulant rodenticides such as warfarin. This has led to the introduction of second-generation rodenticides such as brodifacoum. Unlike the first-generation rodenticides, which took at least 24–48 h to take effect, the second-generation rodenticides act relatively acutely, and clinical signs can be evident within a few hours and have a long residual action.

These anticoagulant rodenticides act by inhibiting vitamin K-dependent blood coagulation factors (VII, IX, and X), by decreasing prothrombin synthesis, and by directly damaging blood capillaries. Animals poisoned by the anticoagulant rodenticides are weak, have swollen joints because of bleeding into the joint cavities, may hemorrhage from the nostrils, and may pass bloodstained feces. Treatment of anticoagulant rodenticide poisoning involves whole blood transfusions if the bleeding and resulting anemia is severe and vitamin K_1 injections for several days. Early intervention requires general decontamination procedures to limit further rodenticide absorption, especially in the case of exposure to second-generation rodenticides, followed by prolonged vitamin K_1 therapy.

The toxicity of zinc phosphide results from the phosphine gas, which is produced by acid hydrolysis of the pesticide in the stomach. Animals with partially filled stomachs are more sensitive to zinc phosphide poisoning than those with empty stomachs because of the greater acid secretion precipitated by the presence of food. The generated phosphine gas is absorbed systemically and exerts its effects in the lungs. Poisoned animals exhibit respiratory difficulties because of the buildup of fluids in the lungs. The cause of death is respiratory failure. Supportive therapy, including respiratory support, is recommended in cases of zinc phosphide poisoning, but the prognosis is poor because no effective antidote is available.

Compound 1080 (sodium monofluoroacetate) is a very lethal toxicant which acts by blocking the Embden–Meyerhoff pathway, thereby depriving vital cells of energy. Fluoroacetate is metabolized to fluorocitrate, which inhibits mitochondrial aconitase. This blocks adenosine triphosphate production. Affected animals are initially uneasy, then they become excitable and will run in various directions in a frenzy, and finally they will fall into seizures and die of anoxia. There is no antidote and, once clinical signs develop, poisoned animals will almost always die within a few hours.

Cholecalciferol is a rodenticide that has been introduced relatively recently and that has been reported to be frequently involved in poisonings of dogs. The compound alters calcium homeostasis by promoting calcium absorption from the gut and also by mobilizing calcium from bone for tissue deposition. Consequently, poisoned animals have increased levels of blood calcium. The calcium is then subsequently deposited in soft tissues like the kidneys, digestive tract mucosa, lungs, heart, liver, and muscle. Mineralization of soft tissues interferes with normal function of these organs.

Clinically, the animals do not show signs until 24–48 or more hours after ingestion of the bait. The affected animals are depressed, have reduced urine production, and the urine is of low specific gravity. Severely poisoned animals have hematemesis, azotemia, and cardiac arrhythmias. Animals with renal impairment are more susceptible to cholecalciferol poisoning than those with normal renal function. Cholecalciferol poisoning requires protracted treatment, which may require as long as 3 weeks in severe intoxications. Appropriate treatment consists of fluid therapy to assist the kidneys in removing the excess calcium, corticosteroids to minimize inflammation, and calcitonin to enhance calcium resorption into the bone. Pamidronate disodium is the new antidote for this poison.

Several other rodenticides can cause poisoning in small animals but do so less frequently because these rodenticides are used less often. Red squill and thallium have been used as rodenticides for many years. Red squill acts as a cardiotoxicant and causes death by cardiac arrest. It also produces convulsions and paralysis. Thallium is a general systemic toxicant. It has a high affinity for sulfhydryl groups throughout the body. Thallium causes cracking at the corners of lips and also causes hair loss. α-Naphthylthio-urea causes death by inducing lung edema and subsequently leading to anoxia. White phosphorus is a hepatorenal toxicant. Animals poisoned by white phosphorus have severe abdominal pain, hepatomegaly, and signs of hepatic insufficiency, such as prolonged bleeding and hypoglycemia.

Cases of rodenticide poisoning in small animals should always be regarded as emergencies. General decontamination procedures, such as inducing

vomiting with hydrogen peroxide or apomorphine, the use of activated charcoal to bind the unabsorbed toxicant(s), and/or enterogastric lavage, should almost always be employed to minimize absorption and the resulting hazard from the toxicant(s).

Herbicides

Herbicides are not often involved in small animal toxicity despite their frequent use around farms and the continual possibility of exposure. However, toxicity in dogs from consumption of herbicide concentrates during mixing is occasionally reported. The triazine herbicides act by inhibiting plant photosynthesis and are generally safe products for mammals. The LD_{50} value of these compounds is at least $1900\,mg\,kg^{-1}$ body weight in the rat. Therefore, toxicity in dogs can only practically occur following the ingestion of large volumes of concentrates. In experimental situations, triazine herbicide-poisoned dogs can become either excited or depressed, develop motor incoordination, and may proceed to have clonic–tonic spasms.

Some inorganic arsenic compounds are also used as herbicides. These inorganic arsenicals are general protoplasmic poisons and are therefore hazardous to both plant and animal life. Affected dogs almost always vomit, have severe abdominal pain, and develop bloody diarrhea. The vomitus may contain mucous shreds and blood from erosion of the gastric and intestinal epithelium.

Paraquat, although restricted from use in Western countries, is a highly toxic herbicide that is still readily available in developing tropical countries. Upon intake, paraquat is rapidly metabolized in the liver and the lungs with the production of secondary oxygen radicals. It is these radicals that cause injury to tissues and especially do so to the lungs. Poisoned animals die acutely of respiratory failure.

Unlike other animals, the dog appears sensitive to chlorphenoxy herbicides, such as 2,4-D; the dog's oral LD_{50} to 2,4-D is $100\,mg\,kg^{-1}$ body weight. Ventricular fibrillation is the cause of death in severely poisoned dogs. Ingestion of sublethal doses induces stiff extremities, ataxia, myotonia, paralysis, coma, and subnormal temperatures.

Chlorates are herbicides that are often used along roadsides. They are rapidly metabolized in the liver to the chlorate ion, which induces methemoglobinemia in both cats and dogs. Cats, however, because of the greater susceptibility of their hemoglobin molecule to oxidation, are more susceptible to chlorate poisoning than dogs.

Organophosphate herbicides (e.g., glyphosate and merphos) are weak cholinesterase inhibitors and are moderately toxic to dogs and cats. Carbamate herbicides are not inhibitors of acetylcholinesterase but are also moderately toxic to dogs. The LD_{50} of most of the carbamate herbicides is at least $5000\,g\,kg^{-1}$ body weight.

Household Chemicals

Antifreeze is the household product most commonly involved in small animal poisonings. The active ingredient in antifreeze is ethylene glycol. The characteristic sweet taste of this compound makes it attractive to small animals. Ethylene glycol is metabolized in the liver by alcohol dehydrogenase to glycolic acid and then to oxalate. Glycolic acid contributes to acidosis, which is characteristic of ethylene glycol poisoning. The oxalic acid binds calcium from blood to form calcium oxalate, which is filtered by the glomerulus into renal tubules where it precipitates into crystals which cause blockage of the tubules. Consequently, severely affected animals have renal failure characterized by anuria and uremia. The binding of blood calcium to the oxalic acid produces hypocalcemia, which, if severe, can also cause death.

Ethylene glycol poisoning is treated by giving ethanol if the animal is presented within 4 h of suspected ingestion and by giving quantities of fluids containing sodium bicarbonate to facilitate flushing the calcium oxalate crystals from the kidneys and also to correct the acid–base imbalance. Alcohol dehydrogenase, the enzyme which reduces ethanol to acetic acid and water, prefers ethanol to ethylene glycol and, in the presence of both substrates will metabolize ethanol, leaves ethylene glycol to be excreted unchanged in urine. 4-Methylpyrazole is newly reported to have effective antidotal properties against ethylene glycol poisoning.

Household products, such as sink cleaners, dishwashing detergents, and toilet cleaners, are also common causes of poisonings in small animals. The majority of cleaning detergents are corrosive compounds, which contain strong alkali, acids, or phenolic compounds. These compounds act as contact poisons, causing coagulative necrosis of the tissues they come in contact with. Following ingestion of these products, the dog or cat will vomit, have severe abdominal pain, and may develop diarrhea. The animal's vomitus and feces may be bloody. Consuming animals may show other signs depending on the specific ingredients of the ingested products. For example, products containing phenolic derivatives will cause acidosis and hepatotoxicity.

In general, treatment following ingestion of household products is symptomatic and involves the administration of adsorbents such as activated

charcoal, gastrointestinal protectants such as Peptobismol, and the correction of any systemic disturbances (such as acidosis) which may accompany the poisoning. Animals should also be provided abundant glucose and a high protein diet.

Garbage

Garbage poisoning is a frequently encountered problem in small animals. This condition is also referred to as enterotoxicosis or endotoxemia, depending on whether poisoning is due to bacterial infection or due to bacterial endotoxins. Dogs and cats not well fed and/or not closely supervised when allowed to roam will eat garbage. Cats may also be affected, but only rarely so, because they are discriminate eaters. The bacteria most commonly involved are coliforms, staphylococci, salmonellae, and occasionally *Clostridium botulinum*. Enterotoxemia-affected animals often develop a bacteremia after eating infected carrion. Clinical signs normally appear at least 24–48 h after ingestion of the infected material.

The condition is characterized by anorexia, vomiting, severe abdominal pain, fever, and a bloody diarrhea. Endotoxemia poisoning is due to bacterial endotoxins, which are normally present in bacterial cell walls. The clinical signs are generally indistinguishable from those of enterotoxemia, except that in the latter there is no bacteremia; this can be evaluated by performing a blood culture. Although rare in occurrence, botulism is a rapidly developing fatal disease resulting from ingesting bones contaminated with *C. botulinum*. In small animals the disease is characterized by an ascending paralysis. At first there is muscle weakness and incoordination in the hindlimbs. As the paralysis progresses anteriorly, dyspnea and convulsions develop.

Garbage poisoning is rarely a severe condition in small animals because the animals usually vomit and thereby reduce the amount of toxicant ingested. However, in severe cases veterinary attention will be required. If the cat or dog is presented early after ingestion, general decontamination procedures should be performed to reduce absorption. Antiinflammatory corticosteroids and antibiotics should be given. Further treatment involves appropriate supportive therapy.

Heavy Metals

Lead and arsenic are the heavy metals most frequently seen in small animal poisonings. Lead poisoning is more commonly reported in the dog than in the cat, but both species are susceptible. The sources of canine lead poisoning include ingested leaded objects, such as lead weights, and paint chips from old houses being renovated. The clinical signs of lead poisoning in the dog involve primarily the CNS. Dogs may also be presented having abdominal pain and diarrhea in addition to the CNS involvement. Lead poisoning is a chronic disease in dogs, but the overt CNS signs may appear suddenly. Lead poisoning also causes blood dyscrasias characterized by reticulocytosis and anemia. Similar clinical signs are elicited in the cat. Treatment consists of giving chelating agents, such as calcium-EDTA, BAL, dimercaptosuccinic acid, or D-penicillamine.

Arsenic is the active ingredient in some insecticides, in rodenticides, and in some industrially used herbicides. Inorganic and aliphatic organic arsenic compounds are rapidly absorbed from the gut, skin, and lungs and are more toxic than the cyclic organic arsenicals used as feed additives. Trivalent arsenic is the proximate toxicant of the pesticide arsenicals which reacts with sulfhydryl groups of proteins throughout the body. It is, therefore, a general poison, inhibiting the sulfhydryl-containing enzymes it comes in contact with. The clinical signs of inorganic arsenic poisoning in dogs include anorexia, severe abdominal pain, bloody diarrhea, and hair loss, as discussed previously for herbicides. Treatment includes thorough decontamination, chelation therapy with BAL, and aggressive supportive therapy.

Toxic Plants and Mushrooms

Although one would not expect dogs and cats to commonly eat plants, plant poisoning is surprisingly often reported in these species. Because of their exploratory behavior, puppies and kittens are most often involved. Boredom and unfamiliarity due to change of environment are some of the predisposing factors that lead to plant ingestions by dogs and cats. Poisonous ornamental plants (e.g., philodendron and rhododendron) and plants growing around fences (e.g., cassia and oak) are often involved. The range of potentially poisonous plants is vast, and the clinical signs are diffuse and similar to those reported for food-producing animals.

Occasionally, dogs or cats will eat poisonous mushrooms or be fed poisonous mushrooms by uninformed owners. *Amanita muscaria* and *A. pantherima* are acutely toxic and induce signs within 15–30 min of ingestion. These two mushroom species cause nervous signs which include salivation, pupillary constriction, muscular spasms, drowsiness or excitement, and, in severe intoxications, coma and death. Ibotenic acid and muscimol are the active chemical components. However, consumption of *A. phaloides*, *A. virosa*, and *A. verna* produces gastrointestinal signs which become evident 6–12 h postingestion. These effects include violent vomition, muscle cramps, diarrhea, and

dehydration. These latter mushrooms also cause hepatic damage that becomes apparent 3–5 days after ingestion. Phalloidin and α- and β-amanitin are the poisonous principles in this group of fungi.

Common Toxicoses in Horses

In comparison to food-producing animals and cats and dogs, horses are less frequently poisoned. The most commonly encountered equine toxicoses are caused by pesticides, snakebites, arsenic, selenium, monensin, cantharidin, and mycotoxins. Most plants that are hazardous to food-producing animals are also toxic to horses, but horses are less frequently affected since owners usually assure the availability of good feed. Horses are very sensitive to monensin and cantharidin poisonings.

Insecticides

The pesticides most frequently responsible for equine poisonings are the organophosphate, carbamate, and chlorinated hydrocarbon insecticides. Both the organophosphates and the carbamates are acetylcholinesterase inhibitors and present clinical pictures similar to those seen in food-producing animals. Affected horses salivate and sweat profusely and have muscle incoordination and ataxia. The chlorinated hydrocarbons are strong CNS stimulants; affected horses become hyperalert, then excited, and, in severe cases, develop convulsions. In almost all instances, the mode of horses being exposed to pesticides is topical.

Monensin

Horses are highly susceptible to monensin poisoning in comparison with the other domesticated animals. Monensin is an ionophore normally added to cattle and poultry feed to provide growth stimulation by enhancing the intestinal absorption of calcium and sodium. Horses are easily poisoned by accidentally consuming cattle or poultry feed containing the recommended amounts of monensin for those species. Affected horses can die suddenly without any other signs. Monensin affects the cardiac and skeletal muscles, and acute cardiac failure is the cause of death.

Blister Beetles

Cantharidin is an irritant toxic agent present in blister beetles. Only a few of the several species of blister beetles contain cantharidin. Blister beetles are abundant in mid-summer and late summer when alfalfa hay containing the beetles is harvested in the central plains of the United States. Horses are poisoned by eating the alfalfa hay containing crushed swarms of blister beetles. Affected horses develop severe colic, abdominal pain, and blood-tinged urine. They will kick at their bellies and roll on the ground; severely affected horses may die of shock. Although recommended treatment involves the use of pain killers, such as banamine hydrochloride, and large volumes of intravenous fluids, there is no effective therapy for affected horses.

Heavy Metals

Lead poisoning in horses is characterized by neurological effects. Affected horses will be either depressed or excited. Colic and diarrhea are also seen. Because of laryngeal nerve paralysis, horses poisoned by lead also present with difficult respirations and a roaring syndrome. Abortions may also occur.

Arsenic poisoning in horses is usually caused by the consumption of foliage which has recently been sprayed with arsenic herbicides. The condition is acute and characterized by intense colic and hemorrhagic diarrhea. As in other animals, inorganic arsenic poisoning does not affect the nervous system, which helps differentiate this poisoning from organophosphate or carbamate poisonings.

Selenium is an essential element but is toxic when excessive quantities are ingested. Exposure of horses is usually through consumption of seleniferous (accumulator or indicator) plants (e.g., *Astragalus* spp.). Exposure to high quantities of selenium over a short time causes diarrhea (which is often foul smelling and contains air bubbles), neurological and cardiovascular effects, and respiratory difficulty. Death in these horses is due to respiratory failure. Chronic exposure to low levels of excessive selenium is characterized by hoof abnormalities at the coronary bands and by discoloration and loss of hair. The hoof deformities are painful and cause lameness.

Toxic Plants

The plant poisonings commonly encountered in horses are those that cause gastrointestinal problems, liver damage, primary or secondary nervous system involvement, and sudden death. Plants such as castor bean and oleander cause colic and diarrhea. Oleander also causes cardiac toxicity. Prolonged ingestion of some plants for several weeks can lead to liver damage and hepatic cirrhosis. This commonly occurs with the hepatotoxic plants *Amsinckia*, *Senecio*, and *Crotolaria*. Liver damage compromises the ability of the horse to detoxify ammonia that accumulates *in vivo,* leading to CNS derangement.

Plants that can cause CNS stimulation include larkspur, locoweed, lupine, water hemlock, and fitweed. Common plants that produce CNS depression are black locust, bracken fern, horsetail, milkweed,

and white snake root. Like ruminants, horses will avoid eating toxic plants because they are usually not palatable. Therefore, consumption of poisonous plants will most often occur during drought conditions or following overgrazing when the animals lack suitable pasture.

Sudden death in horses can be caused by consumption of cyanide-containing plants, such as sorghum. The cyanide ion forms a complex with cytochrome oxidase. This prevents electron transport and the utilization of oxygen by body tissues. As a consequence, the circulating blood is well oxygenated and is bright cherry red in color. This condition is an emergency, and treatment requires the prompt intravenous administration of both sodium thiosulfate and sodium nitrite.

Horses, like other monogastrics, are more resistant to plants capable of causing nitrate–nitrite poisoning than are ruminants. However, horses can modestly reduce nitrates to nitrites in their cecums, but it requires about three times as much nitrate to produce the same toxic effect in horses as in ruminants.

Mycotoxins

Contaminated grains (corn, wheat, and milo) are the sources of mycotoxin exposure for horses. The most commonly involved mycotoxins are aflatoxins, T-2, and fumonisin B_1. Aflatoxins will cause nonspecific effects, such as a poor thriving condition, hemorrhages, and abortions. T-2 is a trichothecene mycotoxin that causes prolonged bleeding times and digestive tract inflammation in affected horses.

A specific mycotoxin which uniquely affects horses is fumonisin B_1, which is produced by the fungus *F. moniliforme*; it has been responsible for causing the condition called equine leukoencephalomalacia. Horses receive the fumonisin by consumption of molded corn. Affected horses become anoretic and depressed after consuming the infested grain for only a few days. The condition then progresses, with the animals becoming blind, walking aimlessly, head pressing, being unable to swallow, and dying in a coma 1–4 days after the initial onset of signs.

Conclusions

The broad discipline of veterinary toxicology has been presented using brief accounts of the common toxicities in different animal species to draw the attention of the reader to similarities and differences in their reactions to toxicants. Because some animals are more sensitive than others receiving the same toxicant, the diagnosis of some poisonings may require the help of toxicologists within the veterinary profession.

This entry was not intended as a detailed reference for diagnosis and treatment of animal poisonings, nor was it meant to be all-inclusive. Rather, it presents the commonly encountered toxicoses in veterinary medicine.

It should be clear that all animals are susceptible to some toxicants and that some toxicants are toxic to all animals (including humans). It is therefore important to be cautious when handling and using chemicals around animals; also, a clean environment must be provided for all animals. Domestic animals particularly are subject to the whims of their owners for hazard-free environments. Animals should be fed well-balanced quality food from reputable sources, and suspect feed should be either avoided or carefully examined for potential toxicants before being given to animals.

It is also vitally important to remember that all chemicals become poisons if the exposure rate is sufficiently high. Therefore, even useful and recommended compounds used routinely around animals (e.g., growth promoters) can be life threatening if used excessively or if given to species for which they were not intended. The susceptibility of sheep to copper-containing cattle feed or the high risk to horses when fed poultry feeds containing monensin are cases in point.

See also: Aflatoxin; Algae; Ammonia; Asbestos; Benzene Hexachloride, Mixed Isomers; Brodifacoum; Carbamate Pesticides; Carbon Monoxide; Castor Bean; Copper; Coumarins; DDT (Dichlorodiphenyltrichloroethane); DEET (Diethyltoluamide); Dichlorvos; Dieldrin; Ethylene Glycol; Hydrogen Sulfide; Lead; Malathion; Methane; Molybdenum; Mushrooms, Coprine; Mushrooms, Cyclopeptide; Mycotoxins; Nitrites; Oleander; Organochlorine Insecticides; Organophosphates; Paraquat; Parathion; Pyrethrins/Pyrethroids; Selenium; Sodium; Strychnine; Sulfur Dioxide; Thallium; Warfarin.

Further Reading

Beasley VR (ed.) (1990) Toxicology of Selected Pesticides, Drugs and Chemicals. *Veterinary Clinics of North America (Small animal practice)*. Philadelphia: Saunders.

Blood DC and Radostits OM (1994) *Veterinary Medicine*. Philadelphia: Saunders.

Cheeke PR (ed.) (1997) *Natural Toxicants in Feeds and Poisonous Plants*, 2nd edn. New York: Prentice Hall.

Hays WJ Jr. and Laws ER Jr. (1991) *Handbook of Pesticide Toxicology*. San Diego, CA: Academic Press.

Kingsbury JM (1964) *Poisonous Plants of the United States and Canada*. Englewood Cliffs, NJ: Prentice Hall.

Osweiler GD, Carson TL, Buck WB, and Van Gelder GA (eds.) (1985) *Clinical and Diagnostic Veterinary Toxicology*, 3rd edn. Dubuque, IA: Kendall Hunt.

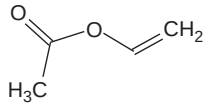
Plumlee KH (ed.) (2004) *Clinical Veterinary Toxicology*. St. Louis: MO: Mosby.

Robinson NE (ed.) (2002) *Current Therapy in Equine Medicine*, 5th edn. Philadelphia: Saunders.
Spoerke DG Jr. and Smolinske SC (1990) *Toxicity of Houseplants*. Boca Raton, FL: CRC Press.
Straw BE, D'Allaire S, Mengelng WL, and Taylor DJ (eds.) (1999) *Disease of Swine*, 8th edn. Ames, IA: Iowa State University Press.

Vinyl Acetate

Heriberto Robles

- Chemical Abstracts Service Registry Number: CAS 108-05-4
- Synonyms: Acetic acid ethenyl ester; Acetic acid ethylene ether; Acetic acid vinyl ester; Ethenyl acetate; Vinyl ethanoate
- Chemical Formula: $C_4H_6O_2$
- Chemical Structure:



Uses

Vinyl acetate is used in the manufacturing of polyvinyl compounds, resins, glues, polymer adhesives, vinyl copolymers, plastics, and latex paints.

Background Information

The International Agency for Research on Cancer has determined that vinyl acetate is possibly carcinogenic. This determination is based on inadequate human epidemiological data and limited evidence of carcinogenicity in laboratory animals.

Exposure Routes and Pathways

Workers handling vinyl acetate may be exposed to it through vapor inhalation and skin contact with its liquid form.

Toxicokinetics

Vinyl acetate is moderately toxic when administered through ingestion, inhalation, and peritoneal injection. At low to moderate doses, it produces irritation at the point of contact. Prolonged dermal exposure may produce severe irritation and skin blistering.

Mechanism of Toxicity

Vinyl acetate is rapidly metabolized in the body to acetaldehyde. *In vitro* studies have revealed similar toxic effects in cell cultures incubated in the presence of either vinyl acetate or acetaldehyde. These results suggest that the acetaldehyde is the ultimate toxic metabolite of vinyl acetate.

Acute and Short-Term Toxicity (or Exposure)

Human

Acute exposures of humans to high concentrations may result in tissue irritation at the point of contact (e.g., skin, eyes, respiratory tract). Signs and symptoms associated with potential routes of exposure include:

- *Inhalation:* Nose and throat irritation with cough and hoarseness. Olfactory fatigue may develop after continuous exposure.
- *Ingestion:* Vinyl acetate seems to have low toxicity when administered by ingestion.
- *Skin:* Skin irritation that may progress to blistering if product is allowed to remain on the skin.
- *Eye:* Eyes irritation and mild (reversible) corneal injury.

Chronic Toxicity (or Exposure)

Animal

Chronic inhalation exposure in rats produces nasal cancer. In addition, thyroid cancers and effects on the male reproductive system have been reported in laboratory animals chronically exposed to vinyl acetate.

Human

Results of an epidemiological study indicated that chronic, occupational exposure to vinyl acetate at concentrations below 22 ppm is not likely to result in irritation of the upper respiratory system. In addition, no chronic adverse effects were reported for chronic exposures to concentrations between 5 and 10 ppm. Chronic exposures to moderate and high doses have been shown to produce damage and

alterations of the central nervous and cardiovascular systems as well as lung and liver damage.

In Vitro Toxicity Data

Vinyl acetate has been shown to be rapidly metabolized to acetaldehyde in cell cultures. The formation of acetaldehyde is believed to be a precursor to the chromosome cell damage, sister chromatid exchange, and DNA crosslinking seen in cell cultures treated with vinyl acetate.

Clinical Management

There is no specific treatment for vinyl acetate toxicity. Supportive and symptomatic treatment is recommended.

Environmental Fate

Vinyl acetate is expected to have a short half-life in environmental media. If released to soil, it will either volatilize or be hydrolyzed in the presence of soil moisture. If released to air, it is expected to degrade by reacting with either hydroxyl radicals or ozone. If released to water, vinyl acetate is likely to either volatilize to the atmosphere or undergo hydrolysis and biodegradation. Biodegradation of vinyl acetate is known to occur under both aerobic and anaerobic conditions.

Exposure Standards and Guidelines

Special precautions must be taken when working with vinyl acetate. Personnel handling this chemical must follow industrial hygiene and health protection requirements for handling potentially carcinogenic substances. A minimum vinyl acetate exposure should be minimized through the use of engineering controls, work practices, and personal protective equipment, including impervious and disposable gowns and gloves as well as eye and respiratory protection. In addition, working areas and working instruments must be especially designed for handling potentially harmful substances.

See also: Polymers.

Further Reading

Klaassen CD (ed.) (2001) *Casarett & Doull's Toxicology, The Basic Science of Poisons*, 6th edn. New York: McGraw-Hill.

Rossoff IS (2002) *Encyclopedia of Clinical Toxicology.* Boca Raton: The Parthenon Publishing Group.

Relevant Websites

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

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

Vinyl Bromide

Karl K Rozman

- Chemical Abstracts Service Registry Number: CAS 593-60-2
- Synonyms: Bromoethylene; Monobromoethylene; Bromoethene
- Chemical Formula: C_2H_3Br
- Chemical Structure: $CH_2 = CH–Br$

Uses

Vinyl bromide is not known to occur as a natural product. It is commercially synthesized either by a metal halide catalyzed reaction of acetylene with hydrogen bromide or from ethylene dibromide with potassium hydroxide. Vinyl bromide is primarily used in flame retardant synthetic fibers, as a copolymer with vinyl chloride in films, laminating fibers, and as a rubber substitute.

Exposure Routes and Pathways

Workers may be exposed occupationally via inhalation during manufacture or use.

Toxicokinetics

Vinyl bromide is readily absorbed by all routes of exposure. Like all other volatile organics, the major route of exposure is via inhalation, though low level exposure in the drinking water and through the skin may also occur. Because of its low solubility in water, its absorption through the lungs will be

ventilation-limited. Due to its lipophilicity it will be preferentially sequestered in tissues of high lipid content. Biotransformation is the critical step in its clearance. Vinyl bromide, like its other halogenated analogues, vinyl chloride and vinyl fluoride, will be converted to its epoxide (2-bromoethylene oxide) by mixed function oxidases. This epoxide reacts with both RNA and DNA to form corresponding covalently bound etheno adducts, which are identical chemical moieties after exposure to any of the vinyl halides. A minor metabolite of vinyl bromide is 2-bromoacetaldehyde which is a rearrangement product of the epoxide. This reactive aldehyde avidly binds to protein. Elimination of vinyl bromide occurs by metabolic clearance and by exhalation of the unchanged parent compound. Its biotransformation is saturated at $\sim$250 ppm. Consequently, at higher concentrations first-order clearance ($18\,l\,h^{-1}\,kg^{-1}$) becomes a zero-order process ($40\,\mu mol\,h^{-1}\,kg^{-1}$). Its kinetic half-life below saturation of metabolic clearance is $\sim$1 h. Since adduct formation involves nucleophilic displacement of bromide, serum levels of bromide will rise to steady state concentrations up to metabolic saturation in accordance with the half-life of bromide (3–5 days in rats, $\sim$12 days in humans).

Absorption, distribution, biotransformation, and excretion of vinyl bromide have not been extensively studied in humans but are unlikely to be significantly different from vinyl chloride which has been studied in humans.

Mechanism of Toxicity

The most sensitive endpoint of toxicity of vinyl bromide is angiosarcoma of the liver. The dynamic half-life of the etheno-DNA-adduct is $\sim$30 days, which is much slower than the kinetic half-life and therefore represents the rate-determining step in its carcinogenic action. Thus, the dynamics of angiosarcoma are not driven by the kinetics of vinyl bromide but by the DNA-adduct which will accumulate for 6.64 half-lives ($\sim$200 days) to a very high steady state concentration even if exposure occurs only sporadically within 6.64 dynamic half-lives. Therefore, it must be understood that in the carcinogenicity of vinyl bromide it is not the time-weighted average of exposure that is important but peak concentrations. In agreement with metabolic saturation is the truncation of the angiosarcoma dose response at an inhalation concentration of 250 ppm. Thus, the mechanism of carcinogenicity of vinyl bromide is one of the best understood among the many compounds studied and the human and animal data base is without internal inconsistency.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} of vinyl bromide was $\sim 500\,mg\,kg^{-1}$. No histopathological changes were found in rats exposed for 7 h to $110\,000\,mg\,m^{-3}$ (25 000 ppm) showing that vinyl bromide is moderately toxic acutely.

In subacute inhalation studies, rats were exposed to $44\,000\,mg\,m^{-3}$ (10 000 ppm) 7 h per day, 5 days per week for 4 weeks; rats, rabbits, and monkeys were exposed to 1100 or $2200\,mg\,m^{-3}$ (250 or 500 ppm) for 6 h and 5 days a week with no change in gross pathology or histopathology, revealing vinyl bromide as a moderately toxic chemical subacutely.

In subchronic studies rats inhaling $8800\,mg\,m^{-3}$ (2000 ppm) vinyl bromide 8 h per day for 5 days a week for 8–15 weeks developed ATP-ase deficient foci in the liver, indicative of its carcinogenic potential, which is also in agreement with its mutagenicity in almost every test system.

Human

Short-term inhalation of high concentrations has been reported to cause loss of consciousness which is not unexpected since all low molecular weight chlorinated aliphatics are known to possess this property. Skin and eye contact with liquid vinyl bromide produced irritation and 'frost-bite' type burn, again, not unexpected of a highly volatile compound.

Chronic Toxicity (or Exposure)

Animal

As expected, based on very strong similarity with vinyl chloride in terms of kinetics and dynamics, vinyl bromide provided a truncated, but up to metabolic saturation, perfect dose response in terms of liver angiosarcoma in both male and female rats inhaling 0, 10, 50, 250, and 1250 ppm (44, 219, 1093, and $5875\,mg\,m^{-3}$) vinyl bromide. Increased tumor incidences were also observed in squamous cell carcinomas of the Zymbal gland and neoplastic nodules and hepatocellular carcinomas.

Human

Effects on reproduction and prenatal toxicity have also not been reported nor mutagenicity or carcinogenicity in human populations. Even though there is no epidemiological evidence that vinyl bromide causes liver angiosarcomas in humans, its close structural analog, vinyl chloride, is a confirmed human carcinogen having caused liver angiosarcomas

in workers first exposed to vinyl chloride prior to 1968. Therefore, and since vinyl chloride, vinyl fluoride, and vinyl bromide all caused angiosarcoma of the liver in all species studied, it can be concluded with certainty that at high enough doses vinyl bromide would also give rise to liver angiosarcomas in humans. Conversely, it can also be concluded from the lack of evidence of liver angiosarcomas in humans that workers were not exposed to cancer-causing concentration/time combinations of vinyl bromide. Since vinyl bromide is commercially available only since 1968, a time at which industrial hygiene practices were already much improved (no liver angiosarcomas in workers first exposed to vinyl chloride after 1968) in fact no angiosarcomas could and should have occurred in vinyl bromide exposed workers. Since humans were shown to be less sensitive to vinyl chloride-induced cancer than were experimental animals, it is highly likely that this would also be the case for vinyl bromide.

Clinical Management

There is no specific antidote known for vinyl bromide. Since most of inhaled vinyl bromide is reexhaled and only a fraction of it is converted to adducts liberating bromide as a metabolite and since this process is saturable, it is unlikely that bromism will ever be a problem in acute or chronic vinyl bromide exposure. Acute inhalation exposure will result in anesthesia which requires immediate removal from enclosed areas and institution of standard emergency room treatment such as general support therapy with oxygenation by nonrebreather mask. Emetics should not be used after ingestion but activated charcoal should be administered. Mouth should be rinsed and up to 200 ml of water or milk should be administered, if patient can swallow. Orotracheal or nasal intubation for airway control may be necessary if patient is unconscious. Decontamination should be done and the skin burns covered with dry sterile dressing. Proparacaine hydrochloride should be used to aid eye irrigation.

Environmental Fate

Vinyl bromide's production and use as a flame retardant for acrylic fiber may result in its release to the environment through various waste streams. It may form in air as a degradation product of 1,2-dibromoethane. Vinyl bromide was detected in fumaroles and lava gas from volcanoes. If released into air, with a vapor pressure of 1030 mmHg at 25°C, vinyl bromide will exist in the gas phase. Gas-phase vinyl bromide will be degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals; the half-life for this reaction in air is estimated at 2.4 days. If coming in contact with soil, vinyl bromide is expected to be adsorbed to suspended solids and to have moderate mobility based upon an estimated K_{oc} of 170. Volatization from moist soil surfaces could be important in the environmental fate of vinyl bromide based upon an estimated Henry's law constant of 1.4×10^{-2} atm m^{-3} mol^{-1}. Estimated volatization half-lives from a model river and a model lake are 1.1 h and 4.1 days, respectively. An estimated bioconcentration factor of 3 suggests that the potential for bioconcentration in aquatic organisms is low.

Exposure Standards and Guidelines

The International Agency for Research on Cancer classified the carcinogenicity of vinyl bromide as having sufficient evidence in experimental animals. The National Toxicology Program Board of Scientific Counselors designated vinyl bromide as reasonably anticipated to be a human carcinogen. Occupational Safety and Health Administration had no formal permissible exposure limit (PEL) for vinyl bromide and accepted the then existing American Congress of Governmental Industrial Hygienists (ACGIH) recommendation of a 5 ppm time-weighted average (TWA) as PEL with final ruling. The National Institute for Occupational Safety and Health has no recommended exposure limit for vinyl bromide. The most recent ACGIH recommendation for vinyl bromide is a threshold limit value-TWA of 0.5 ppm (2.2 mg m^{-3}) with an A2 (suspected human carcinogen) designation. The Environmental Protection Agency's reference concentration for vinyl bromide is 0.003 mg m^{-3}.

See also: Vinyl Chloride.

Further Reading

Benya TJ, Busey WM, Dorato MA, and Berteau PE (1982) Inhalation carcinogenicity bioassay of vinyl bromide in rats. *Toxicology and Applied Pharmacology* 64: 367–379.

Bolt HM, Laib RJ, and Filser JG (1982) Reactive metabolites and carcinogenicity of halogenated ethylenes. *Biochemical Pharmacology* 31: 1–4.

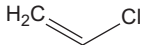
Filser JG and Bolt HM (1979) Pharmacokinetics of halogenated ethylenes in rats. *Archives of Toxicology* 42: 123–136.

Storm JE and Rozman KK (1997) Evaluation of alternative methods for establishing safe levels of occupational exposure to vinyl halides. *Regulatory Toxicology and Pharmacology* 25: 240–255.

Vinyl Chloride

Robert Kapp

- Chemical Abstracts Service Registry Number: CAS 75-01-4
- Synonyms: Chlorethene; Chlorethylene; Chloroethene; Chloroethylene; Ethene, chloro-; Ethylene monochloride; Ethylene, chloro-; Monochloroethene; Monochloroethylene; Monovinyl chloride; Trovidur; VC; VCM; Vinyl C monomer; Vinyl chloride monomer; Vinyl chlorine
- Related Compounds: Vinyl bromide (CAS 593-60-2); Vinyl fluoride (CAS 72-02-5); Vinylidene chloride (CAS 75-35-4); 1,2-Dichloroethene (CAS 540-59-0); Hexachlorobutadiene (CAS 87-68-3)
- Chemical/Pharmaceutical/Other Class: Vinyl monomers; Halogenated hydrocarbons
- Chemical Formula: C_2H_3Cl
- Chemical Structure:



Uses

Vinyl chloride is produced in the following industrial reactions: (1) the thermal cracking of 1,2-dichloroethane, which is produced by the chlorination and/or oxychlorination of ethylene; and (2) the hydrochlorination of acetylene. The vast majority of vinyl chloride is used for the production of polyvinyl chloride (PVC) and the manufacture of copolymers with monomers such as vinyl acetate or vinylidene chloride. A much smaller proportion of vinyl chloride is used in the production of chlorinated solvents – primarily trichloroethanes.

Exposure Routes and Pathways

The main route of occupational exposure to vinyl chloride is by inhalation that can occur in plastics manufacturing plants. Inhalation exposure to the general public is generally quite limited and probably restricted to accidental releases from hazardous waste sites and landfills. Vinyl chloride has been detected in surface and well waters, sediment and soil samples near manufacturing facilities. Some dietary exposure can occur from leaching from certain PVC materials into packaged foodstuffs.

Toxicokinetics

Vinyl chloride is readily absorbed via all routes of exposure and rapidly distributed throughout the body. Following oral administration in male rats, peak blood concentrations are noted in less than 10 min. Approximately 40% of inhaled vinyl chloride is absorbed while as much as 95% is absorbed upon ingestion. Highest concentrations are found in the liver and kidneys.

Storage of vinyl chloride is limited by the rapid metabolism and subsequent excretion. Vinyl chloride is biotransformed by cytochrome P450-mixed function oxidase systems (CYP 2E1), with the two primary metabolites being chloroethylene oxide and chloroacetaldehyde. These materials are further converted to chloroethanol and monochloroacetic acid. Metabolites are primarily excreted in urine. When rats were exposed to vinyl chloride at 100 ppm for 5 h, ~70% of the absorbed dose was excreted as urinary metabolites within 24 h. The half-life for urinary excretion in rats was ~4 h. With an increase in dose via either inhalation or ingestion, the proportion exhaled increased and urinary and fecal elimination decreased.

Mechanism of Toxicity

The mechanism of noncancer toxicity have not been extensively studied. Some immunological changes have been noted suggesting that one of the reactive metabolites binds to IgG, thus initiating immune responses depositing precipitates that can cause blockage in capillaries. It is also suggested that peripheral nervous system symptoms such as paresthesia, numbness, and pain in the extremities may be a direct result of vinyl chloride exposure or may be due to tissue anoxia because of vascular blockage.

Vinyl chloride is also a human and animal carcinogen associated with an increased incidence of hepatic angiosarcoma. Chloroethylene oxide, chloroacetaldehyde, and monochloroacetic acid all react covalently with DNA and RNA. This alkylation results in highly effective base-pair substitutions that can lead to neoplastic transformation. These reactive metabolites might also interact with chromosomes causing clastogenic effects.

Acute and Short-Term Toxicity (or Exposure)

Animal

Vinyl chloride appears to have a low toxicity when administered by inhalation, with LC_{50} values

reported to be in the 130 000–500 000 mg l^{-1} range. The oral LD_{50}, on the other hand, is reported to be 500 mg kg^{-1}. Vinyl chloride is reported to be slightly irritating to the eyes and respiratory tract at high concentrations. Inhalation can cause headache, nausea, central nervous system (CNS) depression, lung and kidney irritation, inhibition of blood clotting, and cardiac arrhythmias in animals.

Human

Vinyl chloride is a CNS depressant; loss of consciousness can occur following exposure to high concentrations (25 000 mg m^{-3}). Acute exposure to high ambient concentrations can lead to dizziness, light-headedness, nausea, headache, irritability, cognitive problems, paresthesia, and irritation of the eyes and respiratory tract.

Chronic Toxicity (or Exposure)

Animal

Chronic animal studies report increased mortality and weight loss, as well as effects on the liver, kidney, and CNS at levels as low as 1.3 mg kg^{-1} day^{-1}. Animal studies have shown increased testicular damage as well as decreased male fertility in rats exposed to low levels of vinyl chloride for 12 months. In addition, some animal studies have shown decreased fetal weights and increased terata at maternally toxic inhalation exposure levels of vinyl chloride. Animal studies have also reported that inhaled vinyl chloride increases the incidence of angiosarcoma of the liver.

Human

Chronic inhalation or oral exposure to low levels of vinyl chloride may cause liver damage in humans. Some individuals occupationally exposed to high levels of vinyl chloride develop a specific syndrome termed 'vinyl chloride disease'. This is characterized by dizziness, numbness, earache, headache, blurred vision, fatigue, nausea, shortness of breath, Raynaud's phenomenon, loss of weight, changes in bone structure at the ends of the fingers, joint, and muscle pain, and scleroderma-type changes in the skin.

Several unsubstantiated case reports have reported reduced male sexual performance upon occupational exposure to vinyl chloride. There have been mixed epidemiological results with respect to teratogenic effects in human exposure.

Epidemiological studies conducted on humans exposed to inhaled vinyl chloride have shown increases in angiosarcoma of the liver. Hepatocellular carcinoma of the liver as well as some brain tumors have been reported; however, the data are not considered definitive. Vinyl chloride has been reported to be mutagenic and clastogenic in human studies. US Environmental Protection Agency (EPA) has classified vinyl chloride as group A, human carcinogen, and group K – known human carcinogen. International Agency for Research on Cancer classifies vinyl chloride as group 1 – carcinogenic to humans. National Institute for Occupational Safety and Health and Occupational Safety and Health Administration (OSHA) both categorize vinyl chloride as Ca – potential occupational carcinogen. US National Toxicology Program categorizes vinyl chloride as K – known to be a human carcinogen. American Conference of Governmental Industrial Hygienists (ACGIH) categorizes vinyl chloride as threshold limit value (TLV)-A1 – confirmed human carcinogen.

Clinical Management

Upon massive exposure, the primary risks are CNS effects and cardiac arrhythmias; therefore, the evaluation of vital functions and life-support measures should be taken and the victim should be decontaminated and removed from the area to minimize further exposure. Vinyl chloride exposure can irritate the skin, eyes, and mucous membranes, and the liquid can cause frostbite. The affected area should be washed with copious amounts of lukewarm or cold water. Hot water should not be used. Oral ingestion of vinyl chloride is unlikely; however, should that occur, it is suggested that water or milk be administered and, in addition, gastric lavage and administration of activated charcoal can be used as a means to reduce absorption.

Upon ocular exposure, the eye should be generously washed with tap water. Refer for medical attention. In case of dermal exposure, contaminated clothing should be removed and the skin should be rinsed with tap water. Titanium tetrachloride ingestion should be referred for medical attention. Vomiting should NOT be induced. Upon inhalation, the victim should be moved to fresh air and given artificial respiration if indicated. The body should be placed in a half-upright position. Refer for medical attention.

Environmental Fate

Anthropogenic sources are responsible for all of the vinyl chloride found in the environment. Vinyl chloride has been identified in at least 493 of the 1416 hazardous waste sites that have been included on the EPA National Priorities List. Of these sites,

491 are located in the United States and two are located in the commonwealth of Puerto Rico. Most of the vinyl chloride released into the environment is eventually transported to the atmosphere, whereas lesser amounts are transported to the groundwater. Vinyl chloride has been detected in the ambient air in the vicinity of vinyl chloride and PVC manufacturing plants and hazardous waste sites. The compound has also leached into groundwater from spills, landfills, and industrial sources. In the atmosphere, vinyl chloride is eliminated by reaction with photochemically generated hydroxyl radicals (half-life = 1–2 days): products include hydrochloric acid, formaldehyde, formyl chloride, acetylene, chloroacetaldehyde, chloroacetylchloranial, and chloroethylene epoxide. Dry deposition is not an important elimination pathway. In photochemical smog, the half-life is reduced to a few hours. In water, volatilization is the primary elimination process. Half-lives for volatilization from a typical pond, river, and lake have been estimated at 43, 9, and 35 h, respectively. In soil, vinyl chloride can volatilize from soil surfaces or leach into groundwater.

Ecotoxicology

Vinyl chloride can bioconcentrate to a limited extent in aquatic organisms. Biomagnification of vinyl chloride in terrestrial and aquatic food chains does not appear to be important because of its high volatility and the fact that it is readily metabolized by higher-trophic-level organisms. Little is known regarding biomagnification in terrestrial food chains.

Exposure Standards and Guidelines

The reference dose for vinyl chloride is 0.003 mg kg^{-1} day^{-1}, the reference concentration is 0.01 mg m^{-3}. The ACGIH TLV, time-weighted average, is 13 mg m^{-3} and the OSHA permissible exposure level is 2.6 mg m^{-3}.

See also: Carcinogenesis; Liver; Occupational Toxicology; Polymers; Respiratory Tract.

Further Reading

Albertini R, Clewell H, Himmelstein MW, *et al.* (2003) The use of non-tumor data in cancer risk assessment: Reflections on butadiene, vinyl chloride, and benzene. *Regulatory Toxicology and Pharmacology* 37: 105–132.

Lemen RA (2001) Unsaturated Halogenated Hydrocarbons. In: Bingham E, Cohrssen B, and Powell CH (eds.) *Patty's Toxicology*, 5th edn., vol. 2, pp. 247–255. New York: Wiley.

Relevant Websites

http://www.epa.gov – Vinyl Chloride (from the US Environmental Protection Agency's Technology Transfer Network Air Toxics Website).

http://risk.lsd.ornl.gov – Toxicity summary for Vinyl Chloride (from the Risk Assessment Information System (RAIS)).

Vinylidene Chloride

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- Chemical Abstracts Service Registry Number: CAS 75-35-4
- Synonyms: 1,1-Dichloroethylene; 1,1-Dichloroethene; Vinylidene dichloride; Sconatex
- Chemical/Pharmaceutical/Other Class: Vinylidene chloride is an unsaturated halogenated hydrocarbon.
- Chemical Formula: $C_2H_2Cl_2$

Uses

The principal use of vinylidene chloride is in the production of polyvinylidene chloride polymers (PVDC). PVDC is used in the food packing industry for making flexible films such as in Saran and Velon wraps and as a barrier coating for paper, cellulose, polypropylene, and other plastics. These polymers are also used in the textile industry for drapery fabric, furniture and automobile upholstery; as flame retardant coatings for fiber and carpet backing; and in piping, coating for steel pipes, and adhesive applications.

Exposure Routes and Pathways

The principal sources of exposure to vinylidene chloride in the environment are ambient air especially near industrial sources and contaminated drinking water. Exposure can occur through inhalation, ingestion, and eye or skin contact. Air releases account for 99% of the total environmental releases. Ambient levels are primarily from emissions from polymer synthesis and fabrication industries, mostly

during manufacture and use, and little during the incineration of polymerized products. Ambient air levels have been reported in the range of 0.005–0.84 ppb, but concentrations were up to 97 ppb at a contaminated waste site. Information on release to soil is limited. Since vinylidene chloride in soil tends to partition into the air or groundwater, soil concentrations are expected to be low. Occupational exposure may occur from inhalation or dermal contact. Low levels have been detected in a number of drinking water supplies in the United States. A US Environmental Protection Agency (EPA) survey reported in 1985 showed that ~3% of the drinking water supplies contained vinylidene chloride with a range of 0.2–0.5 ppb, and an estimated mean of 0.3 ppb. Concentrations of residual vinylidene chloride in household films used for food packaging have been reported at 6.5–10.4 ppm, and in foodstuffs wrapped with commercial films containing residues (average 8.8 ppm), <0.005 to 0.01 ppm.

Toxicokinetics

Vinylidene chloride is well absorbed following oral and inhalation exposure. Dermal absorption is unlikely due to its low molecular weight and hydrophobic nature. After oral exposure to radiolabeled vinylidene chloride, animals showed highest level of radioactivity at 72 h in the liver and kidneys. Following inhalation, highest levels were found at 2 h also in the liver and kidneys.

Biotransformation involves oxidation via the cytochrome P450 system and subsequent detoxification by conjugation with glutathione and cellular macromolecules. The oxidative metabolic pathway is saturable, occurring at an oral dose of ~10–50 mg kg^{-1} in the rat, and at inhalation exposures exceeding 200 ppm. Elimination of vinylidene chloride and its metabolites occurs primarily through the urine and in the expired air at a relatively rapid excretion rate.

Mechanism of Toxicity

The toxicity of vinylidene chloride is related to cytochrome P450-catalyzed metabolism to reactive intermediates that bind covalently to cellular macromolecules. The target organs after acute oral or inhalation exposures are the liver, kidney, and lung. The specific targets are the centrilobular hepatocytes and bronchiolar Clara cells, cell types that are rich in CYP2E1. In rats and mice, vinylidene chloride is oxidized by the liver cytochrome P450 system to the epoxide 2-chloroacetyl chloride and to 2,2-dichloroacetaldehyde. The isozyme CYP2E1 appears to be responsible for the oxidation of vinylidene chloride in the liver and lungs of both species. The epoxide is the principal product, albeit more of the 2,2-dichloroacetaldehyde is produced more in the murine lung than liver. The metabolites then undergo secondary reactions, which are principally conjugation with glutathione and cellular molecules, a detoxification mechanism. The extent of covalent binding is inversely related to the loss of glutathione so that tissue damage increases with a decrease in glutathione level.

Acute and Short-Term Toxicity (or Exposure)

Animal

The following lethal dose (LD) values have been reported:

- Oral LD_{50}, rats 1500 mg kg^{-1}, males; 1800 mg kg^{-1}, females.
- Oral LD_{50}, young or fasted rats 50 mg kg^{-1} (lower glutathione levels).
- LD_{50}, mice 200 mg kg^{-1}, males and females.
- Inhalation LC_{50}, fasted rats 400 ppm, 4 h.
- Inhalation LC_{50}, rats 10 000–15 000 ppm, 4 h.
- Inhalation LC_{50}, mice 40 ppm, males; 200 ppm females; 4 h.

Vinylidene chloride is hepatotoxic, nephrotoxic, and mutagenic in experimental animals. It can affect the developing embryo.

Acute studies in rats showed that the chemical has high toxicity from oral exposure and moderate toxicity from inhalation exposure. For both acute oral and inhalation exposures, young and fasted animals are more sensitive to the acute toxicity of vinylidene chloride than those with access to food, and mice are more sensitive than rats, based on lower median lethal dose values. The principal target organs are the liver and kidney and the most sensitive endpoint is liver damage. Increases in enzyme markers of liver damage (aspartate transminase, AST; alanine transminase, ALT) and histological evidence of centrilobular and midzonal necrosis were observed. Fasting and administration by vehicle that increases the rate of absorption attenuates hepatotoxicity. The liver, kidney, and lung are also the organs most affected by subchronic exposure to vinylidene chloride.

Soft tissue anomalies in rats and skeletal effects in rats, mice and rabbits were observed following exposure to 15–449 ppm vinylidene chloride. Cardiac malformations were observed in fetus from maternal

rats exposed before and during pregnancy in drinking water.

Human

Vinylidene chloride is an eye irritant and can affect the central nervous system (CNS), liver, and kidneys in humans.

Contact with the eyes can cause conjunctivitis and transient corneal injury. Dermal contact causes irritation and there is little effect on the skin if it is allowed to evaporate. Inhalation can cause respiratory effects such as mucous membrane irritation. Exposure to high concentrations (4000 ppm) of vinylidene chloride as seen in workers may show signs of CNS depression with accompanying characteristic signs of intoxication and symptoms including drowsiness, nausea, headache, unsteadiness, or unconsciousness. Inebriation, convulsions, spasms have also been reported.

Chronic Toxicity (or Exposure)

Animal

Consistent with the findings from acute and subchronic exposures, a chronic oral gavage study in rats and mice conducted by the National Toxicology Program (NTP) (1982) also identified liver toxicity as the major toxic effect. Chronic exposures caused effects on the kidneys, liver, CNS, and lungs.

An update in 2004 by US EPA provided an oral reference dose (RfD) of 5×10^{-2} mg kg^{-1} day^{-1}, based on an no-observed-adverse-effect level (NOAEL) of 9 mg kg^{-1} day^{-1} on liver toxicity (hepatocellular midzonal fatty change as the minimal adverse effect) and a lowest-observed-adverse-effect level (LOAEL) of 14 mg kg^{-1} day^{-1} in a 2 year rat chronic and carcinogenicity study via drinking water. The updated evaluation used the benchmark dose (BMD) methodology and calculated a $BMDL_{10}$ of 4.6 mg kg^{-1} day^{-1} for midzonal fatty change in female rats. The BMD_{10} is 4.6 mg kg^{-1} day^{-1}. In this study, vinylidene chloride concentrations were 0, 50, 100, or 200 ppm. The time-weighted average (TWA) exposures were 7, 10, or 20 mg kg^{-1} day^{-1} for males and 9, 14, or 30 mg kg^{-1} day^{-1} for females. There were no significant differences observed among the groups in appearance, demeanor, mortality, body weight, food consumption, water consumption, hematology, urinalysis, clinical chemistry determinations, organ wrights, or organ to body weight ratios. The only treatment-related effect was hepatocellular fatty change and hepatocellular swelling. No exposure related hepatocellular necrosis or neoplastic changes were observed. The minimal hepatocellular swelling was not considered an adverse effect in this study and there were no changes liver weigh, clinical chemistry, or abnormal liver function. The fatty change was considered a minimal effect and might not be considered adverse as there was no evidence of a functional change in the liver of rats and glutathione levels were not reduced. On the other hand, the $BMDL_{10}$ was used to derive the RfD to limit exposure to this $BMDL_{10}$ and protect the liver from more serious damage (fatty liver or necrosis) that could compromise liver function. An uncertainty factor of 10 each was used for interspecies and intraspecies variability.

In a 2 year study by NTP, male and female rats and mice were administered corn oil 5 days week^{-1} for 104 weeks. A nonsignificant increase in adrenal pheochromocytomas was observed in male rats.

Tumor incidence data were used by the US EPA in 1994 for deriving an inhalation slope factor of 1.2 (mg/kg day)$^{-1}$ and a unit risk of 5.0×10^{-5} (μg/m^3)$^{-1}$. An oral slope factor of 6×10^{-1} (mg/kg day)$^{-1}$ and a unit risk of 1.7×10^{-5} (μg/l)$^{-1}$ were derived from the NTP 1982 study.

An update in 2004 by the US EPA determined that quantitative estimate of carcinogenic risk from either oral or inhalation exposure was not applicable. Vinylidene chloride showed equivocal evidence of carcinogenicity by the oral route and suggestive evidence by the inhalation route. Overall, the weight of evidence is not sufficient to justify deriving any unit risk. Male mice developed kidney tumors at one exposure level but not in female mice or male and females rats (i.e., one sex, one species, one exposure level). There were also enzymatic differences (i.e., CYP2E1) between male and female mice, male and female rats, and human kidney cells, and US EPA considered that genotoxicity evidence was limited. Under the draft revised guidelines for carcinogen assessment of 1999, US EPA concluded that vinylidene chloride exhibits suggestive evidence of carcinogenicity but not sufficient evidence to access human carcinogenic potential following inhalation exposure in studies in rodents.

Human

No signs or symptoms of chronic exposure to vinylidene chloride have been reported in humans. However, chronic exposure may lead to liver or kidney damage and the signs and symptoms of such damage may be apparent. There are no adequate human studies available for a quantitative determination of chronic effects. A few existing epidemiological studies involved concomitant exposure to vinyl chloride. There is one epidemiological study

reported in 1976 that investigated the health records of 138 workers exposed to vinylidene chloride in processes not involving vinyl chloride. The subjects had worked in experimental or pilot plant polymerization operations, in a monomer production process as tankcar loaders, or in a production plant manufacturing monofilament fiber. They were designated into three exposure categories: less than 10, 10–24, and greater than 25 ppm. There were no differences between the exposed cohort and the controls in hematology, clinical chemistry, and mortality. The numbers of subjects and endpoints studied were too limited for deriving useful information.

There is no clear evidence that vinylidene chloride exposure poses a carcinogenic risk to humans. Eighteen carcinogenicity or long-term bioassays have been conducted on vinylidene chloride and most were not adequately designed to have the sensitivity for detecting carcinogenic effects. Of the 18 studies, administration was by inhalation in 11 studies, oral route in five studies, skin application in one study, and subcutaneous injection in one study. Only one study showed an increased incidence of tumors (in Swiss mice). There was a significantly increased incidence of kidney adenocarcinomas in the males in the 25 ppm group. The tumors were accompanied by a significant degree of renal pathology (tubular necrosis). However, the relevance of these tumors in Swiss mice to humans is not clearly resolved. In this regard, DNA synthesis associated with tissue regeneration combined with the weak genotoxicity evidence for vinylidene chloride was thought to be a plausible mechanism for the tumors seen in this strain and species only. Additional studies showed that the P450 isozyme (CYP2E1), which is responsible for the metabolism of vinylidene chloride to the predominant reactive species, is not present in the kidneys of nonsusceptible species such as the rat. Also, assay of samples from human kidney showed negative results for the presence of the isozyme. Accordingly, the determination of potential carcinogenicity for humans based on these data is not resolved.

The International Agency for Research on Cancer (IARC) concluded that there is inadequate evidence in humans for its carcinogenicity, that there is limited evidence in experimental animals, and that it is not classifiable as to its carcinogenicity to humans (Group 3).

Clinical Management

The incapacitated worker should be removed from further exposure and appropriate emergency procedures should be implemented (e.g., those listed on the Material Safety Data Sheet, also shown below).

Inhalation. If adverse effects occur, the individual should be removed to an uncontaminated area. Artificial respiration should be given, if not breathing. Immediate medical attention must be sought.

Skin contact. The skin should be washed with soap and water for at least 15 min while removing contaminated clothing and shoes. Medical attention should be sought, if needed. Contaminated clothing and shoes should be thoroughly cleaned and dried before reuse.

Eye contact. The eyes should be flushed with plenty of water for at least 15 min. Then, immediate medical attention should be obtained.

Ingestion. If a large amount is swallowed, medical attention must be obtained.

In Vitro Toxicity Data

Vinylidene chloride has shown positive responses in the bacterial test systems including Salmonella test strains (mutation), *Escherichia coli* (mutation) and *Saccharomyces cerevisiae* (reverse mutation, mitotic gene conversion, aneuploidy) in the presence and absence of metabolic activation. It has produced both negative and positive responses in mammalian and in *in vivo* systems. The positive results were seen in the increase in the incidences of sister chromatid exchange and chromosomal aberration in Chinese hamster cell lines in the presence of an S-9 activation system. Negative results were seen in rat and mouse dominant lethal, micronuclei and chromosomal aberration assays. Following inhalation administration to rats and mice, there was only a minimal increase in DNA alkylation in both species and a minimal increase in DNA repair in kidney of mice. However, tissue damage (kidney nephrosis), an increase in DNA replication and an increase in mitotic figures were observed.

Environmental Fate

Vinylidene chloride is a human-made chemical and is not naturally found in the environment. It can be found from the breakdown of polyvinylidene (PVDC) products, and from the biotic and abiotic breakdown of 1,1,1-trichloroethane, tetrachloroethene, 1,1,2-trichloroethene, and 1,2-dichloroethane. Biotransformation of the chemical in groundwater can form vinyl chloride through reductive dechlorination, which is subsequently mineralized to carbon dioxide. The major transport process from water, soil and sediment is volatilization. Half-lives of

6 days in static pond water and 1 day in mobile river water have been calculated. Vinylidene chloride in soil tends to partition into the air or groundwater. That which is deposited on or near the soil surface is expected to rapidly volatilize into the air, and because soil mobility is quite high it may end up in groundwater. Bioaccumulation is expected to be low.

Atmospheric concentrations are relatively high compared with other environmental compartments because of vinylidene chloride's high vapor pressure and low water solubility. The half-life for the chemical in air has been estimated to be 16 h and 2–3 days. Atmospheric hydroxyl radicals play a major role in its degradation. The major reaction products in air are formaldehyde, phosgene, and hydroxylacetyl chloride.

Ecotoxicology

- Green alga, growth inhibition, 72 h EC_{50} 9.12 mg l^{-1}.
- Bluegill, lethality, 96 h LC_{50} 74 mg l^{-1}.

Little data exist on the effects of vinylidene chloride in the aquatic and terrestrial environments. Bioaccumulation is expected to be low based on the low octanol/water partition coefficient and low water solubility. A bioconcentration factor of 4 and a bioaccumulation factor of 6.9 were reported for fish, and a bioaccumulation factor of less than 13 reported for common carp. Because of the rapid volatilization of the chemical from the aquatic and terrestrial environments and the low concentrations found in surface water (microgram per liter range), no significant risk is expected.

Other Hazards/Sensitive Populations

Persons with higher risk are those who have underlying liver or kidney disease.

Exposure Standards and Guidelines

The World Health Organization (WHO) established a drinking water quality guideline of 0.03 mg l^{-1} in 1993 which is currently under review.

The US EPA has set a federal drinking water standard (called maximum contaminant level, or MCL) and a MCL goal of 7 ppb for vinylidene chloride.

The Office of Environmental Health Hazard Assessment of the California Environmental Protection Agency has established a public health goal of 10 ppb for vinylidene chloride in drinking water. This is based on midzonal hepatocellular fatty changes in female rats observed at all three treatment levels after exposure for 2 years.

The Office of Environmental Health Hazard Assessment of the California Environmental Protection Agency has established a chronic exposure level of 0.02 mg m^{-3} based on liver effects in guinea pigs.

The National Institute for Occupational Safety and Health (NIOSH) has established a recommended exposure limit (REL) for vinylidene chloride of the lowest feasible concentration of 0.4 ppm, the limit of quantitation. NIOSH also considers vinylidene chloride to be a potential human carcinogen based on the risk of potential cancer (liver and kidney tumors in animals).

The American Conference of Governmental Industrial Hygienists (ACGIH) has assigned vinylidene chloride a threshold limit value (TLV) of 5 ppm (20 mg m^{-3}) as a TWA for a normal 8 h workday and a 40 h workweek and a short-term exposure limit (STEL) of 20 ppm (79 mg m^{-3}) for periods not to exceed 15 min. Exposures at the STEL concentration should not be repeated more than 4 times a day and should be separated by intervals of at least 60 min. The limits are based on the risk of renal, hepatic, or other systemic toxicity.

The reportable quantity of vinylidene chloride is 100 lb. If an amount equal to or greater than this quantity is released within a 24 h period in a manner that will expose persons outside the facility, employers are required to notify the National Response Center immediately at (800) 424-8802 or at (202) 426-2675 in Washington, DC. A hazardous substance release is defined by EPA as any spilling, leaking, pumping, pouring, emitting, emptying, discharging, injecting, escaping, leaching, dumping, or disposing into the environment (including the abandonment or discarding of contaminated containers) of hazardous substances. In the event of a release that is above the reportable quantity for that chemical, employers are required to notify the proper Federal, State, and local authorities.

Vinylidene chloride is not currently regulated under the Occupational Safety and Health Administration.

Vinylidene chloride is listed as a hazardous waste under the Resource Conservation and Recovery Act and has been assigned EPA Hazardous Waste No. U078. It is approved for land disposal after treatment and only if the concentration of vinylidene chloride in the waste or treatment residual does not exceed 33 mg kg^{-1}.

Vinylidene chloride is designated as a hazardous substance under the Federal Water Pollution Control Act and further regulated by the Clean Water Act

Amendments of 1977 and 1978. These regulations apply to discharge of this substance.

Miscellaneous

Because of its volatility, determination of vinylidene chloride is best by gas chromatography using a variety of detectors, including flame ionization, electron capture, electrolyte conductivity, and mass spectrometry. The major limitation is interference by other constituents of the media being analyzed. Methods are available for quantifying environmental samples (air, water, soil sediment) and biological samples (breath, food, body tissues).

Volatility should be considered in the estimation of the overall exposure from the presence of vinylidene chloride in water to account for inhalation or dermal absorption due to the use of contaminated tap water.

See also: Vinyl Chloride.

Further Reading

Office of Environmental Health Hazard Assessment (OEHHA) (1999) *Public Health Goal for 1,1-Dichloroethylene in Drinking Water.* Office of Environmental Health Assessment, California Environmental Protection Agency, Oakland/Sacramento, CA.

Quast JF, McKenna MJ, Rampy LW, and Norris JM (1986) Chronic toxicity and oncogencicity study on inhaled vinylidene chloride in rats. *Fundamental and Applied Toxicology* 6: 105–144.

Relevant Websites

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

http://www.osha.gov – Occupational Safety and Health Administration (OSHA 2004). Occupational Safety and Health Guideline for Vinylidene Chloride. Occupational Safety and Health Administration, Department of Labor, Washington, DC.

http://www.epa.gov – US Environmental Protection Agency (US EPA) (last revised 8/13/2002, last updated July 8, 2004).

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

http://www.inchem.org – World Health Organization (WHO) (2003) 1,1-Dichloroethene (vinylidene chloride). Concise International Chemical Assessment Document 51. World Health Organization, Geneva.

Virtually Safe Dose (VSD)

Stephen M DiZio

The various ways that the dose–response relationship may be portrayed includes the simple, intuitive concept of a 'virtually safe dose'. This has its roots in the portrayal of what is termed the 'margin of exposure', a concept itself derived from the pharmaceutical industry when portraying to the physician the ratio between the amount of a drug that produces a harmful effect and that which produces the desired beneficial one.

Margin of exposure is an intuitively simple concept, founded in the premise that any chemical, at some level of exposure, can cause harm. Where that level of exposure, or 'dose' is established, the level of exposure where no harm is present (the 'no-observed-adverse-effect level' or NOAEL) may then be determined. This NOAEL, divided by the dose presented by that chemical due to environmental exposure, represents the margin of exposure. As an example, if chemical 'X' causes mild liver swelling at an oral dose of 10 milligrams (mg) per kilogram (kg) of body weight per day ($mg\,kg^{-1}\,day^{-1}$) when given to a subject for 2 weeks, and no liver problems are found at a dose of $1\,mg\,kg^{-1}\,day^{-1}$, then the NOAEL for that chemical is $1\,mg\,kg^{-1}\,day^{-1}$. If chemical X is present in the public drinking water at 35 μg (0.0035 mg) per liter, and we assume that humans drink 2 l of that water per day, then the environmental exposure is 70 μg per human per day. If that same human weighs ~70 kg, then the human environmental dose is $1\,\mu g\,kg^{-1}\,day^{-1}$, and the margin of exposure is $1\,mg\,kg^{-1}\,day^{-1}/0.001\,mg\,kg^{-1}\,day^{-1}$, or 1000. It has commonly been accepted that a margin of exposure of 1000 or greater is a 'virtually safe dose'.

This, of course, comes with three caveats. The first relates to the experiment where the NOAEL was determined. If animals were the experimental subject, and five animals of each sex were used in the 2 week experiment, then the opportunity of determining an adverse effect at a dose of $1\,mg\,kg^{-1}\,day^{-1}$ (the NOAEL used above) is less than an identical experiment where 100 animals of each sex were used. The second caveat is that harm from a chemical may be time dependent, that an NOAEL found

in a 2 week experiment may (and often is) far greater that one where the animals were exposed on a daily basis for a lifetime. The third caveat is that creatures differ in their sensitivity to chemicals, again intuitively obvious when one realizes that humans are often unharmed when exposed to a single dose of an insecticide, where the insects themselves die. The NOAEL for a mouse may differ from that for a rat, which may differ from that for a human. All these must be taken into account before declaring a level of environmental exposure a 'virtually safe dose'.

In recent years, the concept of a virtually safe dose has been extended to the probability of producing cancer in the exposed subject. Because cancer is assumed to be possible when a subject is exposed to even one molecule of a cancer-causing chemical, then mathematical techniques have been used to extrapolate the probability of cancer occurring in large populations. If, in an experiment where 100 animals per dose are exposed to the cancer-causing chemical Y, and the NOAEL (for cancer) for that experiment is $1\,mg\,kg^{-1}\,day^{-1}$, it is expected that, as stated above, an experiment using 1000 animals per dose would possibly produce a different, and lower, NOAEL. Mathematical techniques that establish a cancer potency for even smaller doses (it is assumed that even one molecule could cause a tumor) are used to extrapolate the dose that could possibly cause cancer in one out of one million exposed subjects. Using these techniques, the United States Environmental Protection Agency, realizing that small amounts of dioxins are found ubiquitously (especially in food), has established a 'virtually safe dose' for these chemicals. This dose is the dioxin level that is assumed to cause cancer in only one out of one million exposed persons. The caveat to the latter is that dose results from an upper bound estimate of cancer potency and the probability of cancer for the exposed population may be far less, or, in fact, zero.

See also: Dose–Response Relationship; Margin of Exposure (MOE); Risk Assessment, Ecological; Risk Assessment, Human Health; Risk Characterization.

Further Reading

Gaylor DW and Gold LS (1995) Quick estimate of the regulatory virtually safe dose based on the maximum tolerated dose for rodent bioassays. *Regulatory Toxicology and Pharmacology* 22: 57–63.

Gaylor DW and Gold LS (1998) Regulatory cancer risk assessment based on a quick estimate of a benchmark dose derived from the maximum tolerated dose. *Regulatory Toxicology and Pharmacology* 28: 222–225.

US EPA Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (TCDD) and Related Compounds. EPA/600/P-00/001Bg, September 2000. National Center for Environmental Assessment Office of Research and Development, US Environmental Protection Agency.

Vision *See* Sensory Organs.

Vitamin A

Allison A Muller

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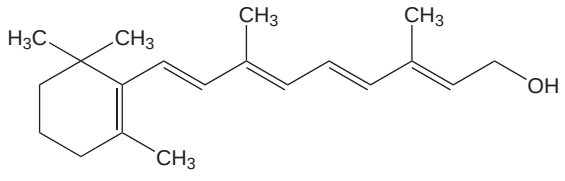
- Chemical Abstracts Service Registry Number: CAS 68-26-8
- Synonyms: Retinol; Retinyl esters; Antiinfective vitamin; 3,7-Dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonaretraen-1-ol
- Chemical/Pharmaceutical/Other Class: Fat-soluble vitamin, derived from retinol
- Chemical Formula: $C_{20}H_{30}O$
- Chemical Structure:



Uses

Vitamin A is used as a dietary supplement and for treatment of deficiency syndromes. It is not an endogenously produced vitamin; thus, it must be provided through dietary or vitamin supplement sources. Vitamin A is essential for normal vision in dim light. Furthermore, it is needed for regulation of all growth and development, for maintaining mucous membrane integrity, and for the reproductive process.

Exposure Routes and Pathways

Ingestion is the most common route of exposure. Available forms include capsules, tablets, topical preparations, and intramuscular solutions. Animal livers are rich in vitamin A.

Toxicokinetics

Vitamin A is readily absorbed from the intestine as retinyl esters. Peak serum levels are reached 4 h after ingestion of a therapeutic dose. The vitamin is distributed to the general circulation via the lymph and thoracic ducts. Ninety percent of vitamin A is stored in the liver, from which it is mobilized as the free alcohol, retinol. Ninety-five percent is carried bound to plasma proteins, the retinol-binding protein. Vitamin A undergoes hepatic metabolism as a first-order process. Vitamin A is excreted via the feces and urine. Beta carotene is converted to retinol in the wall of the small intestine. Retinol can be converted into retinoic acid and excreted into the bile and feces. The elimination half-life is ~ 9 h.

Mechanism of Toxicity

The exact mechanism leading to toxicity is not known. Both acute and chronic toxicity may occur. Daily vitamin A requirements range from 1500 international units (IU) to 4500 IU for children, 5000 IU for adults, and 5000 IU for pregnant women.

Acute and Short-Term Toxicity (or Exposure)

Human

Acute toxicity is uncommon in adults. However, very large exposures to vitamin A have resulted in the development of increased intracranial pressure (symptoms described include headache, dizziness, vomiting, visual changes, and bulging fontanel in infants).

Chronic Toxicity (or Exposure)

Human

Toxicity is more frequently seen with chronic ingestion of high doses of 30 000–50 000 IU day^{-1}. Children have developed acute toxicity following ingestion of 300 000 IU, but more frequently vitamin A toxicity in children develops following chronic ingestion of >10 times the recommended daily allowance for weeks to months. Malnutrition and individual tolerance may also be factors in predisposition to toxicity. Signs and symptoms of toxicity include vomiting, anorexia, agitation, fatigue, double vision, headache, bone pain, alopecia, skin lesions, increased intracranial pressure, and papilledema.

Clinical Management

In massive acute overdose, decontamination is advised. If the ingestion is recent (<30 min) and the patient is asymptomatic, syrup of ipecac is indicated. Activated charcoal may be used to adsorb vitamin A. Plasma vitamin A levels can aid in diagnosis but are not clinically useful in treatment. Upon discovery of a potential overdose, exposure to vitamin A should be immediately discontinued. Young children should be monitored for symptoms of increased intracranial pressure. Elevated intracranial pressure should be treated with mannitol, dexamethasone, and hyperventilation as needed. Vital signs and fluid and electrolyte status should be monitored closely. In general, vitamin A toxicity often resolves itself spontaneously within days to weeks following withdrawal of vitamin A. There are no known cases of vitamin A toxicity associated with beta carotene ingestion.

See also: Dietary Supplements; Liver.

Further Reading

Bendich A and Langseth L (1989) Safety of vitamin A. *American Journal of Clinical Nutrition* 49: 358–371.

Cleland JB and Southcott RV (1969) Illnesses following the eating of seal liver in Australian waters. *Medical Journal of Australia* 1: 760–763.

Hathcock JN, Hattan DG, and Jenkins MY (1990) Evaluation of vitamin A toxicity. *American Journal of Clinical Nutrition* 52: 183–202.

Vitamin B_1 *See* Thiamine.

Vitamin B_2 *See* Riboflavin.

Vitamin B_6 *See* Pyridoxine.

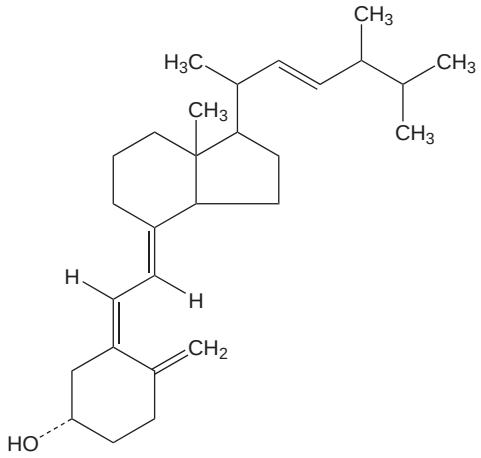
Vitamin B_9 *See* Folic Acid.

Vitamin C *See* Ascorbic Acid.

Vitamin D

Allison A Muller

- Chemical Abstracts Service Registry Number: CAS 50-14-6
- Synonyms: Ergocalciferol (D_2); Cholecalciferol (D_3); α-Calcidol; Calcitriol; Dihydrotachysterol (DHT)
- Chemical/Pharmaceutical/Other Class: Fat-soluble vitamin
- Chemical Structure:



Vitamin D_2

Uses

Vitamin D is a dietary supplement used for the prevention/treatment of deficiency syndromes. It is the only vitamin synthesized by the conversion of 7-dehydrocholesterol to cholecalciferol by exposure to sunlight or shortwave ultraviolet light.

Exposure Routes and Pathways

Ingestion of oral dosage forms is the most common route of exposure in both acute and chronic overdose.

Toxicokinetics

Vitamin D is readily absorbed from the gastrointestinal tract. Cholecalciferol is metabolized in the liver to 25-hydroxycholecalciferol and then to 1-α-25-dihydroxycalciferol in the kidney. This mobilizes stores of calcium from the bone matrix to the plasma. Cholecalciferol is stored in adipose and muscle tissue. The metabolites of vitamin D compounds are excreted primarily in bile and feces.

Mechanism of Toxicity

Excess vitamin D results in hypercalcemia and hypercalciuria, due to increased calcium absorption, bone demineralization, and hyperphosphatemia.

Acute and Short-Term Toxicity (or Exposure)

Animal

No reports of animal toxicity from vitamin D supplements could be found. However, vitamin D has proven fatal to animals when they were exposed to a vitamin D-containing rodenticide.

Human

Acute toxicity is rarely reported. Infants have reportedly tolerated up to 60 000–100 000 IU per kg without ill effect. Chronic toxicity occurs after the recommended daily allowance is excessively exceeded for weeks to months. Common symptoms of toxicity include nausea, flatulence, and diarrhea. Other nonspecific symptoms reported include muscle weakness, fatigue, and bone pain.

Renal failure may also occur due to precipitation of calcium in the kidneys. Vitamin D serum levels may be useful, as well as serum calcium, and alkaline phosphatase levels.

Clinical Management

Exposure to all forms of vitamin D should be stopped. Treatment should be supportive and symptomatic. Hypercalcemia treatment should include a low-calcium diet and prednisone as necessary.

See also: Dietary Supplements; Vitamin A; Vitamin E.

Further Reading

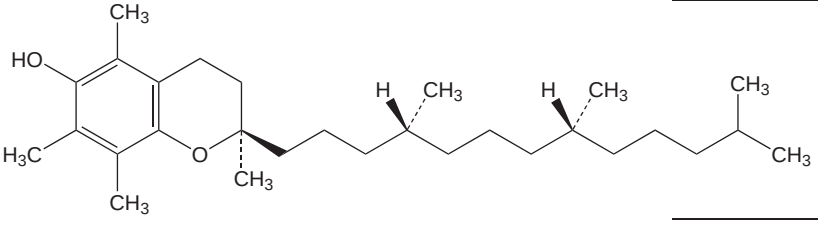
Down PF, Regan RJ, and Polak A (1979) A family with massive acute vitamin D intoxication. *Postgraduate Medical Journal* 55: 897–902.

Pettifor JM, Bikle DD, and Cavaleros M (1995) Serum levels of free 1,25-dihydroxyvitamin D in vitamin D toxicity. *Annals of Internal Medicine* 122: 511–513.

Vitamin E

Allison A Muller

- Chemical Abstracts Service Registry Number: CAS 59-02-9
- Synonyms: Antisterility vitamin; Almefrol; α-Tocopherol
- Chemical/Pharmaceutical/Other Class: Fat-soluble vitamin
- Chemical Formula: $C_{29}H_5O_2$
- Chemical Structure:



Uses

Vitamin E is used as a dietary supplement and for the treatment of deficiency syndromes.

Exposure Routes and Pathways

Ingestion as a supplemental vitamin is the most common route of exposure. Available forms include tablets, capsules, topical preparations, and intramuscular solutions.

Toxicokinetics

Vitamin E is absorbed in the gastrointestinal tract. Bile is necessary for absorption. Vitamin E is metabolized in the liver. The major metabolites of vitamin E are the glucuronides of tocopheronic acid. Vitamin E is distributed to all tissues. Lipid tissues store the vitamin for prolonged periods of time. Up to 30% is excreted in the urine. The half-life after intramuscular injection is ~45 h.

Mechanism of Toxicity

The exact mechanism of toxicity is unknown.

Chronic Toxicity (or Acute Exposure)

Human

The toxidrome of acute and chronic toxicities is no defined. Subjective symptoms include nausea, vomiting, flatulence, fatigue, muscle weakness, headaches, and blurred vision. Controversy exists over whether excessive vitamin E may cause liver and renal damage. The plasma concentration levels for vitamin E vary among individuals.

Clinical Management

Since there is no evidence that acute overdose represents a medical emergency, decontamination is not advised. Once chronic toxicity is suspected, discontinuation of vitamin usage and supportive/symptomatic therapy is recommended.

See also: Dietary Supplements.

Further Reading

Liede KE, Haukka JK, and Saxen LM (1998) Increased tendency towards gingival bleeding caused by joint effect of alpha-tocopherol supplementation and acetylsalicylic acid. *Annals of Medicine* 30: 542–546.

Meyers DG, Maloley PA, and Weeks D (1996) Safety of antioxidant vitamins. *Archives of Internal Medicine* 156(9): 925–935.

Vitamins *See* Vitamin A; Vitamin D; Vitamin E.

Volatile Organic Compounds (VOC)

S Satheesh Anand and Harihara M Mehendale

Background

Volatile organic compounds (VOCs) also referred to as solvents, are chemicals that evaporate easily at room temperature. VOCs are a class of liquid organic chemicals of variable lipophilicity and volatility. These properties, coupled with smaller molecular size and lack of charge, make inhalation the major route of exposure and provide ready absorption across the lungs, gastrointestinal (GI) tract, and skin. VOCs are frequently used to dissolve, dilute, or disperse materials that are insoluble in water. VOCs are classified according to molecular structure or functional group. These include aliphatic hydrocarbons (many of which are chlorinated – halocarbons), aromatic hydrocarbons, alcohols, ethers, esters, aldehydes, etc. Solvents may be utilized individually or as mixtures containing several ingredients. Most VOCs contribute in varying degrees to the formation of ground levels of ozone. This chapter is devoted to describe the uses, health effects, disposition, and factors affecting disposition and toxicity of VOCs in general. For detailed information, readers are advised to refer individual VOCs described elsewhere in this book.

Uses

Billions of pounds of VOCs are produced and utilized annually and there are hundreds of different VOCs used at present. VOCs are widely used as ingredients in household products including paints, paint strippers, varnishes, wax, cleaning, disinfecting, cosmetic, degreasing, aerosol sprays, cleansers, moth repellents, air fresheners, and automotive products. Drinking water is a common source of solvent exposure due to discharge of solvents from industries and household use and the presence of disinfection by-products, such as chloroform ($CHCl_3$), etc. Office equipment such as copiers and printers, correction fluids and carbonless copy paper, graphics and craft materials including glues and adhesives, permanent markers, and photographic solutions also contain some amount of VOCs. Since VOCs are solvents, they are widely employed as degreasers, and as intermediates in chemical synthesis, fuels, and fuel additives.

Environmental Contamination and Exposure

Widespread use of solvents has resulted in their dissemination throughout the environment. Almost everyone is exposed to VOCs, albeit in minute

amounts. VOCs enter the environment through evaporation. The volatilization occurs when the products containing them are used as intended and also during production, processing, storage, and transport activities. The presence of solvents in drinking water has become a major health concern for over three decades due to their potential carcinogenicity. The predominant VOCs present in water are $CHCl_3$ (water disinfection), trichloroethylene (TCE), and tetrachloroethylene (PERC).

People are exposed to solvents in environmental media by inhalation, ingestion, and skin contact. TCE, PERC, benzene, xylenes, and ethylebenzene are most frequently found in highest concentrations in air. Personal activities (smoking, visiting the dry cleaner's, or service station, etc.) and occupational exposures are believed to be largely responsible for relatively high exposures to other VOCs (benzene, toluene, xylene, PERC, etc.). Toluene and benzene are the most commonly found VOCs in indoor air, whereas $CHCl_3$ is most prevalent in water.

Toxicokinetics

The fundamental goal of toxicokinetics (TK) is to delineate the uptake and disposition of chemicals in the body and the subsequent toxicity. Gaining understanding of how the processes that govern VOCs kinetics vary with dose, species, and even different individuals would greatly reduce the number of assumptions that have to be made in the assessment of health risks from the toxicity data.

Absorption

The majority of systemic absorption of inhaled VOCs occurs in the deep part of the lungs, the alveoli, although some absorption has been demonstrated to occur in the upper respiratory tract. Blood:air partition coefficients (PCs) of VOCs are important determinants of the extent of their uptake. Increases in respiration and cardiac output/pulmonary blood flow enhance pulmonary absorption. The percent intake is initially high, but progressively declines as the VOC accumulates in tissues and the level in the blood returning to the lungs increases. A near steady state or equilibrium will soon be reached with inhalation of a fixed concentration of lipophilic solvents. In laboratory animals, VOCs are well absorbed from the GI tract. Peak blood levels are observed within minutes of oral dosing, although the presence of food in the GI tract can delay absorption. It is now assumed that 100% of an oral dose of most solvents is absorbed systematically. The vehicle or diluent in which a solvent is ingested can affect the absorption and TK of the compound. Administration of many VOCs in corn oil delays and prolongs the absorption when compared to aqueous ingestion. This is because of the 'depot' effect where VOCs are retained longer in the liphophilic vehicle (such as corn oil) before being absorbed. Solvents penetrate the stratum corneum, the skin's barrier to absorption, by passive diffusion. Important determinants of the rate of dermal absorption of solvents include the chemical concentration, surface area exposed, exposure duration, integrity and thickness of stratum corneum, lipophilicity, and molecular weight of the solvent.

Transport and Distribution

The VOCs absorbed from the GI tract largely enter the portal circulation and are subjected to first-pass elimination by the liver and lungs. The most volatile and well-metabolized VOCs are most efficiently eliminated before they enter the arterial blood. The efficiency of the hepatic first-pass elimination is thus dependant upon the chemical as well as the rate at which it arrives in the liver. Pulmonary first-pass elimination, in contrast, is believed to be a first-order process irrespective of the chemical concentration in the blood. VOCs are transported by the arterial blood and taken up according to tissue blood flow and mass and tissue:blood PCs. Hence, the extrahepatic organs receive a greater dose following inhalation exposure. This has been shown in rodents receiving the same systemic dose of CCl_4 by inhalation and gastric infusion. The solvents do not bind to plasma proteins or hemoglobin, but partition into hydrophobic sites in the molecules. Mostly, the partition occurs into phospholipids, lipoproteins, and cholesterol present in the blood. VOCs quickly accumulate in the brain after inhalation, producing in the central nervous system (CNS), effects as profound as those of surgical anesthesia in as little as 1–2 min. Adipose tissue accumulates relatively large amounts of VOCs. The pattern of ingestion of VOCs can significantly influence their TK and health effects. The toxic effects seen after administration of VOCs by gavage in acute, subchronic and chronic treatments were not observed when administered via drinking water. For example, $CHCl_3$ has produced hepatotoxicity and carcinogenecity when administered via oral gavage, however, the same dose did not cause any effects when exposed via drinking water except in Wistar and Osborne–Mendel rats.

The rate of systemic elimination of different solvents varies considerably. The two major routes of systemic elimination are metabolism and exhalation. The rate and extent of metabolic clearance are dose- and compound-dependent. Exhalation is

determined largely by the rate of pulmonary blood flow, the chemical's air:blood PC and the alveolar ventilation rate. The more volatile, lipophilic VOCs are exhaled most readily, since they have higher air:blood PCs. Body fat plays an important role in the elimination of lipophilic solvents. Body fat increases the volume of distribution and total body burden of the solvents. Deequilibration from adipose tissue is prolonged due to slow blood flow and high fat:blood PC.

Metabolism

Biotransformation plays a key role in modulating the toxicities of VOCs. Many VOCs are poorly soluble in water and the metabolism converts them to relatively water-soluble derivatives, which may be more readily eliminated in the largely aqueous urine and/or bile. Metabolism either produces inactive metabolite from the parent compound (detoxification) e.g., toluene metabolized to inactive hydroxyl and carboxyl metabolites or produces active metabolite (bioactivation), for example, benzene is oxidized to toxic quinone metabolites. VOCs undergo multiple metabolic pathways to products of varying toxicity. A variety of factors can influence the prominence of the different pathways and hence alter toxic outcomes. Biotransformation of VOCs is catalyzed largely by cytochrome P450 s in liver. Different isozymes can predominate at different doses of the same chemical, for example, TCE and $CHCl_3$ are primarily metabolized by CYP2E1 at low doses, whereas CYP2B1/2 showed to involve at high doses. CYP2E1 is primarily responsible for oxidation of some 16 halogenated and aromatic hydrocarbons. Phase II enzymes such as glutathione-*S*-transferase (GST) involve mostly in the detoxification of VOCs.

Toxicity

The ability of organic chemicals to cause health effects varies greatly from those that are highly toxic, to those with no known adverse health effects. Eye and respiratory tract irritation, headaches, dizziness, visual disorders, and memory impairment are among the immediate symptoms that some people have experienced soon after exposure to some organics. Many organic compounds are known to cause cancer in animals; some are suspected of causing, or are known to cause cancer in humans. The main determinants of VOCs inherent toxicity are: (1) number of carbon atoms; (2) saturated or unsaturated; (3) configuration, that is, straight-chain, branched-chain, or cyclic; (4) presence of functional group; and (5) level of exposure and length of exposure time. Some class-wise generalizations regarding toxicity can be made. For example, amides/amines tend to be potent sensitizers; aldehydes are particularly irritating; hydrocarbons tend to be cytogenic/mutagenic and many unsaturated, short-chain halocarbons are animal carcinogens. The toxicity of VOCs within the same class may vary dramatically. For example, trichloroethane and TCE are both hydrocarbons, yet the latter is an animal carcinogen, but the former is not.

Central Nervous System toxicity

One of the common physiological effects which is associated with high levels of exposure to VOCs is depression of the CNS activity. VOCs have the capacity to cause general anesthetic effects and may ultimately result in unconsciousness, or death, as the most severe consequence. CNS effects due to VOCs increase with an increase in carbon length, double bonds, halogen substitution and functional groups as these increase the lipophilicity. For example, $CHCl_3$ is more potent than methylene chloride and CCl_4 is the most potent in terms of anesthetic effects. Methanol and ethanol are more potent CNS depressants than methane and ethane.

Membrane and Tissue Irritation

Another important adverse effect with VOCs is potential for membrane and tissue irritation. Because membranes are composed principally of a protein–lipid matrix, VOCs at sufficient concentrations may act to dissolve that matrix or extract the fat or lipid components of the membrane. This defatting process, when applied to the skin, may cause irritation and cell damage and by similar processes, may seriously injure the lungs or eyes. Upon accidental or intentional ingestion, irritation in lungs caused by aspiration predisposes lungs from microbial infection to life threatening clinical pneumonias.

Carcinogenicity

Carcinogenicity by VOCs is a paramount public health concern because most of the VOCs are rodent carcinogens and some of them are known to cause cancer in humans. Benzene and vinyl chloride are known human carcinogens under some intense exposure conditions. The carcinogenesis is attributed to the metabolites of these VOCs. Several other chlorinated VOCs (CCl_4, $CHCl_3$, TCE, PERC, etc.) exhibit varying degrees of carcinogenic potential, notably hepatic tumors in animals. Except for leukemogenic effects from extreme benzene exposure and hepatic angiosarcoma in vinyl chloride workers,

no unequivocal human reports are available that document cancer hazard from exposure to VOCs. However, there are a number of epidemiologic observations regarding cancer and exposure to VOCs such as TCE, $CHCl_3$, PERC, etc. Based on the animal studies or the epidemiological reports, VOCs are classified into the following classes: known carcinogen, probable carcinogen, possible carcinogen, unclassifiable carcinogen, and unlikely carcinogen. Determining the human relevance of tumors observed in high-dose rodent studies is the major challenge. Like other chemicals, the toxicity of VOCs depends on several factors: (1) exposure route; (2) amount or rate of exposure; (3) duration of exposure; (4) individual susceptibility; and (5) interaction with other chemicals.

Other Toxic Effects

Apart from CNS effects, hepato-, nephro-, and cardiotoxicity are caused by VOCs. These effects have been reported in animals following acute and chronic exposures at high doses. However, these effects have been rarely reported in humans in occupational or environmental circumstances.

Solvent Abuse

Many solvents are intentionally inhaled in order to achieve a state of intoxication. Solvent inhalation can rapidly produce euphoria, delusions, and sedation as well as visual and auditory hallucinations. Solvent abuse is a unique exposure situation in that participants repeatedly subject themselves to vapor concentrations high enough to produce effects as extreme as unconsciousness. Solvents can be addicting and are often abused in combination with other drugs. Various solvents are present in a wide variety of household and commercial products, which are relatively inexpensive and readily available to children and adolescents. Nearly all hydrocarbon solvents cause CNS depression, but residual organ damage is chemical-dependent. There is concern that chronic solvent abuse can lead to long-term neurologic and psychological sequelae. Some solvents, such as n-hexane, and methyl-n-butyl ketone, cause peripheral neuropathies. Chronic abuse of VOCs such as toluene, which was previously thought to be innocuous, may be responsible for diffuse cerebral and cerebrallar atrophy. Blood dyscrasias, liver damage, kidney injury, and other toxicities are seen in patients who have abused VOCs known to be injurious to those organs. Death, frequently due to arrhythmogenic effects of high concentration of some VOCs (e.g., 1,1,1-trichloroethane, benzene), is sometimes a consequence of solvent abuse.

Factors Affecting Toxicity of VOCs

A number of factors alter the disposition of VOCs and their toxicity. Following is a brief account of such factors:

Age

Systematic data are lacking on age-dependent susceptibility of humans to solvents. The younger and more immature the subject is, the more different its response from that of an adult. There may be developmental periods or 'windows of vulnerability' when the endocrine, reproductive, immune, nervous, and other organ systems are particularly sensitive to certain chemicals. Maturational changes may also substantially affect the kinetics and ensuing toxicity of solvents and other agents. Systemic absorption of inhaled VOCs may be higher in infants and children than adults because of greater cardiac output and respiratory rates, reduced plasma binding, and increased fat content. Poisonings of premature and newborns exposed to benzyl alcohol, hexachlorobenzene, etc. are attributable to initial deficits in metabolic conjugation capacity. It is generally believed that liver P450 activities are greater in infants and children than in adults. A higher P450 would mean that infants and children are either susceptible or resistant depending upon the VOC. Susceptibility of immature subjects may be age-, organ-, chemical-, and species-dependent.

In the elderly, body fat usually increases substantially at the expense of lean mass and body water. Relatively lipid-soluble solvents accumulate in adipose tissue and slowly released to sites of action, metabolism, and elimination. Diminished cardiac output and renal and hepatic blood flows might affect the disposition of VOCs. There are contradictory reports on age-dependent susceptibility to liver damage. While some reports show that aged rats are more vulnerable to CCl_4 and allyl alcohol toxicity, a recent report shows that aged rats are able to mount higher liver regeneration and survive otherwise a lethal combination of chlordecone plus CCl_4 hepatotoxicity. Other major sources of variability and complexity in responses of geriatric populations to solvents include inadequate nutrition, the prevalence of disease states, and the concurrent use of multiple medications.

Gender

Women may differ from men in their responses to solvents, but the differences do not appear to be too

great. Levels of toluene and TCE in expired air are lower in females, reflecting more fat deposition. The major gender differences in P450-mediated metabolism in rats are not seen in humans or most other mammals. Relatively little is known about potential influences of contraceptive, hormone replacement therapy, or pregnancy on the metabolism of VOCs.

Genetics

A variety of genetic polymorphisms for biotransformation have been found to occur at different frequencies in different ethnic groups. Polymorphisms for xenobiotic metabolizing enzymes may affect the quantity and quality of enzymes and the outcomes of exposures to solvents. It is difficult to separate the influences of genetic traits from those of different lifestyles, socioeconomic status, and geographic settings. Ethnic differences in P450 enzyme expression and phase II biotransformation reactions such as GST appears to be associated with variations in VOCs metabolism. The Caucasians have higher total P450 levels and CYP2E1 activities than the Japanese. Some individuals with a null/null genotype for GST-theta lack the ability to conjugate and detoxify metabolites of methyl chloride and methylene chloride. The prevalence of this genotype ranges from 10% in Mexican Americans to 60–65% in Chinese and Koreans. Increased susceptibility to different cancers has been reported to be associated with certain genetic polymorphisms, which occur with different frequencies in different ethnic groups. Significant variations in allelic distributions for isoenzymes including CYP2E1, 2D6, 1A1, and GSTM1 have been observed in different racial groups.

Cytochrome P450 Inducers

Preexposure to chemicals that induce CYP2E1 and/or CYP2B1/2 can potentiate the toxicity of high doses of solvents that undergo metabolic activation. Induction of these enzymes may be of little consequence for low doses of well-metabolized solvents. Ethanol, acetone, ketones, and nicotine are some of the P450 inducers, which alter the PK of VOCs. Many P450 inducers also alter the inducers of detoxifying enzymes.

VOCs are also capable of inducing P450s and alter their, and/or other VOCs', metabolism. For example, repeated exposure to styrene, increased metabolic clearance, and isopropanol has been shown to potentiate CCl_4 hepatotoxicity.

Cytochrome P450 Inhibitors

A number of compounds inhibit P450s and these compounds are expected to enhance the toxicity of solvents that are metabolically inactivated. Conversely, these compounds should protect solvents that undergo metabolic activation. Compounds such as diallyl sulfide inhibit CYP2E1 and protect animals from some carcinogens. Some solvents such as CCl_4, in the course of their own metabolism, destroy P450s and subsequently decrease their metabolism. A reduction of the biotransformation in humans may occur due to metabolic competition between two solvents. Concurrent exposure to ethylene benzene and *m*-xylene and benzene and toluene in humans caused mutual metabolic inhibition.

Physical Activity

Exercise increases two of the major determinants of VOC uptake, alveolar ventilation, and cardiac output/pulmonary blood flow. Blood flow to liver and kidney is diminished with exercise, so biotranformation of well-metabolized solvents may be decreased.

Diet

Dietary habits can influence the TK and toxicity of solvents in several ways. The mere bulk of food in the stomach and intestine can inhibit systemic absorption of VOCs. Solvents in the GI tract partition into dietary lipids, largely remaining there until the lipids are emulsified and digested. This substantially delays the absorption of VOCs such as CCl_4 and its hepatotoxicity. Increased incidences of cancer have been observed in obese humans possibly due to increase in liver CYP2E1 by ketone body formation. Caloric restriction has clearly been shown to reduce the incidence of cancer. Fasting results in increased P450 activities and reduced GSH, which affect the TK and toxicity of VOCs. Food may contain certain natural constituents, pesticides, and other chemicals, which may enhance or reduce the solvent metabolism.

Diseases

Illness can be a major source of variability in response to solvents. Impaired metabolism and clearance are commonly seen in patients with cirrhosis and hepatitis. Lower levels of CYP2E1, 1A2, and GSH are seen in livers of patients with cirrhosis. The CYP2E1 and 2B1 levels were reported to be higher in a diabetic condition. Thus, diabetes may predispose individuals to increased risk of cell injury by solvents, which undergo metabolic activation. However, the final outcome of tissue injury depends upon whether or to what extent repair of injured tissue may occur. After exposure to high doses of VOCs, tissue repair is known to be inhibited causing continued progression of injury leading to organ failure, regardless of

whether initial injury is low or high. Persons with bacterial infection may be more sensitive to cytotoxic actions of solvents. Exposure of animals to small amounts of endotoxins potentiates liver injury by CCl_4 and other VOCs.

Ethanol

Depending upon the size and time of the dose, ethanol may have two opposing effect on the biotransformation of VOCs. Intake of ethanol (short-term) in moderate amounts has a marked inhibitory effect on the biotransformation of several VOCs such as toluene, TCE, styrene, and *m*-xylene. However, chronic ingestion of alcohol induces the liver P450s.

Drugs

Drugs may affect the TK of VOCs by changing pulmonary as well as peripheral blood flow or by inhibiting or stimulating metabolism. The intake of acetaminophen increased the concentration of toluene concentration in volunteers and acetylsalicylic acid decreases the transformation of *m*-xylene. The extent of protein binding may also be affected by drugs.

Metals

Metals can inhibit the hepatic enzyme system and affect the metabolism of VOCs. Rats pretreated with zinc chloride decreased CYP450 and protected CCl_4 liver damage. The effects of exposure to metals on the metabolism of solvents are more pronounced in acute situations than chronic at high doses.

Risk Assessment

VOCs are found in everything from paints and coatings to underarm deodorant and cleaning fluids. The work environment is typically where the highest exposures occur, mainly via inhalation and dermal contact. An estimated 10 million people are potentially exposed to organic solvents in the workplace. The effects ranging from CNS toxicity to carcinogenicity of VOCs have been reported on animals and humans. Regulatory agencies such as the Environmental Protection Agency, Agency for Toxic Substances and Disease Registry, Occupational Safety and Health Organization, etc. use the results of these studies to determine health advisory levels and set limits on the amount of each VOC that is considered safe for human exposure. Health advisory levels are based on a "no-effect level." The no-effect level is the maximum VOC dose that does not produce a known toxic effect in experiments and is further reduced by an additional safety factors for uncertainties such as high to low dose and animal to human extrapolation.

The current risk assessment practices for individual VOCs are generally considered to be protective of potentially vulnerable subgroups, because of the conservative default assumptions. However, most solvent exposures involve a mixture of chemicals, rather than single compounds. Our knowledge of the toxicity of solvent mixtures is rudimentary relative to the toxicology of individual solvents. While the assumption is frequently made that the toxic effects of multiple solvents are additive, solvents may also interact synergistically or antagonistically. The significant data gap that exists in the toxicity of solvent mixtures can preclude accurate risk assessments.

Conclusions

Nearly everyone is exposed to VOCs in the conduct of their normal daily activities due to their wide spread use. Hence, health effects of VOCs have been extensively studied. The common toxicological effects are CNS effects, irritation, hepato- and nephrotoxicity, and carcinogenecity. The health effects of VOCs vary greatly according to the compound, the level of exposure, and the length of exposure. Most studies to date have been conducted on single chemicals and less is known about the health effects of combined exposure of VOCs. Since human exposure often involve multiple VOCs, additional research is needed to characterize the potential health effects associated with mixed exposure.

See also: Benzene; Chloroform; Toluene; Trichloroethylene.

Further Reading

Anand SS and Mehendale HM (2004) Liver regeneration: Critical toxicodynamic response in predictive toxicology. *Environmental Toxicology and Pharmacology* 18: 149–160.

Bruckner JV and Warren DA (2001) Toxic effects of solvents and vapors. In: Klassen CD (ed.) *Casarett and Doull's Toxicology: The Basic Science of Poisonings*, 6th edn. New York: McGraw-Hill.

Lof A and Johanson G (1998) Toxicokinetics of organic solvents: A review of modifying factors. *Critical Reviews in Toxicology* 28: 571–650.

Soni MG and Mehendale HM (1998) Role of tissue repair in toxicologic interactions among hepatotoxic organics. *Environmental Health Perspectives* 106(Suppl. 6): 1307–1317.

Vomiting Agents *See* Arsenical Vomiting Agents.

V-Series Nerve Agents: Other than VX

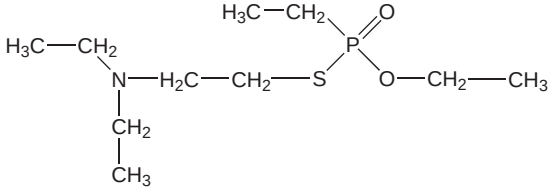
Harry Salem*

Published by Elsevier Inc.

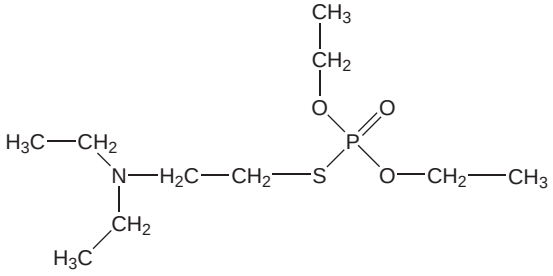
The V-series of nerve agents are less volatile than the G-series but are better able to penetrate the skin. Although they are an inhalation hazard when they are in the air as vapor or aerosol, they are considered more of a percutaneous hazard. This series contains VX, VE, VG (Amiton), VM, VR (RVX, Russian VX), and VS. The V designation is considered to be derived from Victory, Venemous, or Viscous.

Other V-Series Organophosphate Nerve Agents

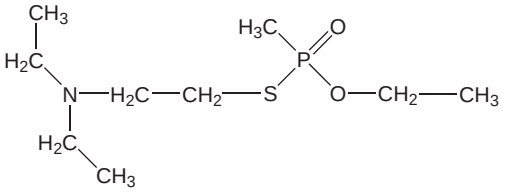
VE – Phosphonothioic acid, ethyl-, *S*-[2-(diethylamino)ethyl]*O*-ethyl ester



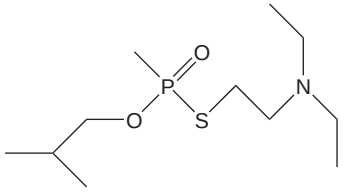
VG (Amiton) – Phosphorothioic acid, *S*-[2-(diethylamino)ethyl]*O*,*O*-diethyl ester



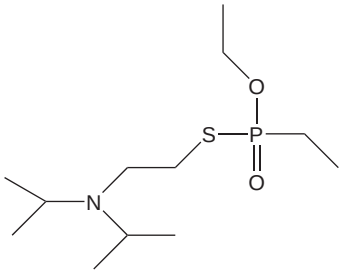
VM – Phosphonothioic acid, ethyl-, *S*-[2-(diethylamino)ethyl]*O*-ethyl ester



VR (RVX, Russian VX) – Phosphonothioic acid, methyl-, *S*-[2-(diethylamino)ethyl]*O*-(2-methylpropyl) ester



VS – Phosphonothioic acid, methyl-, *S*-[2-(diethylamino)ethyl]*O*-(2-methylpropyl) ester



See also: G-Series Nerve Agents; Nerve Agents; Sarin; Soman; Tabun; VX.

Relevant Websites

http://www.bt.cdc.gov – (US) Centers for Disease Control and Prevention, Agency for Toxic Substances and Disease Registry.

http://sis.nlm.nih.gov – (US) National Library of Medicine, Specialized Information Services, Chemical Warfare Agents.

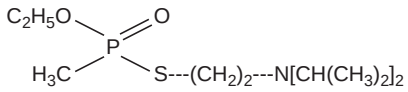
* The views of the author do not purport to reflect the position of the US Department of Defense. The use of trade names does not constitute official endorsement or approval of the use of such commercial products.

VX

Harry Salem and Frederick R Sidell*

Published by Elsevier Inc.

- Chemical Abstracts Service Registry Numbers: CAS 50782-69-9; CAS 51848-47-6; CAS 53800-40-1; CAS 70938-84-0
- Synonyms: Phosphonothioic acid; Methyl-*S*-(2-bis(1-methyl))phosphonothioate; *S*-2-Diisopropylaminoethyl-*O*-ethyl methylphosphonothioate; *S*-2(2-Diisopropylaminoethyl)-*O*-ethyl methylphosphonothioate; *O*-Ethyl-*S*-(2-diisopropylaminoethyl) methylthiolphosphonoate; TX60; Nerve gas; Nerve agent
- Chemical/Pharmaceutical/Other Class: Persistent anticholinesterase compound or sulfonated organophosphorus (OP) nerve agent
- Chemical Formula: $C_{11}H_{26}NO_2PS$
- Chemical Structure:



Uses

VX is a human-made nerve agent used in chemical warfare

Exposure Routes and Pathways

Casualties are caused both by inhalation and by dermal contact. Since VX is an oily liquid with low volatility, liquid droplets on the skin do not evaporate quickly, thus facilitating effective percutaneous absorption. Clothing can release VX for about 30 min after contact with VX vapor, which can lead to the exposure of other people. In addition to inhalation and percutaneous exposure, casualties can also be caused by ocular exposure, ingestion, and injection. Although VX does not mix with water as easily as nerve agents do, it could be released into water and lead to exposures via drinking contaminated water or dermal contact with contaminated water. People can also be exposed by eating food contaminated with VX.

VX is the least volatile of the nerve agents, and can be very persistent in the environment. VX can last for days on contaminated objects and can last for months under very cold conditions. Thus, surfaces contaminated with VX can be a long-term hazard.

*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.

Toxicokinetics

VX is absorbed through the skin and respiratory system. Because it is nonvolatile, it may remain in place for weeks after dispersion and cause casualties. Thus, it is classified as a persistent agent. Although VX does not pose a major inhalation hazard in usual circumstances, by the inhalation route it is estimated to be 10 times as toxic as sarin. It is hydrolyzed by the enzyme organophosphorus (OP) hydrolase.

Mechanism of Toxicity

VX and the other nerve agents are irreversible OP cholinesterase inhibitors. They inhibit the enzymes butyrylcholinesterase in the plasma, the acetylcholinesterase on the red blood cell, and the 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. 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 the nerve agent to the enzyme is considered irreversible unless removed by therapy. The accumulation of acetylcholine in the peripheral nervous system and central nervous system (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 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 rate of return of these enzymes is 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 CNS.

Recently, 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 over stimulation of the cholinergic system or could result from direct action of nerve agent on the enzymes responsible for noncholinergic neurotransmission.

Human Toxicity

Following inhalation of VX, the median lethal dosage (LCt_{50}) in man has been estimated to be 30 mg min m^{-3} at a respiratory minute volume (RMV) of 15 l min^{-1}. Following percutaneous exposure, the LD_{50} was estimated to be 0.315 mg kg^{-1} or 10 mg per 70 kg in humans. The intravenous LD_{50} was estimated to be 0.008 mg kg^{-1} or 0.56 mg per 70 kg humans, and the intramuscular LD_{50} 0.012 mg kg^{-1} or 0.84 mg per 70 kg humans.

The doses that are potentially life-threatening may only be slightly larger than those producing minimal effects. The ECt_{50} for miosis from ocular vapor exposure was estimated to be < 0.09 mg min m^{-3}, and the ECt_{50} for runny nose is also estimated to be < 0.09 mg min m^{-3}. For severe incapacitation for vapor inhalation, the ICt_{50} was estimated to be 25 mg min m^{-3}, while the LCt_{50} was estimated to be 30 mg min m^{-3}.

The permissible airborne exposure concentration for VX for an 8 h workday of a 40 h workweek is an 8 h time-weighted average (TWA) of 0.00001 mg m^{-3}.

These signs and symptoms occur within minutes or hours following exposures. The signs and symptoms following vapor exposure include miosis and visual disturbances, headache and pressure sensation, runny nose and nasal congestion, salivation, tightness in the chest, nausea, vomiting, giddiness, anxiety, difficulty in thinking, difficulty sleeping, nightmares, muscle twitching, tremors, weakness, abdominal cramps, diarrhea, and involuntary urination and defecation. These signs and symptoms may progress to convulsions and respiratory failure. After liquid exposure on the skin, the initial effects are nausea, vomiting, and diarrhea, followed by muscular weakness, seizure, and apnea.

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 intravenously (iv) or intramuscularly (im). 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. This is most apparent in organs with nicotinic receptors. Abnormal activity 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). This may be repeated two or three times at hourly intervals (intravenous or intramuscular). 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.

Supportive therapy may include ventilation via an endotracheal airway if possible and suctioning of excess secretions in the airways.

Animal Toxicity

Small doses of nerve agents in animals can produce tolerance in addition to their classical cholinergic effects. In rats, acute administration of nerve agents in subconvulsive doses produced tumors and hindlimb abduction. In animals nerve agents can also cause effects in behavior, analgesia, 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.

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 are indication 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 organophosphate intoxication.

The available animal toxicity data are presented as follows:

VX animal toxicity

- Subcutaneous LD_{50} ($mg\,kg^{-1}$)
  - Rat 12
  - Mouse 22
  - Rabbit 14
  - Guinea pig 8400
- Intraperitoneal LD_{50} ($mg\,kg^{-1}$)
  - Mouse 50
  - Rabbit 66
- Intramuscular LD_{50} ($mg\,kg^{-1}$)
  - Chicken 30
- Intravenous LD_{50} ($mg\,kg^{-1}$)
  - Cat 5

See also: G-Series Nerve Agents; Nerve Agents; V-Series Nerve Agents: Other than 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.

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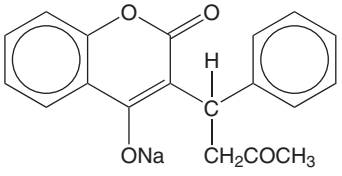
Warfarin

Henry A Spiller

This article is a revision of the previous print edition article by Michael J Hodgman, volume 3, pp. 411–412, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 81-81-2
- Synonyms: Courmadin; Hydroxycoumarin
- Chemical/Pharmaceutical/Other Class: A synthetic derivative of 4-hydroxycoumarin, the hemorrhagic component of sweet clover
- Chemical Formula: $C_{19}H_{16}O_4$
- Chemical Structure:



Uses

Warfarin is used therapeutically as an anticoagulant. It is also used as a rodenticide.

Background Information

In 1921, during a particularly wet year a large number of cattle died from a hemorrhagic disorder. Investigation by the Wisconsin Alumni Research Foundation (WARF) eventually identified the cause of the disorder in a substance found in the rotting sweet clover used as feed by the cattle. The name warfarin is a combination of the acronym for the original patent holder (WARF) and the suffix from coumarin.

Exposure Routes and Pathways

Ingestion is the most common route of exposure. Warfarin is also absorbed transdermally and by inhalation. It is available in oral and injectable forms. Warfarin rodenticides are typically 0.025–0.050% warfarin by weight.

Toxicokinetics

Warfarin is rapidly and nearly completely absorbed by the oral route. Peak plasma levels typically occur within 2–8 h. Warfarin is highly protein bound: 97–99%. The volume of distribution approximates $0.15\,l\,kg^{-1}$. Warfarin is extensively metabolized by hepatic microsomal enzymes. The primary metabolites are 6- and 7-hydroxy warfarin via oxidation and several warfarin alcohols via reduction. The warfarin alcohols retain weak anticoagulant activity. The metabolites undergo enterohepatic circulation. Approximately 85% of warfarin appears in the urine as metabolites. Less than 1% or 2% appears in the urine unchanged. Warfarin metabolites are also excreted in the stool. The plasma half-life varies widely, from 10 to 80 h; it is typically 36–44 h. The duration of clinical effects can significantly exceed the half-life of warfarin. (Note: There are many drug interactions with warfarin; the reader is referred to a standard pharmacology text for further details.)

Mechanism of Toxicity

Warfarin interferes with the hepatic production of a number of proteins involved in hemostasis. These include the coagulation factors II (prothrombin), VII, IX, and X and also proteins C and S, important modulators of coagulation. Vitamin K is a cofactor for the carboxylation of specific glutamic acid groups in these proteins. This is the final phase in activation of these clotting factors. During carboxylation, vitamin K is oxidized to vitamin K 2,3-epoxide. The cyclical regeneration of vitamin K by vitamin K epoxide reductase allows production to continue. However this step is antagonized by warfarin. As a result, vitamin K stores are depleted and vitamin K is unavailable during the carboxylation phase of the coagulation factor activation. Dysfunctional decarboxycoagulation factors are produced and overall synthesis may be reduced. This leads to impaired coagulation.

Acute and Short-Term Toxicity (or Exposure)

Animal

Mammals and birds vary in their sensitivity to warfarin. Horses are resistant to the coumarins and cats are more sensitive than dogs. Signs of toxicity in animals include anorexia, weakness, vomiting, diarrhea, bleeding, and dyspnea. Toxic effects can be monitored by measurement of the prothrombin time (PT) or one-stage PT. Treatment is as for humans. The recommended dose of vitamin K for dogs and cats is 0.25–1 mg kg^{-1} day^{-1} for 5–14 days. There is minimal to no toxicity in aquatic organisms.

Human

Depletion of preformed circulating coagulation factors must occur before any effect by warfarin is apparent. Factor VII has the shortest half-life (4–6 h) and factor II the longest (60 h) of the vitamin K-dependent coagulation factors. The PT may begin to increase by 24 h and be maximal 36–72 h postingestion. Significant toxicity from single-dose ingestion is uncommon; most instances of toxicity are the result of repeated ingestion over time. The most frequent sites of bleeding are mucocutaneous, genitourinary, and gastrointestinal, although bleeding can occur virtually anywhere. Reported effects secondary to overcoagulation are cardiac tamponade, pulmonary hemorrhage, hemothorax, intracranial hemorrhage, gastrointestinal bleeding with hemocult positive stools, lower back/flank pain, hematuria, and retinal hemorrhage.

The more serious events include massive hemorrhage with shock, intracranial bleeding and stroke, and pericardial tamponade. Plasma warfarin levels are not routinely done. The effect of warfarin is best followed by the PT and International Normalized Ratio (INR). Under therapeutic conditions the INR is maintained at 2.0–3.0, except for prophylaxis after artificial heart valve replacement when it may be 2.0–3.5. Specific assays of factor activity can be measured although this is not usually necessary.

Chronic Toxicity (or Exposure)

Animal

Warfarin is used as a rodenticide. Rats exposed chronically to warfarin develop bleeding and death over time. Birds are relatively resistant to the effects of warfarin. Leghorns exposed over 15 days developed no signs of toxic effects. Some birds need to eat half their body weight with feed containing 0.1% warfarin per kg feed in order to develop anticoagulant effects.

Human

Effects other than those outlined previously have not been described. Warfarin use has been associated with birth defects and spontaneous abortion. Toxicity is more likely to occur and at lower doses with chronic exposure.

In Vitro Toxicity Data

Warfarin has been shown to have no impact on Mtln3 rat mammary carcinoma cell growth *in vitro* at concentrations less than 1 mmol l^{-1}.

Clinical Management

For acute single unintentional ingestions, observation at home with poison center follow-up may be acceptable. For substantial recent ingestions, activated charcoal should be administered. Induced emesis should be avoided in the anticoagulated patient as it may cause an increase in intracranial pressure and potentiate a vascular accident. The PT should be monitored for at least the first 48 h for signs of toxicity. Extreme caution should be used with any invasive procedure in the anticoagulated patient. The airway should be protected if compromised. Volume resuscitation should be provided as indicated by clinical status. With active, uncontrolled, or life-threatening hemorrhage, fresh frozen plasma should be administered to provide preformed clotting factors (at least 4–6 units will be necessary in an adult).

Vitamin K_1 (phytonadione) is a specific antidote for warfarin toxicity. In cases with no active bleeding and an INR < 5.0 withdrawal of the warfarin may be sufficient. Pharmacologic doses of vitamin K antagonize the inhibitory effect of warfarin on clotting factor production. The dose and route of vitamin K administration depends on the clinical setting. For rapid reversal, 5–25 mg should be administered intravenously no faster than 1 mg min^{-1}. In children, 0.6 mg kg^{-1} should be used. Clinical effects may be seen within hours. The response and duration of a single dose of vitamin K is variable and dependent on the severity of the toxicity. The half-life of vitamin K is less than 4 h and repeat doses may be necessary. In less acute settings, vitamin K may be given subcutaneously or orally. The PT should be monitored to follow toxicity and response to treatment. Anaphylaxis has been reported with intravenous vitamin K. Vitamin K_3 (menadione) is not effective therapy. In patients therapeutically anticoagulated, rapid reversal can be dangerous and should be done with caution.

Environmental Fate

No information is currently available on breakdown in soil groundwater or surface water.

Exposure Standards and Guidelines

Threshold limit value time-weighted average, $0.1\,mg\,m^{-3}$ (Occupational Safety and Health Administration); short-term exposure limit, $0.3\,mg\,m^{-3}$.

See also: Blood.

Further Reading

Chai SJ and Macik BG (2002) Improving the safety of warfarin. *Seminars in Hematology* 39(3): 179–186.

Montanio CD, Wruk KM, and Kulig KW (1993) Acute pediatric warfarin (coumadin) ingestion: Toxic effects despite early treatment. *American Journal of Diseases of Children* 147: 609–610.

Wasp *See* Hymenoptera.

Water Pollution *See* Pollution, Water.

Wildlife Toxicology *See* Ecotoxicology, Wildlife.

Wisteria

Ann P Slattery

- Synonyms: *Wisteria floribunda* (Japanese wisteria); *Wisteria sinensis* (Chinese wisteria)

Uses

Wisteria is a woody vine or climbing shrub of North America and Eastern Asia. This invasive vine may grow 30 ft or more only limited by the structure on which it is allowed to grow. The pink, white, or blue fragrant flowers bloom in clusters. The seeds are contained in pea-shaped flat pods. Each pod contains three to five seeds.

Exposure Routes and Pathways

Ingestion of seeds, pods, and flowers is the primary route of exposure. Symptoms have developed following exposure to the smoke of this plant when burned.

Mechanism of Toxicity

Although toxins are identified (wistarine and lectin), clear information about their behavior does not exist. As a saponin-containing compound, it is classified as a gastrointestinal irritant.

Acute and Short-Term Toxicity (or Exposure)

Human

All parts of the wisteria plant are considered toxic, especially the pods and seeds. Although serious poisonings are not common, exposures to as few as two seeds have been known to result in serious effects. Symptoms include oral burning, stomach pain, diarrhea, and vomiting. Gastrointestinal symptoms may appear in 1.5–3.5 h. Confusion, syncope, vertigo, and weakness have been described. Increased white blood cells have also been documented.

Symptoms usually resolve within 24–48 h, but one case reported persistent weakness and vertigo lasting 5–7 days. The mitogenic and blood clotting effects of lectins are not seen in toxic exposures. Exposure to smoke from the burning of this plant is known to cause headaches.

Clinical Management

Initial treatment with gastric lavage is indicated. Support with fluid replacement and antiemetics may be indicated.

See also: Plants, Poisonous.

Further Reading

Rondeau ES (1993) Wisteria toxicity. *Clinical Toxicology* 31(1): 107–112.

Wood Dusts

Alan J Weinrich and Paul Demers

Published by Elsevier Inc.

Characteristics

Tree species are classified either as gymnosperms, which generally have needle-like leaves, or as angiosperms, which generally have broad leaves and are deciduous in temperate climates. In practice, trees usually are classified as softwoods, hardwoods, or tropical woods. Softwoods include the temperate gymnosperms or conifers; hardwoods include the temperate angiosperms; and tropical hardwoods primarily are angiosperms, but also include some gymnosperms that thrive in tropical climates.

Wood is composed primarily of cellulose, hemicellulose, and lignin. In addition to these basic components, wood also contains many organic compounds, known as 'wood extractives'. Wood extractives serve to protect trees from bacteria, fungi, and other potentially harmful agents. They also provide grain and color to the wood. These extractives typically make up 5–30% of the wood mass. Softwoods and hardwoods differ in cellular structure as well as chemical composition and there is great variability between species. Biologically active chemicals found in wood include terpenes, lignans, stilbenes, tannins, flavinoids, and quinines.

Human Exposures

Wood dust exposure is common in many types of work. The greatest exposures occur in secondary wood industries, such as furniture and cabinet manufacturing, wood pattern and model shops, and other manufacturing industries. Finished products are created from dried wood using sanders and other high-energy tools that generate fine, inhalable particulate and require detail work that brings the worker's breathing zone close to the source of these wood dust emissions. Substantial exposures also can occur in primary wood industries, such as logging, lumber mills, and pulp mills, and in construction during preparation of the wood including such activities as cutting and sanding.

Wood dusts are generated during woodworking processes, either by shattering the wood cells or by chipping out whole cells or groups of cells. Shattering generally creates more dust and much finer particle sizes than chipping. In general, operations designed to create a smooth wood surface, such as sanding, result in more shattering of cells than rougher woodworking processes. Processes like sawing performed parallel to the natural grain of the wood are less likely to shatter cells than processes performed perpendicular to the grain. Dusts produced from chipped cells cause less concern for human health because the large particles settle quickly from the air and rarely are inhaled.

The dominant route of human exposure to wood dusts is inhalation. In fact, most significant health effects seem to result from direct contact of the inhaled wood dusts with tissues of the respiratory tract. Because of the wide distribution of wood dust particle sizes, there is potential for deposition throughout the respiratory system. However, the majority deposit in the upper airways, primarily in the nose. This correlates well with observations that the most important health effects, such as upper respiratory symptoms and sino-nasal cancer, occur in the upper airways. While ingestion also is common, no adverse health effects were reported. Dermal contact also occurs routinely, occasionally causing dermatitis.

Exposure Measurements

Airborne wood dust exposure is sampled by collecting airborne dust on a filter and measured gravimetrically. A variety of samplers are in use, differing primarily in the size of the particles they collect. Occupational hygiene authorities recommend measuring airborne wood dust concentrations using 'inhalable' dust samplers because they best collect the range of particle sizes that cause health effects. However, most wood dust exposure data have been obtained using methods commonly referred to as

'total' dust samplers. The term 'total' is a misnomer, because typical North American 'total' dust samples have excluded the largest particles that can enter the nose and upper airways and may be responsible for the most important health effects. Wood dust exposure measurements comparing 'total' dust to inhalable dust measurements have demonstrated that 'total' dust samples underestimated worker exposures by factors ranging from 1.2 to 4.

Toxicity

Respiratory Disease

Many studies have considered the risk of respiratory disease among workers exposed to wood dusts. Adverse effects have been observed in the great majority of these studies, although in many cases workers also may have been exposed to bioaerosols, formaldehyde, isocyanates, and other manufactured chemicals with known respiratory effects. A number of reviews of respiratory disease from wood dust exposure have been published and are noted below in the Further Reading list.

Various studies have noted the following respiratory symptoms:

- decreased mucociliary clearance;
- nasal symptoms, such as obstruction, hypersecretion, or irritation;
- chest symptoms, including cough and dyspnea;
- phlegm;
- wheeze; and
- bronchitis.

These effects have been observed among workers exposed to wood dusts from a variety of tree species in many countries and settings, including lumber mills, furniture manufacturing, and cabinet making.

Workers exposed to wood dusts also have demonstrated decreased lung function

- compared to unexposed controls;
- after a workshift;
- decreased air flow (forced expiratory volume in one second (FEV_1), midflow, or peak flow); and
- both decreased FEV_1 and forced vital capacity (FVC).

Asthma

Case reports and epidemiological studies have attributed asthma to exposure to dusts from many different tree species. Several reviews are available, including those noted below in the Further Reading list.

Dusts from several North American and many exotic woods have been identified as allergenic. Included among these are wood types, such as

- African maple,
- ash,
- cedars,
- oak,
- ebony,
- jacaranda,
- mahogany,
- ramin,
- redwood, and
- walnut.

While many case reports and epidemiologic studies on asthma associated with wood dusts have been published, Western red cedar is the most notable and is the only one that has been studied extensively and has dose–response information available.

In addition to Western red cedar, many other tree species have been identified as causing asthma, based on epidemiologic studies and case reports.

Genotoxicity

Researchers have reported the following evidence of genotoxicity from wood dust exposures.

- Significantly ($p < 0.01$) more chromatid breaks among 13 nonsmoking male plywood workers compared to 15 nonsmoking, age-matched controls.
- Significantly ($p < 0.01$) more micronuclei in the peripheral lymphocytes of 298 match factory workers exposed to poplar and linden dusts compared to 45 waiters, with no apparent dose–response relationship.
- Significantly ($p < 0.05$) more DNA single-strand breaks in peripheral lymphocytes of 24 wooden furniture workers than in the 28 controls.

Carcinogenicity of Wood Dusts

An excess of sino-nasal cancer among wood workers was first recognized in the 1960s in England. Workers involved in furniture manufacturing and cabinet making had a 10–20 times increased risk of nasal cancer, and a 100–500 times increased risk specifically for nasal adenocarcinoma. Many case–control studies conducted in different countries have confirmed these findings, with extremely high relative risks observed in European studies. Although the highest risks have been observed among workers in

the wood furniture industry, excesses also have been observed in other wood-related industries, such as sawmills, cabinet making, and carpentry.

In general, the relative risks observed in North American studies have been considerably lower than those observed in European studies. While the reasons for this disparity are unclear, they may be artifacts of the relatively low power of most of the North American epidemiological studies. However, the consistency with which the differences have been observed suggests there may be other causes. US and Canadian studies observed excess risks for all types of sino-nasal cancer, ranging from 1.5 to 4.4. A pooled analysis of data from four cohorts of US wood workers and British furniture workers appeared to show that the sino-nasal cancer mortality excess was restricted to the British cohort. However, the US studies had relatively low power for detecting a two- or threefold excess risk. A cohort study of Canadian softwood sawmill workers that specifically controlled for exposure to chlorophenol fungicides showed a 1.9 times excess of sino-nasal cancer.

Adenocarcinoma has been highly associated with exposure to hardwood dusts while squamous cell carcinoma has been associated with exposure to dusts from a variety of wood types. Based on interviews with sino-nasal cancer patients, exposures to oak and beech clearly have been associated with excess risk. Birch, mahogany, teak, and walnut exposures are strongly suspected of causing sino-nasal cancer. However, because the mechanisms by which wood dust exposures increase the risk of sino-nasal cancer are not clear, other tree species also may be carcinogenic.

No studies were found that compared the risk of sino-nasal cancer based on quantitative estimates of wood dust exposure. However, several studies observed a dose–response relationship using semiquantitative estimates of wood dust exposure based on job title and industry. Because of the long latency of sino-nasal cancer, the effective exposure period is likely to be at least 20–30 years prior to diagnosis, which equates to the 1950s and 1960s for most studies. Unfortunately, there are very few exposure measurements from that period.

Some Carcinogenicity Classifications for Wood Dusts

American Conference of Governmental Industrial Hygienists (ACGIH®): Certain hardwoods as beech and oak: A1 – known human carcinogens.

Germany: Beech and oak wood dust: Group 1 – confirmed human carcinogens; wood (except beech and oak) dust: Group 3 – possible human carcinogens.

International Agency for Research on Cancer: Group 1 – carcinogenic to humans.

US National Institute for Occupational Safety and Health (NIOSH): Hardwood and softwood: Carcinogens.

Some Occupational Exposure Limits for Wood Dusts

1. ACGIH threshold limit value, Western red cedar, 0.5 mg m^{-3}, inhalable particulate mass, sensitizer; all other species, 1 mg m^{-3}, inhalable particulate mass.
2. Australia: Beech and oak, 1 mg m^{-3}, sensitizer; softwoods, 5 mg m^{-3}, sensitizer (both under review).
3. Germany: TRK (technical exposure limit): 2 mg m^{-3} inhalable fraction of the aerosol; airways and skin sensitizer for Western red cedar.
4. Japan Society for Occupational Health: 'total' dust, 4 mg m^{-3}; respirable dust, 1 mg m^{-3}.
5. Netherlands: Beech and oak, 1 mg m^{-3}.
6. South Africa (Department of Minerals and Energy): Hardwood and softwood, 5 mg m^{-3} ('total' dust) respiratory sensitizer.
7. Sweden: 2 mg m^{-3}; 0.5 mg m^{-3}, if dust has unevaluable impregnated substances.
8. United Kingdom: Hardwood and softwood, 5 mg m^{-3}, respiratory sensitizer.
9. US NIOSH: 1 mg m^{-3}.
10. US Occupational Safety and Health Administration: Particulates not otherwise regulated, 15 mg M^{-3}, 'total' dust; 5 mg m^{-3}, respirable dust.

See also: Carcinogenesis; Epidemiology; Respiratory Tract.

Further Reading

ACGIH: Wood dust. In: *Documentation of the Threshold Limit Values for Chemical Substances*, 7th edn. Cincinnati, OH: American Conference of Governmental Industrial Hygienists (latest revision).

Bardana EJ (1992) Western Red Cedar asthma and asthma secondary to other wood dusts and wood or plant related products. In: Bardana EJ, Montanaro A, and Hollaren MT (eds.) *Occupational Asthma*, ch. 8, pp. 87–106. Philadelphia, PA: Hanley and Belfus.

Chan-Yeung M (1993) Western Red Cedar and other wood dust. In: Chan-Yeung M, Malo JL, and Bernstein DI (eds.) *Asthma in the Workplace*. Bernstein, IL: Dekker.

Chan-Yeung M and Malo JL (1995) Occupational respiratory diseases associated with forest products industries. In: Harber P, Schenker M, and Balmes J (eds.) *Occupational*

and Environmental Respiratory Diseases. St. Louis, MO: Mosby.
DECOS (1992) Health-Based Recommended Occupational Exposure Limit for Wood Dust. Dutch Expert Committee for Occupational Standards Report No. RA 8/91. Directorate-General van de Arbeid, Den Haag, The Netherlands.
Demers PA, Teschke K, and Kennedy SM (1997) What to do about softwood dust? A review of respiratory effects and recommendations regarding exposure limits. *American Journal of Industrial Medicine* 31(4): 385–398.
IARC (1995*) Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, vol. 62, Wood. Lyon, France: International Agency for Research on Cancer.
Macbeth RG (1991) Discovery in medicine – Chance or science? The case of woodworkers' nasal cancer. *American Journal of Industrial Medicine* 19: 379–383.
Tatken RL and Browning CA (1987*) Health Effects of Exposure to Wood Dust: A Summary of the Literature*. Cincinnati, OH: US National Institute for Occupational Safety and Health.
Woods B and Calnan CD (1976) Toxic woods. *British Journal of Dermatology* 94(Suppl. 13): 1–97.

Workplace Environmental Exposure Levels (WEELs) *See* Occupational Exposure Limits.

World Health Organization *See* Joint FAO/WHO Expert Meetings (JECFA and JMPR).

Wound Healing *See* Tissue Repair.

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X

Xenobiotics

Midhun C Korrapati and Harihara M Mehendale

The term *xenobiotic* (foreign to the body) was coined to cover all compounds that were foreign to the organism under study. In some situations, this is loosely defined to include naturally present compounds administered by alternate routes or at unnatural concentrations. An examination of the fate of foreign compounds in biological systems is a natural outgrowth of man's curiosity about his environment and how it can affect his health as well as the health of other animals and organisms around him. While the majority of studies concern with the fate of man-made chemicals including drugs used as medicines, in humans and animals there are extensive investigations on the fate of organic compounds in plants, animals and microorganisms.

Classification of Xenobiotics

Humans are exposed throughout their lifetime to a large variety of xenobiotics like drugs and nonessential exogenous compounds by ingestion, inhalation, dermal, or by any parenteral routes of exposure that may pose as a health hazard. Drugs taken for therapeutic purposes as well as occupational or accidental exposure to any man-made or naturally occurring chemicals and the vapors of volatile chemicals or solvents pose possible health risks; smoking and drinking involve the absorption of large amounts of substances with potential health effects. Furthermore, the ingestion of natural toxins in vegetables and fruits, pesticide residues in food, as well as cancer causing pyrolysis products from fats and protein formed during the charbroiling of the meat have to be considered.

Various organ systems like nervous system, cardiovascular, respiratory, and gastrointestinal systems are affected by xenobiotics. Xenobiotics are also classified based upon the mechanism by which they cause toxicity. Immunomodulatory, endocrinomodulatory, antiproliferative, and mutagenic agents are some of the examples of such xenobiotics. These effects are the result of biotransformation of these xenobiotics.

Biotransformation of Xenobiotics

Most of these xenobiotics undergo enzymatic biotransformations by xenobiotic-metabolizing enzymes in the liver and extrahepatic tissues, and are eliminated by excretion as hydrophilic metabolites. In some cases, especially during oxidative metabolism, numerous chemical procarcinogens form reactive metabolites capable of binding covalently to proteins or nucleic acids – a critical step to mutagenicity, cytotoxicity, and carcinogenicity. Therefore, insight into the biotransformation and bioactivation of xenobiotics becomes an undisputable prerequisite for the assessment of drug safety and risk estimation of chemicals and drugs.

Detoxification and toxic effects of drugs and xenobiotics have been studied extensively in various mammalian species. Frequently, differences in sensitivity to these toxic effects were observed and can now be attributed to a difference between species in the isoenzyme/isoforms of cytochrome P450 monoxygenases. The level of expression of the CYP450 enzymes is regulated by a variety of endogenous factors such as hormones, sex, age, diseases, and the presence of environmental factors such as inducing agents. Drugs undergo a variety of chemical changes in the animal organism by enzymes of the liver, intestine, kidney, lung, and other tissues, with consequent alterations in the nature of their pharmacologic activity, duration of activity, and toxicity. Thus, the pharmacologic and toxicologic activity of a drug (or xenobiotic) is in many ways the consequence of its metabolism.

The study of xenobiotic metabolism has developed rapidly during the past few decades. These studies have been fundamental in the assessment of drug efficacy, safety, and design of dosage regimens; in the development of food additives and the assessment of potential hazards of contaminants; in the evaluation of toxic chemicals; and in the development of pesticides and herbicides and their metabolic fate in insects, other animals, and plants. The metabolism of many xenobiotics is fundamental to many toxic processes such as carcinogenesis, teratogenesis, and tissue necrosis. Often the same enzymes involved in

drug metabolism also carry out the regulation and metabolism of endogenous substances. The inhibition and induction of these enzymes by drugs and xenobiotics may consequently have a profound effect on the normal processes of intermediary metabolism, such as tissue growth and development.

The increased knowledge of xenobiotics and their fate in the living organisms, along with the need for greater safety evaluation of drugs and chemicals, has resulted in a proliferation of publications and a series of monographs that represent the current state of knowledge of foreign compound metabolism from biochemical and pharmacologic viewpoints.

See also: Carcinogenesis; Common Mechanism of Toxicity; Mechanisms of Toxicity.

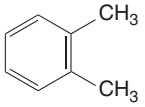
Further Reading

Anders M (ed.) (1985) *Bioactivation of Foreign Compounds*. New York: Academic Press.

Xylene

Stephen R Clough

- Chemical Abstracts Service Registry Numbers: CAS 1330-20-7 (mixed isomers); 95-47-6; 108-38-3; 106-42-3 (*o*-, *m*-, and *p*-isomers, respectively)
- Synonyms: Dimethyl benzene; Lsylen (Polish); Methyltoluene; NCI C55232; UN1307 (DOT); Violet 3; Xiloli (Italian); Xylenen (Dutch); Xylol; Xylole (German); RCRA Waste No. U239
- Chemical/Pharmaceutical/Other Class: Aromatic hydrocarbon. Xylene is a benzene ring with two methyl substitutions which can occur at the *ortho*, *meta*, and *para* positions giving rise to *o*-xylene (1,2-dimethylbenzene), *m*-xylene (1,3-dimethylbenzene), and *p*-xylene (1,4-dimethylbenzene), respectively. The *m*-xylene form is generally the predominant isomer (44–70%) in commercial mixtures.
- Chemical Formula: C_8H_{10}
- Chemical Structure:



Uses

Xylenes are used as thinners; solvents in paints, inks, rubbers, gums, resins, adhesives, and lacquers; paint removers; and as intermediates in the production of plasticizer (phthalic acid and anhydride) and polyester fibers. They are also used extensively as intermediates in the manufacture of perfumes, dyes, insecticides, and pharmaceuticals. The annual production of mixed xylenes varies between 6 and 12 billion pounds.

Exposure Routes and Pathways

Because xylene is fairly volatile, exposure of humans occurs principally by inhalation and is most likely to occur near its principal sources; that is, chemical plants and refineries, gas pumps, painting, and refinishing operations, and automobiles (e.g., in tunnels). Dermal exposure may also be significant, especially in an industrial setting, where skin may be exposed for long periods of time. Oral exposure is the least probable route and occurs primarily as a result of accidental poisoning or suicide.

Toxicokinetics

Xylene is primarily absorbed through the mucous membranes and pulmonary system. In experimental subjects, ~60% of airborne xylene is absorbed from the lung into the bloodstream. Xylene is also readily absorbed from the gastrointestinal tract and through broken or intact skin. Once absorbed, xylene distributes to many tissues in the body, especially lipid-rich organs, although this occurs to a lesser extent than for benzene. Chemical alteration of xylene occurs in the liver and the lung, where the compound is changed to more water-soluble metabolites (the corresponding *o*-, *m*-, and *p*-toluic acids and/or methylhippuric acid) so it can be easily excreted in the urine. In animals, it has been shown that metabolism is qualitatively different in the lung versus the liver. Greater than 95% of absorbed xylene is excreted as a water-soluble metabolite, with the remaining fraction being exhaled unchanged. Excretion appears to occur rapidly; animal studies indicate complete clearance of the compound in 24 h. Xylene will cross the placenta and enter fetal tissue.

Mechanism of Toxicity

Although the exact mechanism of toxicity has not been determined, it is known that the primary toxic

effect of xylene is dysfunction of the brain and central nervous system (CNS narcosis). The main function of neurons is to conduct electrochemical signals to one, several, or thousands of other cells. The normal physiology of neurons is largely dependent on the integrity of the neuronal cell membrane which polarizes and depolarizes during the transmission of these electric signals. Thus, the most probable mechanism of toxicity results from the unique sensitivity of the cell membranes of neurons to the solvent property of xylene, which disrupts the membrane lipid bilayer and thus the normal transmission of nerve impulses.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute CNS effects in animals, such as exaggerated visual disturbances, are similar to those in humans. The median lethal oral dose in rats is $\sim 3\,g\,kg^{-1}$.

Human

Xylene is an irritant to the eyes, nose, throat, and the gastrointestinal tract. Direct contact with the skin is also irritating and will cause defatting, which may lead to dryness, cracking, blistering, or dermatitis. Xylene appears to be more acutely toxic than other structural analogs, such as benzene or toluene. CNS depression, a typical effect seen in solvent exposures, is the primary toxic effect seen following exposure to xylene. At high air concentrations, xylene may cause the following acute signs in humans: flushing and reddening of the skin, feeling of increased body heat, disturbed vision, dizziness, tremors, salivation, cardiac stress, CNS depression, and confusion. Very high exposures may cause anorexia, nausea, vomiting, and abdominal pain; continued exposure may lead to coma and death, which appear to be due to cardiac fibrillation and/or lung congestion and hemorrhage. Females are reported to be more susceptible to the effects of xylene than males.

Chronic Toxicity (or Exposure)

Animal

The results of early subchronic and chronic studies administering xylene to laboratory animals were biased because many of the effects of the solvent were found to be caused by toxic impurities such as benzene. However, later studies showed that xylene does cause a significant change in blood-forming elements and blood chemistry. A National Toxicology Program (NTP) study of rats and mice exposed orally to mixed xylenes showed that exposure resulted only in decreased body weights in both sexes. This occurred at doses of $1000\,mg\,kg^{-1}\,day^{-1}$ given 5 days a week for 13 weeks. No effects were seen at the next lowest dose, $500\,mg\,kg^{-1}\,day^{-1}$. No carcinogenic effects were observed.

In reproductive studies, effects on the fetus have been seen only at oral doses that were associated with concurrent maternal toxicity. In an inhalation study conducted by Biodynamics, pregnant female rats were exposed to mixed xylenes $6\,h\,day^{-1}$ for 190 days. Toxicity to the fetus was apparent in the group exposed to 500 ppm. Rat pups born to dams exposed to 500 or 250 ppm displayed reduced weight of ovaries, but the effect was transient. Developmental toxicity was seen in another inhalation study in rats.

Human

Effects from chronic exposure to xylene are similar to those from acute exposure but are systemically more severe. Repeated, prolonged exposure to xylene may result in conjunctivitis of the eye and dryness of the nose and throat. Repeated exposure of the skin will cause dryness, flaking, and/or dermatitis. Inhalation may cause CNS effects, such as excitation, then depression characterized by signs such as paresthesia, tremors, apprehension, impaired memory, weakness, nervous irritation, vertigo, headache, anorexia, nausea, and flatulence. Clinical findings may include moderate but reversible changes such as bone marrow hyperplasia, liver enlargement, and kidney nephrosis. Based on the weight of evidence, the US Environmental Protection Agency (EPA) has classified xylene as a class D carcinogen (insufficient evidence to classify human carcinogenicity).

Clinical Management

Persons who have been overcome by xylene fumes or gases should be removed from the area of exposure and exposed to fresh air. Should breathing become labored or shallow, medical intervention (e.g., artificial respiration) may be necessary. Following accidental or intentional ingestion, vomiting should not be induced; stomach lavage should be initiated as soon as possible. Liquid xylene spills on exposed skin should be immediately dried with an absorbent towel; next, the affected area should be washed with soap and water. In cases of eye exposure, the eyes should be irrigated immediately.

Environmental Fate

Xylenes are ubiquitous in the environment and the vast majority ultimately end up partitioning into the

atmosphere. Once in the air, xylene is transformed into other products; for example, substituted aldehydes and phenols. The estimated half-life for the photooxidation of xylenes in the atmosphere is between 0.5 and 1.0 day. Automobile and industrial emissions contribute the majority of xylene found in the atmosphere. Concentrations are lowest in remote areas (average levels of <0.5 ppb) and highest in urban areas (levels ranging from 0.5 to 21 ppb). Xylene is also found in plants and is present in their combustion products; for example, forest fire smoke and tobacco smoke. Xylene has also been detected in surface water and treated wastewater effluents, with average levels below $1\,\mu g\,l^{-1}$. It has been detected in 3% of groundwater and 6% of surface water supplies sampled in a US EPA survey. It is typically found in groundwater impacted by gasoline releases and, when found in concert with benzene, toluene and ethylbenzene, is generally a good indicator of a gasoline spill. Xylene is readily biodegradable and will not concentrate to a great degree.

Exposure Standards and Guidelines

The no-effect level from the NTP study has been used to calculate a safe oral dose for xylene in humans of $2\,mg\,kg^{-1}\,day^{-1}$. This is 10 times higher than the oral reference dose published by the US EPA on its IRIS database, $0.2\,mg\,kg^{-1}\,day^{-1}$ based on the rat no-observed-adverse-effect level of $250\,mg\,kg^{-1}\,day^{-1}$ adjusted for duration of exposure and then divided by an uncertainty factor of 1000. The inhalation reference concentration for xylene is currently $0.1\,mg\,m^{-3}$, derived from a duration adjusted rat lowest-observed adverse-effect level of $39\,mg\,m^{-3}$ divided by an uncertainty factor of 300. Under the Safe Drinking Water Act, the maximum contaminant level (MCL) is the standard criterion for drinking water and the maximum contaminant level goal (MCLG) is the ideal. The proposed MCL and the MCLG for mixed xylenes are both $10\,mg\,l^{-1}$.

The current occupational exposure limit (threshold limit value time-weighted average) recommended by the American Conference of Governmental Industrial Hygienists and enforced by the US government, as an Occupational Safety and Health Administration permissible exposure limit time-weighted average, is 100 ppm ($434\,mg\,m^{-3}$). The ceiling limit is 150 ppm ($651\,mg\,m^{-3}$).

Miscellaneous

Xylene compounds are lighter than water and only slightly soluble ($\sim 130\,mg\,l^{-1}$ for mixed isomer solution).

See also: Benzene; Neurotoxicity; Petroleum Hydrocarbons; Pollution, Air Indoor; Toluene.

Relevant Websites

http://www.epa.gov – US Environmental Protection Agency (2003) Toxicological Review of Xylenes (CAS No. 1330-20-7) in Support of Summary Information on the Integrated Risk Information System (IRIS). EPA 635/R-03/001.

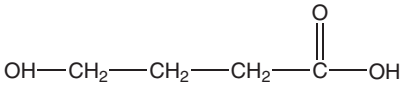
http://risk.lsd.ornl.gov – Oak Ridge National Laboratory (2003) Xylene. Risk Assessment Information System, Oak Ridge, TN.

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

Xyrem

Arezoo Campbell

- Chemical Abstracts Service Registry Number: CAS 502-85-2
- Synonyms: Gamma hydroxybutyric acid (GHB); Sodium oxybate; 4-Butanediol, Gamma-butyrolactone (Slang terms: G-riffic; Home Boy; Grievous Bodily Harm; Liquid-X)
- Chemical/Pharmaceutical/Other Class: Drug of abuse
- Chemical Formula: $C_4H_8O_3$
- Chemical Structure:



Uses

Xyrem is used as an oral medication, available in solution form, for the treatment of cataplexy in patients with narcolepsy. Gamma hydroxybutyric acid (GHB) is also used as a drug of abuse.

Background Information

Xyrem is manufactured by Orphan Medical as an oral solution approved in 2002 by the US Food and Drug Administration (FDA) for the treatment of cataplexy associated with narcolepsy. GHB is the active component of Xyrem. It is a metabolite of the neurotransmitter gamma aminobutyric acid (GABA). Because of its role as a central nervous system (CNS) depressant, it causes sleep and ataxia. GHB has been used as a 'date-rape drug' and many hospitalizations and deaths are associated with it. Therefore, until recently, it was classified as a schedule I controlled substance. This meant that GHB had a high potential for abuse and no medical purpose. However, in 2002, because of a series of clinical trials that demonstrated that the drug reduces cataplexy attacks, it was approved for use under the trademark Xyrem. Because it had previously been used as a 'date-rape drug', Xyrem is considered a schedule III controlled substance and will be distributed only in accordance with strict FDA regulations. Illegal use of Xyrem will be punishable under Federal and state laws.

Exposure Routes and Pathways

The gastrointestinal tract is the route of exposure for Xyrem.

Toxicokinetics

GHB is absorbed very quickly. After ingestion, it is detected in the serum after 10 min. The maximum plasma concentration is achieved $\sim$1–2 h after exposure. The elimination of the drug is also very rapid and the elimination half-life is $\sim$1 h. The removal of GHB occurs via expired carbon dioxide and very little ($\sim$4%) of it is eliminated unchanged in the urine. If Xyrem is taken with food, the bioavailability is greatly reduced while the excretion remains the same. GHB can readily cross the blood–brain barrier and the placenta.

Mechanism of Toxicity

Xyrem is a CNS depressant because it is an analog of the inhibitory neurotransmitter, GABA. GHB has high affinity binding sites in the mammalian brain. These are found in the basal ganglia, cortex, midbrain, and the hippocampus. It is endogenously present in the brain and thought to have a function although the specificity of its role in the CNS is at present unknown. The highest concentrations of GHB are in the basal ganglia. GHB dose-dependently decreases the release of enkephalins in the brain of rats. This function appears to be modulated by the nigrostriatal dopaminergic pathway. However, a high affinity receptor for GHB has not been identified. Some of its negative effects may be mediated by the $GABA_B$ receptors. The maximum stimulation of these receptors by GHB is $\sim$69% when compared to the binding of a $GABA_B$ receptor agonist. Therefore, the drug appears to be a weak agonist of the GABA binding site of $GABA_B$ receptors. GHB has been shown to prevent cell damage. The exact mechanism of this protection is unknown. However, it seems to be mediated by antiinflammatory and antioxidative properties. The precise mechanism by which Xyrem produces an effect on cataplexy is also unknown.

Acute and Short-Term Toxicity (or Exposure)

Animal

Intracerebroventricular injection of GHB causes generalized seizures in animals. Therefore, the compound has been used as a model for petit mal epilepsy.

Human

Primary effects of Xyrem are dose related and include CNS depression, amnesia, and hypotonia ($10\,mg\,kg^{-1}$). Exposures in the range of 20–$30\,mg\,kg^{-1}$ cause somnolence, drowsiness, dizziness, and euphoria. At levels of 50–$70\,mg\,kg^{-1}$ common symptoms are bradycardia, nausea, and vomiting. Higher exposures can lead to coma. Xyrem may cause neuropsychiatric side effects even at recommended doses. Oral doses as low as 5 g have caused CNS depression. Concurrent alcohol use can delay the onset of symptoms.

Chronic Toxicity (or Exposure)

Animal

There are no data indicating chronic toxicity due to Xyrem. The potential for abuse of GHB in rhesus monkeys is low. Rats show mild withdrawal symptoms when injected every 3 h for 3–6 days with concentrations GHB that do not cause seizures.

Human

Symptoms of withdrawal similar to other sedatives have been documented in adults using Xyrem daily. Patients who become dependent on the drug will need supportive care for up to 15 days.

Clinical Management

Because GHB is rapidly absorbed, it will not be detected in most routine toxicology screenings. Further, gastric lavage with activated charcoal will not be helpful. Intubation and mechanical ventilation may be needed in patients with CNS depression. There is no antidote for Xyrem intoxication and treatment is based on the symptoms present.

Exposure Standards and Guidelines

Xyrem is a liquid with a GHB concentration of $0.5\,g\,ml^{-1}$. The total daily dose for patients who are taking the medication as an anticataplexy drug should be in the range of 4.5–9.0 g. It should not be taken with alcohol, sedatives, or other CNS depressants.

See also: Drugs of Abuse.

Relevant Website

http://www.fda.gov – Xyrem (sodium oxybate) Information Page.

Y

Yew

Ann P Slattery

This article is a revision of the previous print edition article by Lanita B Myers, volume 3, pp. 419–420, © 1998, Elsevier Inc.

- SYNONYMS: *Taxus baccata*, *Taxus cuspidata*, *Taxus brevifolia*; *Taxus canadensis*; *Taxus floridana*; American Yew; Chinese yew; English yew; Japanese yew; Ground hemlock; Pacific yew; Western yew

Uses

Yews are evergreen shrubs or trees with alternate branch and reddish brown, thin, scaled bark. They are common as ornamental hedging and ground covers. The yew is often brought indoors at Christmas and used as decoration. Aqueous extracts of the yew have been used for years in Native American folk medicine for the cardiotonic, expectorant, antispasmodic, diuretic, and antiseptic properties. Experiments are being conducted on the potential for some extracts to possess central nervous system depressant, analgesic, antipyretic, cytotoxic, and antileukemic properties. Paclitaxel (Taxol) is an antineoplastic agent derived from various *Taxus* species used to treat numerous types of cancer including ovarian, breast, nonsmall cell lung and Kaposi's sarcoma.

Exposure Routes and Pathways

Ingestion of any part of the plant is the common route of exposure. The aril is not toxic. The hard seed and leaves are toxic and have the potential to release taxine. The plant parts are toxic whether green or dry.

Toxicokinetics

Taxine can be absorbed orally or by injection. Inhalation absorption is unlikely because it is not highly volatile. The onset of symptoms may be within 1 h or delayed for several hours. Systemic symptoms are expected within 1–3 h.

Mechanism of Toxicity

The main toxins of the yew species are the alkaloids taxine A and taxine B, which are present in all parts of the shrub except the fleshy part of the berry. These compounds are capable of causing symptoms similar to digitalis poisoning including hypotension, bradycardia, and depressed myocardial contractility and conduction delay. The mechanism appears to involve a block of the distal part of the conduction tissue of the heart, which can result in fatal arrhythmias. Atrioventricular conduction is particularly susceptible to yew alkaloids.

Acute and Short-Term Toxicity (or Exposure)

Human

Serious poisoning is uncommon. Most cases of yew berry ingestions result in no symptoms, because the seed must be chewed to release the taxine. One chewed berry may be potentially fatal in a child. Persons who ingest other parts of the plant or multiple berries should have gastric decontamination performed.

Symptoms initially expected after ingestions of leaves or a chewed seed are dizziness, dry mouth, and mydriasis, which develop rapidly. Nausea, vomiting, and abdominal pain follow these symptoms. A rash may appear, and facial pallor and cyanosis or reddish discoloration of the lips may occur. This is followed by generalized muscle weakness and drowsiness leading to coma. Seizures are also possible.

The primary action of these alkaloids is bradycardia and various other life-threatening arrhythmias with hypotension and decreased respiratory function. Death is due to cardiac and/or respiratory failure. Anaphylactoid reactions have been reported from chewing yew needles. If the seeds are ingested and not masticated, there is a likelihood they will pass without releasing the taxine.

Severe contact dermatitis can result from cutting yew wood. Taxines are water soluble, so drinking teas or water in which leaves are soaking is potentially dangerous.

Chronic Toxicity (or Exposure)

Human

Chronic ingestion of the yew species has revealed liver and kidney fatty degeneration on autopsy.

Clinical Management

Animal

In animals, ingestion of large amounts of any part of the yew often causes sudden death without previous symptomatology or signs of struggle. Survival after yew poisoning is uncommon. Smaller ingestions would be expected to cause gastroenteritis. Clinical signs in a herd of 35 yew-poisoned cattle included lethargy, recumbency, dyspnea, jugular pulsation and distension, and death. Most cattle died within 4 h. EKG changes and seizures were noted in dogs. Toxicity symptoms in a horse included weak pulse, ataxia, lower lip and tail limp, leg muscle trembling, respiratory grunt, collapse, seizures, and death within 15 min.

Induction of emesis should not be attempted. Lavage may be used if possible. Activated charcoal and a cathartic should be administered. Life support and respiratory function should be maintained as needed. Diagnosis of yew poisoning is based on the presence of yew plant in the gut on necropsy.

Lethal toxic doses reported in specific animal species are as follows:

- Horse: 2 g leaves per kg body weight or 100–200 g.
- Sheep: 10 g leaves per kg body weight or 100–200 g.
- Dog: 30 g of leaves.
- Swine: 3 g leaves per kg body weight or 75 g.
- Fowl: 30 g of leaves.
- Oxen: 10 g leaves per kg body weight or 500 g.
- Goats: 12 g leaves per kg body weight.

Surprisingly, deer are able to eat the foliage of *Taxus cuspidata* and apparently suffer no harm.

Human

Basic and advanced life-support measures should be utilized as needed. Several studies have demonstrated that the vast majority of unintentional exposures result in either no effects or only minor gastrointestinal symptoms. However, intentional exposures require management in an emergency department or other critical care environment. There are no antidotes. No laboratory test identifies taxine specifically.

Serious ingestions require cardiac monitoring in an intensive-care setting. Hypotension may be resistant to dopamine and dobutamine. Norepinephrine can also be used. Bradycardia can be treated with atropine and a temporary pacemaker as needed. Digoxin-specific FAB antibody fragments have been used with some success for cardiac conduction abnormalities after a yew exposure. If no contraindication, lidocaine, amiodarone, or procainamide may be used for ventricular dysrhythmias.

See also: Digitalis Glycosides.

Further Reading

Krenzelok E, Jacobsen T, and Aronis J (1998) Is the yew really poisonous to you? *Clinical Toxicology* 36(3): 219–223.

Olin BR (1993) Yew. *The Lawrence Review of Natural Products* May.

Wax PM, Cobaugh DJ, and Lawrence RA (1999) Should home ipecac-induced emesis be routinely recommended in the management of toxic berry ingestion? *Veterinary and Human Toxicology* 41(6): 394–397.

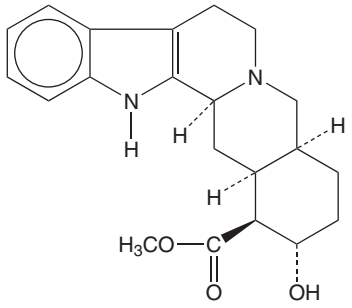
Wilson CR, Saur JM, and Hooser SB (2001) Taxines: A review of the mechanism and toxicity of yew (*Taxus* spp.) alkaloids. *Toxicon* 39(2–3): 175–185.

Yohimbine

Rebeca Gracia

- Chemical Abstracts Service Registry Numbers: CAS 146-48-5 (Yohimbine); CAS 65-19-0 (Yohimbine hydrochloride)
- Synonyms: Aphrodine; *Pausinystalia yohimbe* (*Corynanthe yohimbe*); Cotyine; *Rauwolfia serpentina* (roots only); YoYo; Quebrachine; Actibine; Aphrodyne; Dayto Himbin; Revervyl; Reverzine; Yobine; Yocon; Yohimex; Yohydrol (also available in various combination products); (16α,17α)-17-Hydroxyyohimban-16-carboxylic acid methyl ester
- Chemical/Pharmaceutical/Other Class: Rubiaceae family and α-2 antagonist

- Chemical Formula: $C_{21}H_{26}N_2O_3$
- Chemical Structure:



Uses

Yohimbine is used in the treatment of impotence, and as an aphrodisiac and mild hallucinogen. It is also used as a mydriatic and sympatholytic and has been suggested as an antidote to clonidine overdose.

Background Information

Yohimbine has been on the US Food and Drug Administration (FDA) unsafe herb list since March 1977, it is approved for use in veterinary medicine, as a reversal agent for xylazine overdose. Yohimbine has FDA approval as a mydriatic and sympatholytic in humans. It is also available as a herbal supplement without a prescription.

Exposure Routes and Pathways

Ingestion is the most common route of accidental and intentional exposure to yohimbine. The substance is extracted from the bark of the tree *Corynanthe yohimbe*. This tree is found in western Africa. The powder form may also be smoked or steeped into a tea. It is available in an intravenous form for veterinary purposes.

Toxicokinetics

Oral absorption is rapid, with an absorption half-life of 7–11 min. Peak plasma levels occur at 45–60 min. The volume of distribution is highly variable, demonstrated to be 2.24 ± 1 to $1.25\,l\,kg^{-1}$ after oral administration but $0.26\,l\,kg^{-1}$ after intravenous exposure. Yohimbine is excreted via the kidneys. Less than 1% of the unchanged drug was recovered in the urine after 24 h. Yohimbine is rapidly eliminated from the plasma with a half-life of less than 1 h.

Mechanism of Toxicity

Yohimbine is a competitive α-2 antagonist causing increased sympathetic outflow and enhanced release of norepinephrine. In fact, a two- to threefold increase in plasma norepinephrine has been reported after intravenous doses of $0.016\,mg\,kg^{-1}$. Yohimbine may also have effects at α-1 adrenoceptors and, in high concentrations, serotonin and dopamine receptors. It also has been shown to inhibit monoamine oxidase.

Acute and Short-Term Toxicity (or Exposure)

Animal

Yohimbine has US FDA approval to reverse the effects of xylazine in dogs. Toxic effects are similar to those observed in humans. Hypertension, tachycardia, central nervous system stimulation, and antidiuresis may occur.

Human

Although overdoses are rare, oral doses of 15–20 mg have produced hypertension. Oral doses as little as $0.1\,mg\,kg^{-1}$ may produce stimulant effects but reported therapeutic doses vary and up to $100\,mg\,day^{-1}$ has been given. Daily doses of 18 mg divided three times a day are generally well tolerated. Toxic manifestations usually involve tachycardia, diaphoresis, mydriasis, salivation, nausea, vomiting, and facial flushing. Neurological signs include dizziness, anxiety, 'squeezing headache', incoordination, and paresthesias.

Clinical Management

Basic and advanced life-support measures should be performed as needed. Gastric decontamination with activated charcoal may be beneficial if performed within the first hours of ingestion. Treatment is focused on decreasing hypertension and anxiety. Nitroprusside is preferred for severe hypertension although labetalol, nitroglycerin, and phentolamine are possible alternatives. Clonidine may be effective to reverse the α-adrenergic antagonism. Diazepam is useful to decrease anxiety.

See also: Clonidine.

Further Reading

Friesen K, Palatnick W, and Tenenbein M (1993) Benign course after massive ingestion of yohimbine. *Journal of Emergency Medicine* 11: 287–288.

Roberge RJ, McGuire SP, and Krenzelok EP (1996) Yohimbine as an antidote for clonidine overdose. *American Journal of Emergency Medicine* 14: 678–680.

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Z

Zinc

Shayne C Gad

This article is a revision of the previous print edition article by Arthur Furst and Shirley B Radding, volume 3, pp. 423–424, © 1998, Elsevier Inc.

- Representative Chemicals: Zinc chloride ($ZnCl_2$); Zincochromite ($ZnCrO_4$); Zinc sulfate ($ZnSO_4$)
- Chemical Abstracts Service Registry Number: CAS 7440-66-6
- Synonyms: LS6; Blue powder; Merrillite
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formula: Zn^{2+}

Uses

Zinc is an essential trace element and is commonly ingested as a nutritional supplement. Divalent zinc is one of the most important of the micronutrients. More than 100 enzymes are zinc dependent; for example, carboxypeptidase, carbonic anhydrase (which is responsible for the exchange of carbonic acid in the blood and the exhalation of carbon dioxide), and the alcohol dehydrogenase (which metabolizes alcohol). Deficiency of zinc, especially in newborns, results in impaired growth, loss of hair, skin eruptions, and often impaired or delayed sexual maturation. Many medical problems are also associated with zinc deficiencies (e.g., ulcerative colitis, chronic renal disease, and anemia).

Commercially, zinc is used in galvanized iron and in various alloys (e.g., brass and bronze). It is also used in dry cell batteries, electrical fuses, fungicides, and construction materials (e.g., roofing and gutters). Zinc chloride is used in electroplating, soldering fluxes, burnishing and polishing compounds for steel, and in antiseptic and deodorant solutions. Zinc chloride is used as yellow pigment. Zinc oxide is used in ointments, rubber, and paints (for white pigments).

Exposure Routes and Pathways

Ingestion and inhalation of zinc are possible exposure pathways. Zinc is readily absorbed by most plants and, hence, is found in most foods (especially grains, nuts, legumes, meats, poultry, and most seafood). The concentration of zinc in drinking water depends on the composition of water pipes and vessels. Inhalation is a significant exposure pathway in industrial areas, where zinc levels in air are high.

The concentrations of zinc in various foods and human tissues have also been determined. In a 1980–82 survey of total diet samples, the Food and Drug Administration (FDA) estimated that the average intake of zinc from food (including water) for an adult was $0.23\,mg\,kg^{-1}\,day^{-1}$. The FDA concluded that the daily intake of zinc from the inhalation of ambient air is negligible compared to the daily intake from food. Certain population groups may be exposed to higher concentrations of zinc than the general population. People who work in coal mines, people who work with the refining and smelting of nonferrous metals, and people who live near waste sites and metal smelting operations may be exposed to high levels of zinc. People who consume large amounts of foods high in zinc content, such as oysters and mussels, may also be exposed to high levels of zinc. The higher exposure may not always be manifested as increased body burden in the exposed individuals.

Toxicokinetics

Up to 30% of ingested zinc is absorbed from the small intestine; however, a homeostatic mechanism controls the absorption. Nutritional status also influences zinc absorption; deficiency of pyridoxine or tryptophan somewhat inhibits absorption. Zinc induces a zinc metallothionein, the form in which it is bound to the liver and other tissues. The pancreas is high in zinc, and in males the prostate gland contains the greatest store of zinc. Zinc is excreted in the feces.

Mechanism of Toxicity

Excessive zinc interferes with iron and copper metabolism; the latter leads to copper-deficiency anemia. Salts of strong mineral acids are corrosive to skin and intestine.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute oral toxicity in rodents exposed to zinc is low, with LD_{50} values in the range 30–600 mg kg^{-1} body weight, depending on the zinc salt administered. Acute effects in rodents following inhalation or intratracheal instillation of zinc compounds include respiratory distress, pulmonary edema, and infiltration of the lung by leukocytes.

Human

Zinc is a skin irritant. It is difficult to ingest too much zinc from foodstuffs. Consumption of beverages stored in galvanized containers or pipes, use of zinc utensils, or ingestion of too many zinc supplements can result in nausea, cramps, vomiting, and diarrhea.

In the industrial setting, inhalation of fumes of zinc, zinc oxide, or zinc chloride leads to pulmonary edema and metal fume fever. Onset occurs within 4–6 h and may be delayed up to 8 h. Symptoms include chills alternating with fever, sweating, and weakness, which can last from 24 to 48 h.

Zinc salts (e.g., zinc chloride and zinc sulfate) are corrosive to the skin and the gastrointestinal tract and can cause acute tubular necrosis and interstitial nephritis.

Chronic Toxicity (or Exposure)

Animal

Zinc is not carcinogenic; however, testicular tumors were induced by direct injection of zinc chloride into the testes of experimental animals (copper chloride produced the same effect).

Human

Since the zinc–copper ratio is important, intake of too much zinc can lead to symptoms of copper deficiency. However, patients have taken 10 times the recommended daily allowance for zinc with no adverse reaction.

Chronic inhalation of zinc compounds can lead to liver damage, which can be fatal.

Clinical Management

Clinical management is supportive. Chelating agents such as British Antilewisite (2,3-dimercaptopropanol) or D-penicillamine are not effective.

Environmental Fate

Zinc enters the air, water, and soil as a result of both natural processes and human activities. Most zinc enters the environment as the result of human activities, such as mining, purifying of zinc, lead, and cadmium ores, steel production, coal burning, and burning of wastes. These releases can increase zinc levels in the atmosphere. Waste streams from zinc and other metal manufacturing and zinc chemical industries, domestic wastewater, and run-off from soil containing zinc can discharge zinc into waterways. The level of zinc in soil increases mainly from disposal of zinc wastes from metal manufacturing industries and coal ash from electric utilities. In air, zinc is present mostly as fine dust particles. This dust eventually settles over land and water. Rain and snow aid in removing zinc from air. Most of the zinc in bodies of water, such as lakes or rivers, settles on the bottom. However, a small amount may remain either dissolved in water or as fine suspended particles. The level of dissolved zinc in water may increase as the acidity of water increases. Some fish can collect zinc in their bodies if they live in water containing zinc. Most of the zinc in soil is bound to the soil and does not dissolve in water. However, depending on the characteristics of the soil, some zinc may reach groundwater. Contamination of groundwater from hazardous waste sites has been noticed. Zinc may be taken up by animals eating soil or drinking water containing zinc. If other animals eat these animals, they will also have increased amounts of zinc in their bodies.

Ecotoxicology

Bivalves and other sessile estuarine organisms are often used as a measure of contamination of estuarine water because they usually contain higher levels of metals than fish. The arithmetic mean concentration of zinc in oysters (*Crassostrea virginica*) from the Mississippi Sound collected in 1988 was 640 mg kg^{-1} (wet weight). In a nationwide mussel watch program, the mean concentrations of zinc in molluscs (*Mytilus edulis*) around the coast of the United States during 1976–88 ranged from 67 to 3700 mg kg^{-1} (dry weight). Although the concentration on a nationwide basis varied depending on sampling sites, the level of zinc showed little evidence of statistically significant change during 1976–88. The mean concentration of zinc in oysters (*Crassostrea virginica*) collected from the US coastline of the Gulf of Mexico during 1986–88 was 2150 mg kg^{-1} (dry weight). In the National Contaminant Biomonitoring Program, the geometric

mean concentration of zinc in various whole fish was 21.7 mg kg^{-1} (wet weight). Of all fish tested (e.g., bloater, sucker, white perch, bass, and catfish.), common carp showed the highest level of zinc. No significant trend in the level of zinc in whole fish was observed during 1978–84. The concentration of zinc in yellow perch (*Perca flavescens*) from six acidic lakes in northwestern New Jersey ranged from 26.1 to 66.2 mg kg^{-1} (dry weight). Although the concentrations of mercury and lead in fish from acidic lakes were higher compared to fish collected from non-acidic lakes, the concentrations of zinc showed no significant difference. Similarly, high concentrations of zinc were not found in white suckers (*Catostomus commersoni*) and brown bullheads (*Ictalurus nebulosus*) collected from two acidic Adirondack lakes in New York.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value–time-weighted average (TLV – TWA) for zinc chloride (as fume) is 1 mg m^{-3}. The TLV – TWA for zinc oxide (as fume) is 5 mg m^{-3}.

Miscellaneous

For hundreds of years before being recognized as a distinct element, zinc ores were used to make brass. The pure metal was isolated in India in the thirteenth century. Zinc occurs at 0.02% in the Earth's crust.

See also: Metallothionein; Metals.

Further Reading

Jakubowski M (2001). Zinc and cadmium. In: Bingham E, Cohrssen B, and Powell CH (eds.) *Patty's Toxicology*, 5th edn., vol. 2, pp 253–269. New York: Wiley.

Zatta P, Lucchini R, van Rensburg SJ, and Taylor A (2003) The role of metals in neurodegenerative processes: Aluminum, manganese, and zinc. *Brain Research Bulletin* 62(1): 15–28.

Relevant Websites

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

http://www.inchem.org – Zinc (Environmental Health Criteria 221 from the International Programme on Chemical Safety).

Zinc Oxide

Rebeca Gracia

- Chemical Abstracts Service Registry Number: CAS 1314-13-2
- Synonyms: Chinese white; Zinc white; Flowers of zinc; Philosopher's wool; Calcine; Amalox; Calamine; Felling zinc oxide; Zincite; AZO 22; Emar; Outmine; Pasco
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formula: ZnO

Uses

Zinc oxide is used in various pharmaceutical products such as ointments, powders, creams, bandages, gauze, and pastes. It is commonly found in combination with other topical agents. Medicinal-grade zinc oxide is a concentration of no less than 99.5% and calamine is no less than 98%. Zinc oxide is used in industry as an accelerator, rubber reinforcing agent, in paints (white pigment, mold-growth inhibitor), plastics (ultraviolet absorber), feed additive, cosmetics, as a photoconductor, and in piezoelectric devices.

Exposure Routes and Pathways

Exposure to zinc oxide can occur through inhalation, ingestion, and eye or skin contact. Ingestion of zinc oxide ointments is most common in household settings and is generally considered nontoxic due to relatively low product concentrations. Inhalation of zinc oxide in industrial areas, as particulate matter or fumes, may lead to potentially toxic exposures.

Toxicokinetics

Zinc oxide is not absorbed to any significant amount when applied to intact skin. It is absorbed slowly when ingested; only 20–30% of dietary zinc is absorbed from the small intestine. Inhalation may result in minimal systemic absorption. Zinc is widely distributed throughout the body with increased concentrations of zinc metallothionein being found in

the liver, pancreas, muscles, and bone. It is highly protein bound and excess zinc may also be stored in erythrocytes. Zinc is excreted predominately in the feces.

Mechanism of Toxicity

Topical and inhalational exposures to zinc oxide primarily produce irritation. Excessive systemic absorption of zinc may result in altered iron and copper metabolism with resultant toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

Topical administration of zinc oxide in animals failed to cause toxicity. The oral LD_{50} in mice is $7950\,mg\,kg^{-1}$. The LC_{50} in mice is $2500\,mg\,m^{-3}$ (duration not provided). Rats exposed to $2500\,mg\,m^{-3}$ for 3–4 h died either during or immediately after the exposure. Guinea pigs exposed to $0.7\,mg\,m^{-3}$ over 1 h showed no change in pulmonary airway resistance, but did demonstrate progressive diminution in lung compliance. Guinea pigs exposed to 5 or $7\,mg\,m^{-3}$ zinc oxide fumes for $3\,h\,day^{-1}$ for 5 and 6 days, respectively, had transient changes in pulmonary function with small airway inflammation and edema. These animals showed reduced total lung capacity, vital capacity, and carbon monoxide diffusion capacity. No adverse effects were observed in guinea pigs exposed to zinc oxide fumes at a concentration of $2.7\,mg\,m^{-3}$.

Chronic Toxicity (or Exposure)

Animal

Zinc oxide administered to rats at $200\,mg\,kg^{-1}\,day^{-1}$ for 21 days prior to mating and throughout pregnancy resulted in increased fetal deaths and reduced fetal body weights. No adverse effects were observed at $100\,mg\,kg^{-1}\,day^{-1}$.

Human

Ingestion of large amounts of zinc oxide may cause nausea, cramps, vomiting, and diarrhea. Zinc oxide is often used in ointments along with vitamins A and D, and toxicity usually develops to these added constituents rather than the zinc oxide. It would require an ingestion of greater than 60 g of a typical ointment to result in vitamin A toxicity. Smaller amounts could also cause gastrointestinal disturbances such as diarrhea due to the emollient base. As much as $2\,g\,kg^{-1}$ of zinc oxide has been tolerated. Zinc oxide dust is an irritant at high concentrations can result in respiratory system effects. Acute inhalation of zinc oxide can result in coughing, substernal pain, upper respiratory tract irritation, rales, chills, fever, nausea, and vomiting. Inhalation of zinc oxide fumes can result in metal fume fever.

In Vitro Toxicity Data

Zinc oxide has been demonstrated to be mutagenic, causing alterations in cell lines.

Human

Prolonged, recurring zinc oxide exposures to the skin may cause papular-pustular eruptions. This skin condition may be referred to as oxide pox. Studies of zinc refinery workers found no correlation between exposures and lung or other types of cancer. Chronic inhalation of zinc compounds has been implicated in cases of fatal liver damage.

Clinical Management

Clinical management is supportive. Gastric decontamination should be considered only in the case of massive ingestions. Normal zinc levels in the blood are between 68 and $136\,\mu g\,dl^{-1}$. Chelating agents such as BAL (British Antilewisite; 2,3-dimercaptopropanol) or calcium EDTA will enhance removal of zinc, but are not likely indicated unless the unusual case of massive chronic exposure. Hemodialysis and other methods of extracorporeal elimination are not necessary.

Environmental Fate

Zinc oxide poses no inherent risk to ecosystems, but it may be dissociated and release a zinc ion that can then result in aquatic toxicity.

Exposure Standards and Guidelines

Occupational Safety and Health Administration permissible exposure limit: $15\,mg\,m^{-3}$ of air for total zinc oxide dust and $5\,mg\,m^{-3}$ for the respirable fraction as an 8 h time-weighted average (TWA) concentration.

National Institute for Occupational Safety and Health recommended exposure limits: $5\,mg\,m^{-3}$ for total zinc oxide dust as a TWA for up to a 10 h workday and a 40 h workweek and a 15 min ceiling of $15\,mg\,m^{-3}$ (based on the risk of metal fume fever).

American Conference of Governmental Industrial Hygienists threshold limit value (TLV): $10\,mg\,m^{-3}$ for total zinc oxide dust (containing no asbestos and $<1\%$ crystalline silica), as a TWA for a normal 8 h workday and a 40 h workweek; TLV – TWA of $5\,mg\,m^{-3}$ and a TLV – STEL (short-term exposure limit) of $10\,mg\,m^{-3}$ for zinc oxide fume (based on providing reasonable control of this nuisance dust).

See also: Cosmetics and Personal Care Products; Zinc.

Further Reading

Meerdink GL, Reed RE, and Perry D (1986) Zinc poisoning from the ingestion of pennies. *Proceedings of American Association of Veterinary Laboratory Diagnosticians* 29: 141–150.

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APPENDIX 1
SELECTED TOXICOLOGY-RELATED INSTITUTIONS

Academy of Toxicological Sciences*

Sachin S Devi and Harihara M Mehendale

This article is a revision of the previous print edition article by David M Krentz and Harihara M Mehendale, volume 1, p. 7, © 1998, Elsevier Inc.

The Academy of Toxicological Sciences was established in 1981 for the purpose of recognizing and certifying toxicologists in order to ensure the competence and experience of professional practitioners whose work affects the public welfare. Recognition and certification are accomplished by the peer-review process, a time-honored mechanism for scientists to evaluate one another. The academy bases certification on formal training, proven ability, and experience. Demonstrated achievement, rather than the potential for achievement, is the substance of the academy's evaluation process. Thus, an individual certified as a fellow in toxicology by the Academy of Toxicological Sciences is a qualified person who actively practices toxicology and who has been evaluated by the peer-review process by the academy according to its bylaws.

*Adapted from information supplied by the Academy of Toxicological Sciences.

Candidates for certification must have broad knowledge of toxicology and demonstrate substantive involvement in toxicological activities. To apply, an applicant submits an application form and supporting documentation to the secretary-treasurer of the academy. The board of directors reviews the credentials of applicants twice a year in the spring and fall. The criteria for certification in toxicology by the academy are divided into three sections: (1) education and training, (2) professional experience, and (3) demonstration of scientific judgment and recognition. Following review by the board of directors, candidates are notified in writing of the board's decision.

Successful candidates are certified as fellows of the academy for a period of 5 years. Every 5 years, each fellow is re-certified by submitting a current *curriculum vitae* for the board's review and vote.

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American Academy of Clinical Toxicology

Christopher P Holstege

This article is a revision of the previous print edition article by Harihara M Mehendale, volume 1, p. 50, © 1998, Elsevier Inc.

History

The American Academy of Clinical Toxicology (AACT) was founded in 1968 by a group of physicians and scientists with the specific goal of advancing the diagnosis and treatment of poisonings. In 1974, the AACT established the American Board of Medical Toxicology (ABMT) to certify physicians in the specialty of clinical toxicology. This subspecialty was recognized by the American Board of Medical Specialties in 1992. In 1985, a second certifying board, the American Board of Applied Toxicology (ABAT) was established for nonphysician peer recognition.

Mission

The AACT was established in 1968 as a not-for-profit multidisciplinary organization uniting scientists and clinicians in the advancement of research, education,

and prevention and treatment of diseases caused by chemicals, drugs, and toxins.

Purpose

The AACT unites scientists and clinicians in the advancement of research, education, and prevention of diseases caused by chemicals, drugs, and other toxins. Today, the AACT is an international organization whose membership comprises clinical and research toxicologists, physicians, veterinarians, nurses, pharmacists, analytical chemists, industrial hygienists, poison information center specialists, and allied professionals.

Objectives

The founders of AACT established the academy to:

- Promote the study of health effects of poisons on humans and animals.
- Unite into one group scientists and clinicians whose research, clinical, and academic experience focus on clinical toxicology.
- Foster a better understanding of the principles and practice of clinical toxicology.
- Encourage development of new therapies and treatment in clinical toxicology.
- Facilitate information exchange among individual members and organizations interested in clinical toxicology.
- Define the position of clinical toxicologists on toxicology-related issues.

Key Activities

The ABAT was established by the AACT to provide special recognition to professionals (other than practicing physicians) who demonstrate exceptional knowledge, experience, and competence in applied clinical toxicology. An examination is administered periodically and is open to AACT members who meet the qualifications. Candidates who pass the examination are awarded the status of Diplomate of the American Board of Applied Toxicology.

Publications

Members receive AACTion, the Academy's newsletter, to keep them current with the organizational activities of the AACT. The Academy seeks to be active on issues that affect the membership and the discipline of clinical toxicology. As the need arises, an *ad hoc* committee is appointed to develop an Academy position paper. A directory that identifies the members of the Academy is available only to AACT members. The Journal of Toxicology – Clinical Toxicology is the official journal of AACT.

Meetings

The AACT, affiliated with many professional organizations, holds annual meetings in conjunction with both the American and Canadian Associations of Poison Control Centers and the American College of Medical Toxicology.

Awards and Grants

AACT offers a Multicenter Research Award, the Lampe-Kunkle Research Award, and the Micromedex International Travel Scholarship.

Related Organizations

The AACT was a charter member of the World Federation of Associations of Clinical Toxicology Centers and Poison Control Centers sponsored by the World Health Organization. The Academy supports the efforts of other toxicology organizations worldwide.

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American Association of Poison Control Centers

Christopher P Holstege

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Mission

The American Association of Poison Control Centers (AAPCC) is a nationwide organization of poison centers and interested individuals. (For a complete listing of poison centers please see the website.)

Purpose

The AAPCC provides a forum for poison centers and interested individuals to promote the reduction of morbidity and mortality from unintentional poisonings through public and professional education and scientific research. It sets standards for poison center operations.

Membership Criteria

There are several membership categories of the AAPCC including US Poison Center Member, Poison Prevention Education Center, Associate Institutional Member, Canadian Associate Institutional Member, Animal Poison Center, Industry Product Surveillance Service of Industry Poison Center, Individual Member, Emeritus Individual Member, and Sustaining Member. Each membership category has its own criteria and related entitlements (see Website).

Key Activities

The activities of the AAPCC include the following:

- Certification of regional poison centers and poison center personnel.
- Interaction with private and governmental agencies whose activities influence poisoning and poison centers.
- Development of public and professional education programs and materials.
- Collection and analysis of national poisoning data.

AAPCC policies and programs are determined by the Board of Directors, composed of the officers of the Association (president, past-president, president-elect, secretary, treasurer) and eight directors. Elected at-large, Board of Directors members serve 3 year terms.

Publications

The AAPCC publishes the following:

- Annual Report of the American Association of Poison Control Centers Toxic Exposure Surveillance System (TESS) published every September in *The American Journal of Emergency Medicine*.
- The Association's newsletter, *The Poison Line*, published six times a year.
- Hosts an online discussion forum called 'Patient Management Guidelines for Poisonings'.

Meetings

Each year, the Association holds a meeting that includes scientific presentations, business meetings, and committee meetings.

Awards and Grants

The AAPCC offers a Recognition Award to individuals who have made significant contributions to poison control and offers Research Awards to educators and specialists in poison information.

Contact Details

American Association of Poison Control Centers
3201 New Mexico Avenue, Suite 330
Washington, DC 20016, USA
URL:http://www.aapcc.org/
Tel.: 202-362-7217
Email: info@aapcc.org
Toll Free Emergency Number: The AAPCC has established the following national toll-free number for poisoning emergencies: 1-800-222-1222.

American Board of Toxicology

Sachin S Devi and Harihara M Mehendale

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The American Board of Toxicology, Inc. (ABT) certifies individuals in general toxicology through a process that evaluates expert knowledge as demonstrated by education, experience, and passage of a comprehensive written examination. Certified individuals are initially recognized by being designated as Diplomats of the American Board of Toxicology for a period of 5 years.

Other ABT objectives are to encourage the study of the science of toxicology and to stimulate its advancement by promulgation of standards for professional practice. It is ABT policy that Diplomats demonstrate a continual commitment to excellence in the science of toxicology. Successful achievement of these goals as outlined by the Board will result in an individual maintaining recognition as a Diplomat by the ABT.

The ABT has identified three performance criteria by which a Diplomat will be evaluated pursuant to recertification. These criteria are: (1) Active Practice of Toxicology, (2) Continuing Education, and (3) Maintaining Expert Knowledge in General Toxicology. Each Diplomat, at the beginning of the fourth year of their current certification, will be required to apply for recertification. ABT will review activities in each of the three performance areas and notify the Diplomat of acceptable progress or deficiencies that need to be addressed. If, in the opinion of the Board, a Diplomat is not compliant with each of the three criteria at the end of the fifth certification year, that Diplomat may be required to successfully pass the formal certification examination. Diplomats who are compliant with each of the three performance criteria will be certified for an additional 5 years.

Active Practice of Toxicology: Active practice is defined as performing, directing, or managing toxicology activities such as research, testing, teaching, clinical practice, or regulation.

Continuing Education: A successful program of continuing education may encompass a myriad of diverse activities. The study of published texts, periodicals, or scientific journals germane to toxicology is a means by which Diplomats routinely maintain or expand their knowledge of toxicology. Other evidence of a commitment to continued education is attendance at specific programs where toxicology themes are presented in a comprehensive or in-depth manner. Such programs are often held during general or annual meetings of the Society of Toxicology, American College of Toxicology, FASEB, Environmental Mutagen Society, Teratology Society, American Association for Cancer Research, or Chapter Meetings of the Society of Toxicology. Attendance Forum or Target Organ Conferences also provide opportunities to maintain or expand a Diplomat's knowledge of toxicology.

Maintaining Expert Knowledge of General Toxicology: It is held that an objective mechanism is required for the Diplomat and ABT to gauge the success of their efforts to maintain expert knowledge in general toxicology. A recertification examination prepared by the ABT is to serve in this evaluation process. Diplomats will have the opportunity to privately complete the recertification examination during the fourth year of their certification period using their own reference material as needed. The completed examination will be graded by ABT and returned to the Diplomat. The Diplomats will be furnished a comparison of their results with the performance of peers for each subject area. Stimulated by these results the Diplomat would be expected to tailor a continuing education program that addresses those subject areas in which their knowledge appears to have diminished. The ABT may ask a Diplomat to complete specific portions of the recertification exam to assess the success of their focused continuing education program.

Summary of Recertification Process: Each Diplomat maintains a personal file of activities germane to the Active Practice and Continuing Education criteria for certification, that is, name of meeting attended, number of hours, title, topics, faculty, etc. Each Diplomat is required to be recertified every 5 years in order to maintain the Diplomat status. In addition to maintaining active practice of toxicology during the first 3 years, this procedure involves submission of credentials during the fourth year and fulfilling other requirements during the fifth year.

Contact Details

American Board of Toxicology
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URL: http://www.abtox.org

American College of Medical Toxicology

Christopher P Holstege

The American College of Medical Toxicology (ACMT) is a professional association of physicians with recognized expertise in the field of medical toxicology. The purpose of the ACMT is to advance the science, study, and practice of medical toxicology by fostering the development of medical toxicology in its provision of emergency, consultation, forensic, legal, community, and industrial services. ACMT is a nonprofit organization that is not involved in authorizing or designating any political lobby action. ACMT members elect a Board of Directors (nine members), including executive officers.

History

The ACMT was formerly known as the American Board of Medical Toxicology (ABMT). The ABMT offered specialty certification in medical toxicology at a time when the American Board of Medical Specialties (ABMS) did not recognize subspecialty certification in toxicology. When the ABMS approved formal recognition of medical toxicology as a subspecialty in September 1992, the ABMT discontinued its function as a certifying body. It was reincorporated in September 1993 as the ACMT.

Membership

Active members of the ACMT are physicians who have been certified by the ABMT and/or by the Sub-Board in Medical Toxicology of the ABMS. In addition to active members, the ACMT accepts applications for international and associate membership. International members are physicians licensed to practice medicine in countries outside the United States, who practice medical toxicology as a substantial portion of their professional activities. Associate members are physicians licensed to practice medicine, who have completed a residency training program in a primary medical specialty, and who are enrolled in or have completed a training program in medical toxicology. Active members, international members, and members emeritus of the ACMT who have met additional criteria may be designated as 'Fellow of the American College of Medical Toxicology', and are entitled to use the title 'FACMT'.

Contact Details

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11240 Waples Mill Road, Suite 200
Fairfax, Virginia 22030, USA
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American College of Toxicology

Harihara M Mehendale

Introduction

The American College of Toxicology (ACT) is dedicated to providing an interactive forum for the advancement and exchange of toxicologic information between industry, government, and academia. In the international arena, ACT supports and participates in the efforts of the International Union of Toxicology (IUTOX), which allows ACT to advance these goals on a global scale. The goal of the college is to bring together people having common interests in the broad field of toxicology. This includes not only those individuals involved in toxicology but also those from related disciplines: analytical chemistry, biology, biological statistics, computer science, physiology, toxicokinetics, pathology, teratology, genetic toxicology, molecular biology, experimental psychology, immunology, cancer biology, and animal husbandry. The college recognizes that application of the science of toxicology is multifaceted, encompassing many disciplines. All of these relate to modern toxicology and attempt to address present and future problems. Toxicology can be defined in the classical sense as the scientific study of the effects of toxicants on biological systems. However, the explosion of scientific knowledge has made infinitely more complex the statement of the toxicological problem and understanding the solution. Modern toxicology offers a unique challenge since it involves quantitative interpolation of data from high to low dose

and its eventual extrapolation from simple life forms and animals to humans and the environment. The prediction of toxic effects upon all stages of development and the use of computer models represent frontiers of knowledge in toxicology that in some cases are in their infancy.

ACT also recognizes that the interests and problems of its members are disparate and not only stem from the performance of their responsibilities but also are significantly impacted by government regulations, industrial practices, and societal perception.

ACT is committed to addressing the toxicological issues of the day and those it anticipates will arise in the future. Its interests lie in disseminating information to and among its members so that their combined talents and creative insights may further the practice of their science. To do so, the college brings together the necessary experts to debate and discuss unique and creative approaches to problems that hopefully will better serve the needs and interests of the communities in which we live and the society we serve.

Mission Statement

Mission

The mission of the ACT is to educate and lead professionals in industry, government, and related areas of toxicology by actively promoting the exchange of information and perspective on the current status of safety assessment and the application of new developments in toxicology.

Strategic Objectives

- Focus on interdisciplinary exchange of scientific information, especially as scientific information is used in regulation.
- Sponsor scientific and educational programs in toxicology.
- Present the ideals and opinions to its membership.
- Disseminate information of the results of toxicological research, standards, and practices through the College journal and newsletter.
- Serve in other capacities in which the College can function more efficiently as a group than as individuals.

Activities

Activities include annual meetings and workshops. ACT publishes a newsletter (quarterly) and the *International Journal of Toxicology* (formerly *Journal of the American College of Toxicology*). The *International Journal of Toxicology* publishes fully refereed papers covering the entire field of toxicology, including research in risk assessment, general toxicology, carcinogenicity, safety evaluation, reproductive and genetic toxicology, epidemiology and clinical toxicology, mechanisms of toxicity, new approaches to toxicological testing, and alternatives to animal testing. Reviews and major symposia in the field are included.

Membership

ACT membership is by election after submission of an application and supporting documentation. There are three types of individual membership: full, associate, and student. Full membership is for qualified individuals who have conducted and published original research in toxicology. Associate membership is for individuals with critical interests in toxicology who have not reached full membership status. Student membership is for qualified predoctoral students. Honorary membership and Fellow status are also awarded periodically. Corporate membership is available for corporations, associations, and other organizations that support the activities of the College.

Contact Details

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American Conference of Governmental Industrial Hygienists

Andrew Maier

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History

The American Conference of Governmental Industrial Hygienists (ACGIH®) is a private, not-for-profit, nongovernmental organization headquartered in Cincinnati, OH. The ACGIH has played an important role in the area of occupational health and safety with a history in the field of over 60 years.

Mission and Purpose

The ACGIH is a member-based organization with a mission and purpose to advance worker health and safety through education and the development and dissemination of scientific and technical knowledge.

Membership Criteria

There are over 4000 members of the organization worldwide. Membership categories include regular membership for those who are occupational hygiene, occupational health, environmental health, or safety professionals whose primary employment is with a government agency or an educational institution. Associate memberships are for those who are engaged in the occupational hygiene, environmental health, occupational health, or safety professions, but are not eligible for regular membership. Other membership categories include student, retired, honorary, or organizational.

Key Activities, Publications, Databases, and Services

The ACGIH supports its objectives by developing professional guidelines and technical documents and sponsoring professional conferences and seminars. The ACGIH publishes jointly with the American Industrial Hygiene Association the *Journal of Occupational and Environmental Hygiene*, a monthly peer-reviewed technical journal. In addition, the organization, through the work of its numerous technical committees, publishes professional guidelines and technical documents. An important example of this activity includes the Threshold Limit Values (TLVs®) for Chemical Substances and physical agents and Biological Exposure Indices (BEIs®). These occupational exposure criteria are widely used around the world as the basis for occupational health protection. The organization has published over several hundred other documents on a variety of occupational health and safety topics. The ACGIH also supports its mission through the sponsorship of conferences and seminars, including as a sponsor for the annual American Industrial Hygiene Conference and Exposition (AIHCE), and through focused seminars, workshops, and lectures on topics of current interest.

Related Organizations

- American Industrial Hygiene Association.
- (US) National Institute for Occupational Safety and Health.
- (US) Occupational Safety and Health Administration.

Contact Details

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American Industrial Hygiene Association

Andrew Maier

History

The American Industrial Hygiene Association (AIHA) is a nonprofit organization founded in 1939 and headquartered in Fairfax, VA, USA.

Mission and Purpose

The mission of AIHA is that it 'promotes, protects, and enhances industrial hygienists and other occupational health, safety and environmental professionals in their efforts to improve the health and well-being of workers, the community, and the environment'.

Membership Criteria

The AIHA has ~12 000 members, making it one of the largest international associations of occupational and environmental health professionals. Members include professionals practicing industrial hygiene in industry, government, labor, academic institutions, and independent organizations. A variety of membership categories exist, based on area or practice and level of experience in the field.

Key Activities, Publications, Databases, and Services

The AIHA supports this mission through a variety of different activities and membership services. AIHA publishes jointly with the American Conference of Governmental Industrial Hygienists (ACGIH), a monthly peer-reviewed technical journal – *Journal of Occupational and Environmental Hygiene*. In addition, the organization, through the work of its numerous technical committees, publishes professional guidelines and technical documents. Important examples of this activity include the Emergency Response Planning Guidelines and Workplace Environmental Exposure Level Guides. The emergency and occupational exposure criteria published in these documents are widely used for assessing health risk during emergency exposure situations and for occupational health protection. The organization publishes numerous other documents on a variety of occupational health and safety topics. The AIHA also supports its mission through the sponsorship of conferences and seminars, including as a sponsor for the annual American Industrial Hygiene Conference and Exposition (AIHCE), as well as numerous focused seminars, workshops, and lectures, and online training courses on topics of current interest. AIHA also provides laboratory accreditation programs to help ensure a high standard of quality in the analysis of exposure and bulk material sampling data used in making occupational health decisions.

Related Organizations

- American Conference of Governmental Industrial Hygienists (ACGIH);
- (US) National Institute for Occupational Safety and Health (NIOSH); and
- (US) Occupational Safety and Health Administration (OSHA).

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CIIT Centers for Health Research

Sachin S Devi and Harihara M Mehendale*

The CIIT Centers for Health Research (CIIT) is a private, not-for-profit research organization whose purpose is to advance industry and public interest in the acquisition, evaluation, and dissemination of information on the potential impact of exposure to chemicals and other substances on human health. CIIT's core research program on human health risks is funded by member companies of the American Chemistry Council through the Long-Range

*Compiled from the information provided by CIIT.

Research Initiative (LRI). CIIT also receives funding from federal grants and industry contracts.

CIIT uses a systems biology approach to human health effects research. The hallmark of systems biology is the seamless integration of functional genomics, computational biology, and bioinformatics to guide biomedical research and provide integrative, quantitative tools for hypothesis testing and experimental design. CIIT's LRI-funded research program on human health risks is concentrated in four major areas: (1) toxicological and physiological studies to assess responses of the organism and cell to chemical exposures; (2) high-throughput genomics with *in vivo*, *in vitro*, and *ex vivo* preparations to catalog and evaluate tissue and cell responses; (3) computational analysis of biological data using simulation and bioinformatics to interpret these responses; and (4) quantitative modeling of dynamic systems that predict dose–response behavior of these biological responses under realistic exposures. In 2004, CIIT expanded its endocrine biology program to provide a foundation for human disease research.

CIIT is administratively organized into the Division of Biological Sciences and the Division of Computational Biology. In keeping with the systems biology model, CIIT is also organized into technology-based research centers that support multiple projects and serve as a focus for external funding. The centers target areas relevant to environmental health issues and will change as new issues emerge. CIIT currently has three research centers. The Center for Computational Biology and Extrapolation Modeling develops biologically based computer simulation models and uses them as the basis for human health risk assessments. The Center for Developmental Dosimetry brings together the expertise needed to evaluate the fate of xenobiotics in developing organisms. The Center for Integrated Genomics provides centralized equipment, expertise, and training for studies of gene expression.

CIIT faculty hold advanced degrees in analytical chemistry, biochemistry, biology, cell biology, chemical engineering, environmental engineering, environmental sciences, inhalation toxicology, mathematics, mechanical engineering, molecular biology, pharmacology, physiology, statistics, toxicology, and veterinary biosciences. In the 27 years since CIIT began operations, faculty have established a strong presence in the research community through publication in the peer-reviewed literature and the formal presentation of research results at scientific meetings. CIIT scientists have published more than 3800 scientific documents, including over 1200 research articles. CIIT faculty have served in a number of scientific advisory positions for organizations representing public health concerns over the years. They have contributed to the quality of a wide variety of research publications by serving as peer reviewers and in various editorial capacities. Faculty are also involved in the education of numerous college students in the Research Triangle through adjunct faculty appointments at Duke University, North Carolina State University, and the University of North Carolina at Chapel Hill.

CIIT has a strong commitment to training and education in health effects research. CIIT awards postdoctoral fellowships to scientists who have recently obtained advanced degrees, predoctoral fellowships to PhD students at area universities, and summer internships to undergraduate students. Since 2001, CIIT has been sponsoring workshops to introduce middle and high school teachers to biomedical research. As a result of CIIT's strong commitment to education, alumni of the postdoctoral and predoctoral programs have been major contributors to health effects research. During the 27 years since CIIT began operations, 201 postdoctoral fellows and trainees, 67 predoctoral fellows, and 121 summer interns have participated in CIIT's education programs.

CIIT was founded in 1974 as the Chemical Industry Institute of Toxicology by farsighted leaders of 11 major chemical companies in the United States to address growing concerns about the effects of chemicals on environmental and human health. CIIT began operations in 1976 under the leadership of first President Dr. Leon Golberg, an internationally known toxicologist who had founded and headed the British Industrial Biological Research Association. Dr. Robert A. Neal, who was Director of the Center in Environmental Toxicology and Professor of Biochemistry at Vanderbilt University School of Medicine, was appointed CIIT's second President in 1981. Dr. Roger O. McClellan, President and Director of the Inhalation Toxicology Research Institute in Albuquerque, New Mexico, was recruited to be CIIT's third President in 1988. Dr. William F. Greenlee, Chair of the Department of Pharmacology and Molecular Toxicology at the University of Massachusetts Medical School, was appointed fourth President in 1999 as CIIT entered the final stages of its transition from an institute primarily sponsored by CIIT member companies to an organization receiving its major funding from member companies of the American Chemistry Council. Dr. Greenlee refocused CIIT's research vision on the key human health issues of global concern, restructured CIIT's core research program using a systems biology approach, and developed a multifaceted approach to funding. In keeping with Dr. Greenlee's vision, the institute changed its name in December 2000 to the CIIT Centers for Health Research.

Contact Details

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Consumer Product Safety Commission*

Michael A Babich

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The US Consumer Product Safety Commission (CPSC) is an independent federal regulatory agency created by Congress in 1972. The agency's mission is to protect the public against unreasonable risks of injuries and deaths associated with consumer products. The CPSC has jurisdiction over 15 000 types of products used in and around the home, in schools, and in recreation. Products under the jurisdiction of CPSC include clothing, children's articles, household appliances, home furnishings, cleaners, and consumer fireworks. CPSC is directed by three Commissioners, each of whom is appointed by the President of the United States, with one of the Commissioners nominated by the President to the position of Chairman.

Statutes Administered by CPSC

To carry out its mission, CPSC administers five statutes. They are: (1) the Consumer Product Safety Act (CPSA), (2) the Federal Hazardous Substances Act (FHSA), (3) the Flammable Fabrics Act (FFA), (4) the Poison Prevention Packaging Act (PPPA), and (5) the Refrigerator Safety Act (RSA). Toxicological issues arise most frequently under the CPSA, FHSA, and PPPA. CPSC regulations implementing these statutes may be found at Title 16 of the Code of Federal Regulations (CFR) and are available on the Commission's website.

CPSA regulations include a ban of paint containing more than 0.06% lead, as well as children's products that bear lead-containing paint (16 CFR part 1303). Certain products that contain respirable, free-form asbestos are also banned under the CPSA (16 CFR part 1304).

In 1992, the Commission issued guidelines for assessing chronic hazards under the FHSA, including carcinogenicity, neurotoxicity, reproductive/developmental toxicity, exposure, bioavailability, risk assessment, and acceptable risk (57 FR 46626-46674). The chronic hazard guidelines are intended to assist manufacturers in complying with the FHSA. A summary of the chronic hazard guidelines appears in the CPSC regulations at 16 CFR § 1500.135, and is available on the Commission's website.

In 1998, the Commission issued guidance requesting manufacturers, importers, distributors, and retailers to eliminate lead that may be accessible to children from consumer products (16 CFR § 1500.230). In 1998, the Commission also issued guidance requesting manufacturers to eliminate the use of hazardous liquid chemicals (e.g., methanol, methylene chloride, and petroleum distillates) from children's products, such as rolling balls, necklaces, pens, and liquid timers (16 CFR § 1500.231).

The Labeling of Hazardous Art Materials Act (LHAMA) amended the FHSA to provide additional requirements for arts and crafts materials. Under regulations implementing LHAMA, each producer or repackager of an art material must describe in writing, and submit to the Commission, the criteria used to determine whether an art material has the potential for producing chronic adverse health effects (16 CFR § 1500.14 (b)(8)). The producer or repackager must also submit a list of art materials requiring chronic hazard labeling (16 CFR § 1500.14 (b)(8)(ii)(C)). In addition, the CPSC regulations require art materials to have a statement of conformance and bear an emergency management telephone number (16 CFR § 1500.14 (b)(8)(ii)(C)).

To require child-resistant packaging under the PPPA the Commission must find that special packaging is needed to protect children from serious personal injury or illness from handling, using, or ingesting a substance and that special packaging can be developed and mass produced that will protect the integrity of the product. Chemicals are regulated under the PPPA on a case-by-case basis.

Contacting CPSC

Consumers may contact CPSC to report an unsafe product or product-related injury, find out whether a

*This information has been prepared by CPSC staff; it has not been reviewed or approved by, and may not reflect the views of, the Commissioners. Because this material was prepared by CPSC staff in their official positions, it is in the public domain and may be freely copied or reprinted.

product has been recalled, request injury data, or obtain CPSC publications, including press releases, staff reports, and regulations.

Contact Details

Mailing address

US Consumer Product Safety Commission
Washington, DC 20207, USA

Street address

4330 East-West Highway, Bethesda, MD 20814, USA
Tel.: +1-800-638-2772 (Call to obtain product safety information and other agency information and to report unsafe products. Available 24 h a day, 7 days a week.)
URL: http://www.cpsc.gov

Regional Offices

Eastern
201 Varick Street, Room 903, New York, NY 10014, USA
Tel.: +1-212-620-4120
Central
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Tel.: +1-312-353-8260
Western
1301 Clay Street, Suite 610-N, Oakland, CA 94612, USA
Tel.: +1-510-637-4050

Further Reading

US Consumer Product Safety Commission (CPSC) (1992) Labeling requirements for art materials presenting chronic hazards; guidelines for determining chronic toxicity of products subject to the FHSA; supplementary definition of 'toxic' under the Federal Hazardous Substances Act; final rules. Federal Register 57: 46626–46674 (1992).

Relevant Websites

http://www.access.gpo.gov – Code of Federal Regulations. Volume 16, Chapter II. Consumer Products.

Department of Defense, US

Ruth Custance

Introduction

The Department of Defense (DoD) is responsible for providing the military forces to prevent war and protect the security of the United States. The DoD includes the Office of the Secretary of Defense, Joint Chiefs of Staff, three Military Departments, nine Unified Combatant Commands, the DoD Inspector General, 15 Defense Agencies, and seven DoD Field Activities. The three Military Departments include the Army, Navy, which includes the Marine Corps, and the Air Force. The Department has an annual budget of ~$370 billion and has ~1.5 million active duty personnel and employs 650 000 civilian employees.

History

The Army, Navy, and Marine Corps were established by the Second Continental Congress in 1775 in support of the American Revolutionary War. The War Department was established in 1789 to administer these military forces. The armed forces were reorganized under a secretary of defense by the National Security Act of 1947, which also created the US Air Force as an independent service from the Army. In 1949, the services were brought together in a single Department of Defense.

Support of basic research within the military establishment has a long history. The Lewis and Clark expedition to explore the Northwest Territory was funded by the Army in 1804. However, prior to World War II, the Army and Navy Departments were conducting little basic research, which resulted in a military force that was not aware of the engineering and scientific opportunities available. During the early stages of World War II, it was realized that new technologies were needed and a large effort was expended to develop such technology as radar for early warning, surveillance, nuclear weapons, homing torpedoes, jet aircraft, rockets, and cryptology. The importance in World War II of these technologies developed from basic research programs caused the

Congress to formalize the DoD support of basic research by establishing the Office of Naval Research (ONR) in 1946. The National Science Foundation (NSF) was established shortly after in 1950, the Army Research Office (ARO) in 1951, the Air Force Office of Scientific Research (AFOSR) in 1952, and following Sputnik, the first spaceship launched into orbit by the Russians, the Defense Advanced Research Projects Agency (DARPA) in 1958. Postwar DoD contributions include the DARPA-NET, which developed into the Internet and Global Positioning System developed by the Naval Research Laboratory. The DoD basic science research budget is targeted more heavily in the area of physical sciences and engineering. Of all the Federal agencies, DoD is one of the largest funders of research in electronics, computers, mathematics, aeronautics, material science, mechanics, and environmental sciences. More than half of the DoD's basic research budget is spent at universities.

Mission

The mission of the DoD is to "support and defend the Constitution of the United States; to provide for the common defense of the nation, its citizens, and its allies; and to protect and advance US interests around the world." To accomplish this mission, the Department maintains trained forces ready to respond to threats to US security.

The DoD has established two corporate-level goals:

- *Goal 1*. Shape the international security environment and respond to the full spectrum of crises by providing appropriately sized, positioned, and mobile forces.
- *Goal 2*. Prepare now for an uncertain future by pursuing a focused modernization effort that maintains US qualitative superiority in key warfighting capabilities. Transform the force by exploiting the Revolution in Military Affairs and reengineer the Department to achieve a twenty-first century infrastructure.

In support of the goals of the DoD, scientific research is conducted under many of the defense agencies and military departments.

Department of Defense Basic Research

The DoD supports a major Basic Research Program across science and engineering fields that are important to defense needs. Historically, DoD research programs have introduced innovative capabilities such as radar, digital computers, cryptology, wireless mobile communications, multimedia connections, lasers and fiber optics in communications and in medicine, composite materials, satellite navigation, and environmental technologies.

Defense research is conducted in the following disciplines (approximate percentages of total funding are shown in parentheses): physics (9%), chemistry (9%), mathematics (7%), computer science (6%), electronics (13%), materials science (8%), mechanics (13%), terrestrial sciences (3%), ocean sciences (13%), atmospheric and space sciences (6.0%), biological sciences (9%), and cognitive and neural science (4%).

The Director of Research reports to the Director of Defense Research and Engineering (DDR&E) in the Office of the Secretary of Defense. The responsibilities of the Director of Research include: Providing leadership, policy guidance, and scientific oversight of basic research and serving as the DoD advocate for the Research (budget category 6.1) Program, which is managed by the Service Research Offices, namely the ARO and other Army organizations, the ONR, and the AFOSR, and by the DARPA, and through smaller research programs in the Ballistic Missiles Defense Organization, the National Security Agency, and the Army Corps of Engineers (COE). The Director of Research coordinates DoD basic research activities with the NSF and other federal departments and agencies, and with interagency groups such as those under the National Science and Technology Council chaired by the President's Science Adviser.

Most of the science and engineering work comprising the Defense Research Program is organized in the following 12 disciplinary areas, which are grouped under five categories:

- The physical sciences
  - Physics
  - Chemistry
- Mathematics and computer science
  - Mathematics
  - Computer science
- Engineering
  - Electronics
  - Materials sciences
  - Mechanics
- The environmental sciences
  - Terrestrial sciences
  - Ocean sciences
  - Atmospheric and space sciences
- The life sciences
  - Biological sciences
  - Cognitive and neural sciences

Some research interests in the Environmental Sciences and Life science disciplinary areas include:

- Terrestrial sciences
  - Weather related behavior of solid earth
  - Hydrodynamic and sedimentary processes for logistics over the shore
  - Pollution prevention and conservation
  - Structures research for survivability of airfields, pavements, buildings
  - Geodesy, seismology, remote sensing, terrain analysis, and modeling
- Ocean sciences
  - Ocean engineering and instrumentation
  - Physical oceanography
  - Marine chemistry, biology, and meteorology
  - Underwater acoustics
  - Littoral underwater visibility and target recognition
- Atmospheric and space sciences
  - Atmospheric effects on electromagnetic propagation
  - Atmospheric sensing and probing
  - Aerosol research
  - Solar and space physics
  - Upper atmospheric and ionospheric research
- Biological sciences
  - Biotechnology for novel materials enhancing survivability and mission effectiveness, such as more sensitive and accurate sensors against biological warfare agents, and protective materials against them
  - Biomolecular processes and materials for biosensors and biodegradation
  - Cellular biology, for example, to improve the healing of wounds sustained in combat
  - Treatment of infectious diseases more common in military service
  - Laser safety and eye protection

The principal points of contact for each discipline are in the Service Research Organizations, the ARO, the ONR, and the AFOSR, each of which is generally organized internally by the 12 disciplinary areas presented above, and the DARPA.

Examples of specific research programs or focus areas within the DoD that relate to toxicology include BioSystems administered under the Under Secretary of Defense Science and Technology, the Army Center for Environmental Health Research (USACEHR), the Armed Forces Institute of Pathology, the Naval Health Research Center, and the Navy Environmental Health Center.

The BioSystems program is responsible for guidance and oversight in the technology areas of Human systems, biomedical, chemical/biological defense, environmental quality, and civil engineering. Research topics include developing risk knowledge, vaccines, and therapeutic agents for infectious disease protection and providing advanced technologies for DoD to operate in an environmentally sound manner, for example, through the Strategic Environmental Research and Development Program, the DoD's corporate environmental research and development program.

The USACEHR developed from the toxicology program that existed as part of the US Army of Biomedical Research and Development Laboratory and is realigning under the Army Medical Research Institute of Chemical Defense. Research programs include participation in the Tri-Service Toxicology, which is responsible for developing the biochemical data needed to characterize the toxicity of materials used by the Armed Forces and to use these data to conduct health-hazard evaluation and risk assessment and the Toxicology Research program and Reproductive Hazards Program, which conducts research in the area of the carcinogenicity, immunotoxicology, and reproductive and development toxicology in fish and other nonmammalian systems.

The Naval Health Research Center Environmental Health Effects Laboratory conducts research in the areas of reproductive toxicology, cardiac toxicology, risk assessment, neurobehavioral toxicology, inhalation toxicology, and environmental and molecular toxicology in support of the Tri-Service toxicology needs. Research topics include evaluating the acute toxicity of jet fuel in occupationally exposed humans, inhalation toxicity of combustion of composite materials, and evaluating neurobehavioral effects at the neuromolecular level resulting from exposure to compounds and stressors such as physical fatigue.

The Navy Environmental Health Center administers the occupational health program for the Department of the Navy. Support is provided to the Naval Facilities Engineering Command in support of the Navy's Installation Restoration Program, the Base Realignment and Closure Program, and other related environmental projects. Specific services include health and safety, health and environmental risk communication, public health assessment and toxicology. The Navy was selected to serve as the lead agent for health and environmental risk communication training for the DoD.

Basic Science Research Funding

Defense research programs are organized within two types of budget categories: Funding Offices (offices

responsible for certain application areas, e.g., ships, space, communications) and Performing Organizations (usually DoD laboratories). The budget category out of which a contract or grant is funded indicates whether the work is considered basic research, applied research, prototype development, manufacturing technology, or something else. Basic Research is known as 'Budget Category 6.1'. Each of the above Service Research Organizations conducts 6.1 research. Three additional organizations within the ARO that support 6.1 research are the Army Research Institute for the Behavioral and Social Sciences (ARI), the Army Medical Command, and the COE.

ARO, ONR, and AFOSR regularly publish brochures and Broad Agency Announcements (BAAs) describing their research interests in general terms. The BAAs, published in the *Commerce Business Daily*, include instructions regarding proposal content and submission, as well as the criteria used to evaluate proposals.

In addition to research within the DoD Service Organizations, the DoD supports research within universities in the University Research Initiative (URI). The URI is a program funded out of the Office of the Director of Research, in the Office of the ODDR&E. It is jointly administered by the ARO, ONR, and AFOSR. The URI consists of several component programs including:

- Multidisciplinary University Research Initiative, which provides funding for research groups from different disciplines working for a common objective.
- The Defense University Research Instrumentation Program, which provides funding for badly needed, relatively expensive instrumentation for scientific and engineering research of special interest to the DoD.
- The National Defense Science and Engineering Graduate Fellowships, which support outstanding students to undertake graduate research in areas of strong interest to DoD.
- The Defense Experimental Program to Stimulate Competitive Research, which funds research in States underrepresented in Federal research support.

Contacts Details

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- Defense Technical Information Center
  URL: http://www.dtic.mil
- United States Army Center for Environmental Health Research (USACEHR)
  URL: http://www.usacehr.detrick.army.mil
- The Naval Health Research Center Environmental Health Effects Laboratory
  URL: http://www.navy.al.wpafb.af.mil

Department of Energy, US

Ruth Custance

Introduction

The Department of Energy (DOE) is primarily a national security agency with all of its missions related to its core mission to support national security. The DOE is responsible for energy security, maintaining the safety, security and reliability of the nuclear weapons stockpile, cleaning up the environment from past practices during the Cold War, and advancing science and technology. The DOE has been in existence for ~25 years and operates 24 research laboratories and facilities, and manages the environmental cleanup related to nuclear defense activities conducted over the last 50 years. The DOE has an annual budget of ~$23 billion and employs ~14 500 Federal and 100 000 contractor employees.

History

The founding of the DOE can be traced to the Manhattan Project and the race to develop the atomic bomb during World War II. Following the war, there was much debate in Congress regarding whether control of the atom should be under civilian or military control. The Atomic Energy Act of 1946 settled the debate by creating the Atomic Energy Commission, which took over the Manhattan Project's extensive scientific and industrial complex.

The Atomic Energy Commission was specifically established to maintain civilian government control over the field of atomic research and development. During the early Cold War years, the Commission focused on designing and producing nuclear weapons and developing nuclear reactors for naval propulsion. The Atomic Energy Act of 1954 ended exclusive Government use of the atom and began the growth of the commercial nuclear power industry, giving the Atomic Energy Commission authority to regulate the new industry.

In the 1970s, the Atomic Energy Commission was abolished and the Energy Reorganization Act of 1974 created two new agencies: the Nuclear Regulatory Commission to regulate the nuclear power industry and the Energy Research and Development Administration to manage the nuclear weapon, naval reactor, and energy development programs. As a result of the prolonged energy crisis of the 1970s, the Department of Energy Organization Act joined the Federal Government's agencies and programs into a single agency, the Department of Energy. Established on October 1, 1977, the Department of Energy assumed the responsibilities of the Federal Energy Administration, the Energy Research and Development Administration, and parts and programs from several other agencies.

Mission

Over its 25 year history, the DOE has changed its emphasis and focus as the needs of the Nation have changed. During the late 1970s, the DOE emphasized energy development and regulation. In the 1980s, nuclear weapons research, development, and production took a priority. Since the end of the Cold War, the DOE has focused on environmental cleanup of the nuclear weapons complex, nuclear nonproliferation and nuclear weapons stewardship, reliable energy supplies and delivery, energy efficiency and conservation, and technology transfer.

The DOE's principal tools in the pursuit of its national security mission are science and technology. The DOE has developed great scientific and technical capabilities which have served America in ways never anticipated. Those capabilities will be applied to the overarching mission of ensuring the national security.

The DOE has four strategic goals:

- *Defense strategic goal*: To protect our national security by applying advanced science and nuclear technology to the Nation's defense.
- *Energy strategic goal*: To protect our national and economic security by promoting a diverse supply and delivery of reliable, affordable, and environmentally sound energy.
- *Science strategic goal*: To protect our national and economic security by providing world-class scientific research capacity and advancing scientific knowledge.
- *Environment strategic goal*: To protect the environment by providing a responsible resolution to the environmental legacy of the Cold War and by providing for the permanent disposal of the Nation's high-level radioactive waste.

Office of Science

The Office of Science within the DOE manages fundamental research programs in basic energy sciences, biological and environmental sciences, and computational science. In addition, the Office of Science is the Federal Government's largest single funder of

materials and chemical sciences, and it supports important parts of US research in climate change, geophysics, genomics, life sciences, and science education.

The Office of Science manages research through five interdisciplinary program offices: Advanced Scientific Computing Research, Basic Energy Sciences, Biological and Environmental Research, Fusion Energy Sciences, and High Energy Physics and Nuclear Physics.

The Office of Science also manages the 10 laboratories within the national laboratory system that was created over half a century ago. Five of the laboratories are multiprogram facilities: Argonne National Laboratory, Brookhaven National Laboratory, Lawrence Berkeley National Laboratory, Oak Ridge National Laboratory, and Pacific Northwest National Laboratory. The other five laboratories are single-program national laboratories: Ames Laboratory, Fermi National Accelerator Laboratory, Thomas Jefferson National Accelerator Facility, Princeton Plasma Physics Laboratory, and Stanford Linear Accelerator Center.

The Office of Science also funds research and development projects conducted at the following national laboratories which are overseen by other DOE offices: Idaho Engineering and Environmental Laboratory (DOE's Office of Nuclear Energy, Science and Technology), Lawrence Livermore National Laboratory (DOE's National Nuclear Security Administration), Los Alamos National Laboratory (DOE's National Nuclear Security Administration), National Energy Technology Laboratory (DOE's Office of Fossil Energy), National Renewable Energy Laboratory (DOE's Office of Energy Efficiency and Renewable Energy), and Sandia National Laboratory (DOE's National Nuclear Security Administration).

The Biological and Environmental Research (BER) program within the Office of Science is involved in developing environmental and biomedical knowledge that is needed to identify, understand, anticipate, and mitigate the long-term health and environmental consequences of energy production, development, and use. As the founder of the Human Genome Project in 1986, BER continues to play a major role in biotechnology research and also invests in basic research on global climate change and environmental remediation. DOE's Genomes to Life program will use new genomic data and high-throughput technologies to explore the diverse natural capabilities found in microbes. This research will play an important role in helping solve DOE's mission challenges in energy production and environmental cleanup.

The BER program supports fundamental research in climate change, environmental remediation, genomics, systems biology, and medical sciences. BER funds research at public and private research institutions and at the DOE laboratories. BER supports research facilities used by public and private sector scientists across a range of disciplines: structural biology, DNA sequencing, functional genomics, climate science, the global carbon cycle, and environmental molecular science. Specific long-term goals in scientific advancement that the BER program is committed to, include:

- *Life sciences*: Characterize the multiprotein complexes (or the lack thereof) involving a scientifically significant fraction of a microbe's proteins. Develop computational models to direct the use and design of microbial communities to clean up waste, sequester carbon, or produce hydrogen.
- *Climate change research*: Deliver improved climate data and models for policy makers to determine safe levels of greenhouse gases for the Earth system. By 2013, substantially reduce differences between observed temperature and model simulations at subcontinental scales using several decades of recent data.
- *Environmental remediation*: Develop science-based solutions for cleanup and long-term monitoring of DOE contaminated sites. By 2013, a significant fraction of DOE's long-term stewardship sites will employ advanced biology-based clean up solutions and science-based monitors.
- *Medical applications and measurement science*: Develop intelligent biomimetic electronics that can both sense and correctly stimulate the nervous system and new radiopharmaceuticals for disease diagnosis.
- *Facilities*: Manage facilities operations to the highest standards of overall performance using merit evaluation with independent peer review.

The Life Sciences Division within the BER manages a diverse portfolio of research to develop fundamental biological information and to advance technology in support of DOE's missions in biology, medicine, and the environment. Specific research areas include:

- *Genomes to life research* – to underpin biotechnology solutions for energy, the environment, carbon sequestration, and biothreat defense. This program will develop high throughput, genome-scale technologies needed to understand the workings of biological systems from the nature of multiprotein 'molecular machines' to the regulatory networks that control them to the complex workings of natural microbial communities. A key

aspect is the development of the computational capabilities and systems that will be needed to model complex biological systems. This is a joint program with the Office of Advanced Scientific Computing Research.

- *Human genome research* – to create and apply new technologies and resources in comparative genomics, the use of model systems, and information management for identifying the genes and their regulatory elements within the human genome.
- *Microbial genome research* – to characterize and exploit the genomes and diversity of microbes with potential relevance for energy, bioremediation, or global climate.
- *Low dose radiation research* – to understand and characterize the risks to human health from exposures to low levels of radiation.
- *Structural biology user facilities* – to develop and support DOE national user facilities for use in fundamental structural biology.
- *Structural biology research* – to develop novel technologies for high throughput determination of protein structure and function.
- *ELSI research* – to anticipate and address ethical, legal, and social implications (ELSI) arising from genome research.

The Environmental Remediation Sciences Division (ERSD) within the BER supports research that will provide the fundamental scientific knowledge needed to address the challenging environmental problems that hinder the remediation of contaminated environmental sites and treatment of stored waste and contaminated waters across the DOE complex. The Division is currently made up of two research programs (the Environmental Management Science Program and the Natural and Accelerated Bioremediation Research program), one research laboratory (the Savannah River Ecology Laboratory), and a DOE user facility (the William R. Wiley Environmental Molecular Sciences Laboratory). The goal in bringing these programs together in one Division is to increase their effectiveness through coordination and integration of the research supported in the individual programs.

In addition to the programs run by the ERSD, it is involved in the following multiagency programs:

- Environmental Molecular Science Institutes (NSF-DOE Partnership). The ERSD, together with the Chemical Sciences, Geosciences, and Biosciences Division of the DOE Office of Basic Energy Sciences have teamed with the National Science Foundation to establish several Environmental Molecular Science Institutes (EMSIs). The EMSI program is aimed at increasing the fundamental understanding of molecular-level process in natural environments, including those impacted by human activities. Five-year grants are awarded competitively to universities and National Laboratory partners. NSF funding is used to support the university researchers and DOE funding is used to support National Laboratory participation. ERSD currently supports two EMSIs, one based at the University of Notre Dame and the other based at the State University of New York at Stony Brook.

The Medical Science Division (MSD) within the BER supports fundamental research and technology development in medicine, particularly in the fields of nuclear medicine, imaging sciences, and neurosciences.

The goal of the research and development programs conducted by the MSD is to utilize current advances in science and technology to develop innovative diagnostic and treatment solutions to important human health problems. The DOE is uniquely capable of advanced technological solutions to medical problems because of its unsurpassed expertise in the physical sciences, particularly in physics, chemistry, engineering, and computational sciences.

The current programs of the MSD are an extension of the original charge of the Atomic Energy Commission (AEC), "to exploit nuclear energy to promote human health." From the production of a few medically important radioisotopes in 1947, to the development of production methods for radiopharmaceuticals used in standard diagnostic tests for millions of patients throughout the world, to the development of ultrasensitive diagnostic instruments, for example, the positron-emission tomography scanner, the DOE medical sciences program leads progress in the field on nuclear medicine. Today, the MSD program has incorporated recent developments in radiochemistry, genomic sciences, and structural biology to establish a new era in mapping the human brain, and is using highly specific radiotracers and instruments to more precisely diagnose neuropsychiatric illnesses and cancer.

The DOE National Laboratories have great expertise in development of both large instruments (neutron and light sources, high field magnets, lasers, and supercomputers) as well as very small instruments (microengineering labs on a chip). Coordinated programs in the DOE National Laboratories, universities, and industry are directed to developing an artificial retina to restore sight to the major causes of blindness; development of clinical instruments to

image a moving patient; and using techniques developed in astronomy, visualize cells in the far reaches of the eye without distortion.

In addition, the DOE and Environmental Protection Agency (EPA) collaborate on research and computing resources. For example, the linking of two national supercomputers, will take place under a Memorandum of Understanding (MOU) signed by EPA and the DOE. High performance computing will allow better and faster runs of environmental models such as the Community Multi-Scale Air Quality model, an important tool for states to meet upcoming deadlines for their air quality attainment plans.

Work in computational toxicology, the application of computer-based statistical techniques, and molecular genetics that allow chemical testing based on a chemical's molecular structure and its effects on genes, will also be accelerated by this agreement. Computational toxicology can reduce animal testing and provide better toxicity information for chemicals in a faster manner.

EPA will also benefit under the MOU from access to DOE's Joint Genome Institute. Genomics is a new area of biology, derived from the large-scale DNA sequencing efforts of the human genome, and holds the potential to reveal molecular pieces of the toxicity pathway and improve chemical risk assessments and the evaluation of the health of ecosystems.

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Environmental Protection Agency, US

Patricia M Nance

In July 1970, the White House and Congress worked together to establish the Environmental Protection Agency (EPA) in response to the growing public demand for cleaner water, air, and land. Before the establishment of the EPA, the federal government was not structured to make a coordinated attack on the pollutants that harm human health and degrade the environment. The EPA was assigned the daunting task of repairing the damage already done to the natural environment and to establish new criteria to guide Americans in making a cleaner environment a reality. EPA's mission is to protect human health and to safeguard the natural environment – air, water, and land – upon which life depends.

The EPA employs 18 000 people across the country, including the headquarters offices in Washington, DC, 10 regional offices, and more than a dozen labs. EPA's staff is highly educated and technically trained; more than half are engineers, scientists, and policy analysts. In addition, a large number of employees are legal, public affairs, financial, information management, and computer specialists. The Administrator, who is appointed by the President of the United States, leads EPA. The organizational chart for the EPA is given below.

EPA Offices

Each of the EPA's Offices is responsible for specialized areas involved with the protection of the environment and human health. The Office of the Administrator provides executive and logistical support for the EPA Administrator and the staff offices that directly support the Administrator. The Administrator is responsible to the President, and is assisted by the Deputy Administrator and staff offices. The Office of the Administrator supports the leadership of EPA's programs and activities to protect human health and safeguard the air, water, and land upon which life depends.

The Office of Administration and Resources Management's mission is to provide management, infrastructure, and operations support to EPA's ~150 offices and laboratories nationwide. EPA strives to make its buildings as energy-efficient and sustainable as possible to serve as models of healthy workplaces with minimal environmental impacts. From the award winning Science and Technology Center in Kansas City, Kansas, and New England Regional Laboratory in Chelmsford, Massachusetts, to green power purchases at the Region 2 Office in New York City, EPA facilities are demonstrating the principles of sustainable design.

The Office of Air and Radiation oversees the air and radiation protection activities of the Agency including national programs, technical policies, and regulations.

The American Indian Environmental Office coordinates the Agency-wide effort to strengthen public health and environmental protection in Indian Country, with a special emphasis on building Tribal capacity to administer their own environmental programs.

The Chief Financial Officer manages and coordinates EPA's planning, budgeting, analysis, and accountability processes as well as provides financial management services.

The Office of Enforcement & Compliance Assurance delivers compliance with US environmental laws while inspiring the regulated community to employ methods that focus on pollution prevention.

The Office of Environmental Justice serves as a focal point for ensuring that communities comprised predominately of people of color or low-income populations receive protection under environmental laws.

The Office of Environmental Information is responsible for establishing an innovative center of excellence that advances the creation, management, and use of information as a strategic resource at EPA. The History Office preserves the Agency's institutional memory and provides background information and publications to the public.

The Office of General Counsel provides legal service to all organizational elements of the Agency with respect to Agency programs and activities. The Office of General Counsel provides legal opinions, legal counsel, and litigation support. In addition, the Office assists in the formulation and administration of the Agency's policies and programs as legal advisor.

The Office of Inspector General conducts audits and investigations of Agency programs and operations.

The Office of International Affairs manages Agency involvement in international policies and programs that cut across Agency offices and regions. It provides leadership and coordination on behalf of the Agency and acts as the focal point on international environmental matters.

The Office of Prevention, Pesticides, and Toxic Substances develops national strategies for toxic substance control and promotes pollution prevention and the public's right to know about chemical risks.

The Office of Research and Development is responsible for the research and development needs of the Agency's operating programs and the conduct of an integrated research and development program for the Agency. The Science Policy Council is responsible within the Agency to address and resolve cross-media, cross-program, and cross-disciplinary science policy issues. The Deputy Administrator chairs the Council.

The Office of Solid Waste and Emergency Response provides policy, guidance, and direction for the land disposal of hazardous wastes, underground storage tanks, solid waste management, encouragement of innovative technologies, source reduction of wastes, and the Superfund Program.

The Office of Water is responsible for the Agency's water quality activities including development of national programs, technical policies, and regulations relating to drinking water, water quality, groundwater, pollution source standards, and the protection of wetlands, marine, and estuarine areas.

EPA Activities

EPA leads the nation's environmental science, research, education, and assessment efforts.

Develop and Enforce Regulations

EPA works to develop and enforce regulations that implement environmental laws enacted by Congress. EPA is responsible for researching and setting national standards for a variety of environmental programs, and delegates to states and tribes the responsibility for issuing permits and for monitoring and enforcing compliance. Where national standards are not met, EPA can issue sanctions and take other steps to assist the states and tribes in reaching the desired levels of environmental quality.

Offer Financial Assistance

In recent years, between 40% and 50% of EPA's enacted budgets have provided direct support through grants to State environmental programs. EPA grants to States, nonprofit organizations, and educational institutions support high-quality research that will improve the scientific basis for decisions on national environmental issues and help EPA achieve its goals. EPA provides research grants and graduate fellowships. The Agency supports environmental education projects that enhance the public's awareness, knowledge, and skills to make informed decisions that affect environmental quality. The Agency also offers information for state and local governments and small businesses on financing environmental services and projects. EPA also provides other financial assistance through programs as the Drinking Water State Revolving Fund, the Clean Water State Revolving Fund, and the Brownfields program.

Perform Environmental Research

At laboratories located throughout the nation, EPA works to assess environmental conditions and to identify, understand, and solve current and future environmental problems. Further, it integrates the work of scientific partners such as nations, private sector organizations, academia, and other agencies; and provides leadership in addressing emerging environmental issues and in advancing the science and technology of risk assessment and risk management.

Sponsor Voluntary Partnerships and Programs

The Agency works through its headquarters and regional offices with over 10 000 industries, businesses, nonprofit organizations, and state and local governments on over 40 voluntary pollution prevention programs and energy conservation efforts. These partners set voluntary pollution-management goals; examples include conserving water and energy, minimizing greenhouse gases, slashing toxic emissions, re-using solid waste, controlling indoor air pollution, and getting a handle on pesticide risks. In return, EPA provides incentives like vital public recognition and access to emerging information.

Further Environmental Education

EPA advances educational efforts to develop an environmentally conscious and responsible public, and to inspire personal responsibility in caring for the environment.

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Relevant Website

http://www.epa.gov – US EPA website (for more information about EPA or specific EPA offices).

European Centre for Ecotoxicology and Toxicology of Chemicals

Michael Gribble

History

The European Centre for Ecotoxicology and Toxicology of Chemicals AISBL (ECETOC) is a scientific, nonprofit, noncommercial association founded in 1978. ECETOC is financed by over 45 companies with interests in the manufacture or use of chemicals. A stand-alone organization, it was established to provide a scientific forum in which the extensive specialist expertise of the European industry associated with chemicals, specialty chemicals, pharmaceuticals, agrochemicals, consumer products, and food could be harnessed to research, review, assess, and publish studies on the ecotoxicology and toxicology of chemicals. The main objective of these activities is to identify, evaluate, and minimize any potentially adverse effects on health or the environment that might arise from the manufacture and use of chemicals.

A Scientific Committee (SC) comprising leading industry scientists in the field of health and environmental sciences is appointed to direct and peer-review the work program and outputs of ECETOC. In 2001, leading scientists from academia joined the industry scientists in ECETOC's SC and peer-review panel, reinforcing and extending the range of expertise available to guide and test the ECETOC science program and its outputs.

Vision

ECETOC supports the safe manufacturing and use of chemicals, pharmaceuticals, and biomaterials through sound science.

Mission

ECETOC acts as an independent, credible peer-reviewed technical resource to all concerned with the identification of research needs and provision of scientific rationale for the assessment of health effects and environmental impact, and thereby to

justify industry's license and freedom to operate. Strategic objectives include: (1) promoting the use of sound science in both industry and regulatory decision-making and report on the results; (2) in close consultation with ECETOC members, defining the scope, managing the progress, and interacting with research programs; (3) providing a forum for regulators, academic and industrial scientists for the evaluation of the safe use of chemicals and their associated products; (4) contributing to understanding of the societal issues associated with health assessment and environmental safety of substances; and (5) identifying emerging issues that are of importance to ECETOC member companies.

Membership Criteria

Membership is based on the principle of scientific participation. Any company that is legally constituted according to the laws and customs of its country of origin and has a registered office in a European country can be a Member of the Association, provided it is engaged in the industrial manufacture, processing, or use of chemicals and has appropriate expertise that enables it to contribute to ECETOC's strategic objectives.

Partnerships

ECETOC's relationship with academia was established and reinforced through partnerships in the Task Force activities, collaborative European Commission-sponsored research projects, and in successful joint projects such as symposia organized with the European Environmental Mutagen Society (EEMS).

In 1997, drawing on its networks with leading scientists in academia and regulatory agencies, ECETOC was actively engaged in the founding of the chemical industry's Long-range Research Initiative (LRI). ECETOC continues to provide the essential scientific input to the development and management of many of the program areas funded by European Chemical Industry Council (Cefic).

Key Activities

ECETOC facilitates the networking of suitably qualified industry scientists with relevant skills and expertise, complemented, where appropriate, with experts from academia and/or regulatory agencies. The output includes workshops, technical reports, and monographs, reflecting the current state of the science for the issues under review.

ECETOC operates by coordinating efforts by chemical manufacturers, processors, and users to study and attempt to resolve the ecotoxicological and toxicological problems that may result from the manufacture, processing, and use of chemicals.

ECETOC aims to act as a scientific advisor to organizations such as the Cefic and other industry organizations with related interests. Commercial, political, and advocacy activities are strictly excluded from the modus operandi of the Association.

ECETOC cooperates in a scientific context with intergovernmental agencies, governments, health authorities, and other public and professional institutions with interests in ecotoxicological and toxicological issues relating to chemicals. ECETOC has become a valued partner with the European Commission and with many other regulatory bodies in the development of European Union chemicals legislation. For example, recognizing the ongoing need for improved approaches for evaluating the risks to humans and the environment arising from exposure to chemicals, ECETOC has supported a range of activities of direct relevance to the European Union's REACH (Registration, Evaluation, Authorization of Chemicals) chemicals legislation. REACH-related activities have included a task force on Targeted Risk Assessment, and other task forces have addressed informed testing strategies, appropriate application of human data, and alternative methodologies including quantitative structure–activity relationships.

A Specific Substances program reflects a steady demand from participating companies for ECETOC hosting and peer-review of their consortia-driven projects.

Workshops staged in support of the Environmental program have included the 'Water Framework Directive Awareness Workshop', the 'Availability, Interpretation and Use of Environmental Monitoring Data' Workshop, and the 'Ecological Quality' Workshop. In addition, a Stakeholder Event was held to share the developing methodology and associated web tool for ECETOC's 'Targeted Risk Assessment' focused on improving the eventual workability of the REACH Regulations.

Examples of other workshops include one on the 'Use of Human (Epidemiology) Data in Risk Assessment' workshop in conjunction with World Health Organization's International Programme of Chemical Safety (IPCS), and an 'Influence of Maternal Toxicity on Developmental Toxicity' workshop.

Publications

Technical documents are the major work product of the ECETOC organization and a total of 226 have been published and distributed as of the end of 2003.

The full list is available through the ECETOC Secretariat and originals are available for purchase through the ECETOC website.

ECETOC began building its scientific credentials with the preparation of critical reviews, guidance documents, and issue papers, embracing the fundamental aspects of toxicology and ecotoxicology and their interpretation and extrapolation to effects in humans and the environment. The first monographs, published in the early 1980s, dealt with the complex issue of chemical carcinogens. Other publications have covered topics such as mutagenicity, reproductive toxicity, neurotoxicity, skin sensitization, and respiratory allergy. In parallel, in the environmental sciences, other publications have dealt with aspects such as atmospheric and aquatic phototransformation, biodegradation, and bioaccumulation followed the first report on photodegradation of chemicals in the environment.

By 1990, ECETOC had published over 100 reports and was recognized officially by the WHO IPCS and the International Agency for Research on Cancer (IARC). Liaison with these and other agencies such as the European Commission's European Chemicals Bureau (ECB) and European Centre for Validation of Alternative Methods (ECVAM) confirmed and developed ECETOC's role as a key contributor to the development of sound scientific approaches to the safety assessment and consequential responsible environmental management of chemicals.

Data Bases

Databases are frequently part of the contents of the Technical Documents and some are also being provided electronically on CD. Examples of the databases developed by ECETOC include ones on aquatic toxicity, eye and skin irritation, and skin and respiratory sensitizers.

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European Society of Toxicology

Ankur V Dnyanmote and Harihara M Mehendale

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European Society of Toxicology (EUROTOX) is the Federation of national societies of toxicology in Europe, which together have ~7000 members. In addition, EUROTOX counts some 500 individual members who come from 50 countries, mostly from Western Europe. According to its statutes, EUROTOX aims to foster toxicology, both scientifically and educationally, in all countries of Europe. For this, EUROTOX organizes an annual scientific congress, workshops, and postgraduate training courses. Specific activities are organized by the EUROTOX Specialty Sections.

EUROTOX is actively harmonizing toxicology education and training, having established the European Register of Toxicologists in 1994. It participates in the worldwide recognition of toxicologists as recently started under the auspices of the International Union of Toxicology (IUTOX). Furthermore, EUROTOX honors annually a distinguished European toxicologist by its Merit Award. Important recent research contributions are honored by inviting an outstanding toxicologist to present the Gerhard Zbinden Memorial Lecture at the annual congress.

Young toxicologists are encouraged by the annual Young Scientist Award, which is awarded every year to the best presentation at the EUROTOX Congress. Finally, EUROTOX members (i.e., the individual members and all members of the affiliated national societies) are entitled to attend the scientific meetings at a reduced fee, and are given a discount on the subscription rates of *Archives of Toxicology* (published by Springer-Verlag, Heidelberg).

Historically, EUROTOX has its roots in the European Society for the Study of Drug Toxicity, which was founded in 1962 in Zürich. The first annual scientific meeting was held in 1963 in Paris, during which the Statutes of the new society were adopted. As the Society's interests started to extend into toxicology areas other than drug toxicology, it was

decided to change the name into European Society of Toxicology (EST).

This was done at the scientific meeting in 1974 in Carlsbad. In the late 1970s and early 1980s national toxicology societies grew rapidly, both in number and in membership. Thus, 14 national societies of toxicology (Finland, France, the German Federal Republic, the German Democratic Republic, Hungary, Ireland, Italy, The Netherlands, Norway, Poland, Spain, Sweden, Switzerland, and the United Kingdom) and EST decided to found the Federation of European Societies of Toxicology (FEST), which was done at the EST congress in Kuopio, 1985.

As this soon turned out to create unnecessary duplications, EST changed its Statutes, adopted the name EUROTOX, and EST and FEST merged at the 5th IUTOX Congress in Brighton, 1989. In the years thereafter EUROTOX grew steadily, now encompassing also Austria, Belgium, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Greece, Latvia, Macedonia, Portugal, Romania, Russia, Slovak Republic, Slovenia, Turkey, and Ukraine.

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European Union and Its European Commission

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The year 2004 was a significant year for the European Union (EU) and its European Commission as the number of EU member countries (or 'Member States') expanded from 15 to 25. The 25 Member States include Austria, Belgium, Cyprus, the Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, the Netherlands, Poland, Portugal, Slovakia, Slovenia, Spain, Sweden, and the United Kingdom. In addition, other countries such as Bulgaria and Romania may be joining the EU before 2010.

The EU was established as a family of democratic European countries, committed to working together for peace and prosperity. It is a unique international organization in that its member states have set up common institutions to which they delegate some of their sovereignty so that decisions on specific matters of joint interest can be made democratically at the EU level. The EU's beginning came after World War II, with the idea of the EU based on the desire to prevent such killing and destruction from ever happening again.

EU-Wide Institutions

There are five EU-wide institutions, each with a specific role:

- *The European Parliament* (EP) is elected by the people within Member States every 5 years. Parliament's principal roles include examining and adopting European legislation (under the codecision procedure, Parliament shares this power equally with the Council of Ministers), to approve the EU budget, to exercise democratic control over the other EU institutions, and to assent to important international agreements such as the accession of new EU Member States and trade or association agreements between the EU and other countries. The EP has parliamentary committees to deal with particular issues, for example, foreign affairs, budgets, and the environment.
- *The Council of the European Union*, representing the governments of the Member States, is the main legislative and decision-making body in the EU. It brings together the representatives of the Member State governments, which are elected at the national level. It is the forum in which the representatives of Member State governments can assert their interests and reach compromises. The members of the Council meet regularly at the level of working groups, ambassadors, and ministers, or, for deciding the major policy guidelines, at the level of presidents and prime ministers.

The Council, together with the EP, sets the rules for all the activities of the European Community (EC), which forms the first 'pillar' of the EU. It covers the single market and most of the EU's common policies, and guarantees freedom of movement

for goods, persons, services, and capital. In addition, the Council is the main EU institution responsible for the second and third 'pillars', that is, intergovernmental cooperation on common foreign and security policy and on justice and home affairs.

- *The European Commission*, the driving force and executive body of the EU: it is the institution where much the of the EU's day-to-day work is done. It drafts proposals for new European laws, which it presents to the EP and the Council. The Commission makes sure that EU decisions are properly implemented and supervises the way EU funds are spent. It also sees that everyone abides by the European treaties and European law. The president is chosen by the governments of the EU Member States and must be approved by the European Parliament. The other members are nominated by the member governments in consultation with the incoming president and must also be accepted by Parliament.
- *The Court of Justice* ensures compliance with the common rules in the EU, and settles disputes over how the EU treaties and legislation are interpreted. Member State courts must ask the Court of Justice when they are in doubt about how to apply EU rules. Individual persons can also bring proceedings against EU institutions before the Court.
- *The Court of Auditors* controls the sound and lawful management of the EU-wide budget. The funds available to the EU must be used legally, economically, and for the intended purpose.

Other Important EU Bodies

These five institutions described above are flanked by five other important EU bodies, that is, the European Economic and Social Committee (expresses the opinions of organized civil society on economic and social issues), the Committee of the Regions (expresses the opinions of regional and local authorities), the European Central Bank (responsible for monetary policy and managing the euro), the European Ombudsman (deals with citizens' complaints about misadministration by any EU institution or body), and the European Investment Bank (helps achieve EU objectives by financing investment projects).

How Environmental and Safety Needs and Issues are Addressed within the EU

Many environmental and safety issues in Europe could not be tackled without joint action by all EU countries. For example, the EU's European Environment Agency gathers information on the state of the EU environment, enabling protective measures and laws to be based on solid data, and the European Chemicals Agency is being created to work on and implement the EU-wide effort on the human and environmental safety of the uses and exposures to 'existing' and 'new' chemicals called the Registration, Evaluation, Authorisation of CHemicals (REACH).

In all, the EU has adopted over 200 environmental protection directives that are applied in all Member States. Most of the directives are designed to prevent air and water pollution and encourage waste disposal. Other major issues include nature conservation and the supervision of dangerous industrial processes. The EU wants transport, industry, agriculture, fisheries, energy, and tourism to be organized in such a way that they can be developed without destroying natural resources and leading to sustainable development. For example, the EU has cleaner air because of the EU decisions in the 1990s to put catalytic converters into all cars and to get rid of the lead added to gasoline.

The General Product Safety Directive

The safe uses of chemicals in consumer products and articles (e.g., in toys, clothing, and furniture) are covered in part by the General Product Safety Directive (GPSD), revised in 2004. The GPSD includes a rapid exchange (RAPEX) notifications system to quickly share suspected or known safety issues about nonfood consumer products between Member States. The European Commission typically receives several safety alerts each week via RAPEX. Among the dangers presented are the risk of choking and suffocation, electric shock, and fire. The types of products most often found in these alerts are toys, followed by electrical appliances. The EU has a separate rapid alert system on food safety, which also makes weekly summaries of alerts available.

The European Commission's Nonfood Scientific Committees

In 2004, the European Commission reorganized its nonfood scientific committees following the creation of the European Food Safety Authority (EFSA) and the transfer to the Authority of responsibilities for risk assessment on food-related issues previously carried out by some of the scientific committees. The Commission reviewed and refocused the work of its three remaining nonfood scientific committees and created three new committees: Scientific Committee on Consumer Products (SCCP), Scientific Committee

on Health and Environmental Risks (SCHER), and Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR). The reorganized committees provide the EU with a more proactive and flexible approach to risk assessment. The restructuring also ensures appropriate cooperation and coordination between these committees and other Community bodies responsible for scientific advice such as the EFSA, the European Agency for the Evaluation of Medicinal Products, and the European Centre for Disease Prevention and Control.

Specifically, the SCCP advises on questions related to the safety of consumer products (other than food). Examples of the type of issues within the scope of the SCCP include the safety and allergenic properties of cosmetic products and issues relating to the safety of toys, textiles, clothing, domestic products, and consumer services such as tattooing. The SCHER examines issues relating to the toxicity and ecotoxicity of chemical, biochemical, and biological compounds whose use may have harmful consequences for human health and the environment. The SCENIHR advises on emerging or newly identified risks and on broad issues requiring a comprehensive assessment of risks to consumer safety or public health. It will also give an opinion on human health issues not covered by other EU risk assessment bodies. Examples of the type of issues within the scope of the SCENIHR include antimicrobial resistance, nanotechnology and other new technologies, medical devices including those incorporating substances of animal and/or human origin, tissue engineering, physical hazards such as noise and electromagnetic fields (from mobile phones, transmitters, and electronically controlled home environments), and methodologies for assessing new risks.

The European Commission's Joint Research Centre

The European Commission's Joint Research Centre (JRC) is a source of independent scientific and technical reference for European policy makers, serving the European Commission, the EP, the Council, and the Member States. The JRC's seven scientific institutes carry out research of direct concern to EU citizens, working with industry, universities, other research institutes, and Member States. The JRC is among the European Commission's 36 Directorates-General (DGs). The DGs are specialized services within the European Commission. Examples of other DGs include the Brussels, Belgium-based Directorate General (the JRC's central coordination and administrative body), the Institutional and Scientific Relations Directorate, the Programme and Resource Management Directorate, and the DG Health and Consumer Protection, with the overall goal of promoting a better quality of life by ensuring a high level of protection of consumers' health, safety, and economic interests as well as of public health. The seven JRC institutes are the Institute for Health and Consumer Protection (IHCP), the Institute for Environment and Sustainability (IES), and the Institute for Protection and Security of the Citizen (IPSC) in Ispra, Italy, the Institute for Reference Materials and Measurements (IRMM) in Geel, Belgium, the Institute for Transuranium Elements (ITU) in Karlsruhe, Germany, the Institute for Energy (IE) in Petten, the Netherlands, and the Institute for Prospective Technological Studies (IPTS) in Seville, Spain.

The JRC's IHCP includes the European Centre for the Validation of Alternative Testing Methods (ECVAM), an international reference center for the development and scientific acceptance of alternative testing methods to replace, reduce, and refine the use of laboratory animals. IHCP also includes the Physical and Chemical Exposure (PCE) Unit providing scientific understanding, information, and assessment tools to support the Commission services in evaluating and quantifying exposure and risk assessments for environmental stressors such as chemicals, biological contaminants, radiation, and noise. The PCE's work includes a new European information system on risks of exposures to chemicals in consumer products and articles (EIS-ChemRisks). This effort serves as a European-wide expert and stakeholders 'network of networks' to systematically exchange and assess information on risks from chemicals released from consumer products/articles. EIS-ChemRisks will support the GPSD and may provide technical support to the relevant aspects of REACH. Further, as part of EIS-ChemRisks, the PCE is establishing a single Web-based gateway to all major European initiatives in the field of human exposure to chemicals contained and released from products/articles. This gateway is being designed to act as an interactive EU-wide information source and a common communication tool for the user society to develop and continuously update reference data and tools, and includes the 'European Exposure Assessment Toolbox' as a set of tools and reference data to enable harmonized exposure assessment procedures within the EU.

In addition, the IHCP includes the European Chemicals Bureau (ECB). Current working areas of the ECB, at least until the new European Chemicals Agency officially begins operation, are collecting information on new and existing chemicals, and providing scientific and technical support to the conception, development, implementation, and

monitoring of EU policies on dangerous chemicals. Further, the ECB supports the development and harmonization of testing methods such as quantitative structure activity relationships, the legal classification and labeling of substances; the management of risk assessment of substances; the notification of new substances; the authorization of biocides; and the information exchange on import and export of dangerous substances.

The European Chemical Substances Information System (ESIS) serves as a portal to the existing chemicals data sets maintained by ECB. ESIS includes information related to the European Inventory of Existing Chemicals (EINECS), the European List of Notified Chemical Substances (ELINCS), High Production Volume Chemicals (HPVCs) and Low Production Volume Chemicals (LPVCs), Classification, and Labeling, IUCLID (International Uniform Chemical Information Data Base) Chemical Data Sets, and the EU's chemical risk assessment process. IUCLID is the basic tool for data collection and evaluation within the EU Risk Assessment Programme, as well as under the OECD Existing Chemicals Programme. The Risk Assessment reports are extensive documents written in first draft by EU member states, and the ECB also mediates meetings that attempt to reach consensus on the conclusions of the risk assessments.

Further, the IHCP's Biomedical Materials and Systems (BMS) Unit conducts applied and exploratory research studies in the area of bioengineering, materials and surface sciences, medical photonics, and nuclear technology for health application. Finally, IHCP's 'Biotechnology and GMOs' unit provides scientific and technical support to EU legislation in biotechnology through the development, validation, and harmonization of detection methods of GMOs (genetically modified organisms) and genetically modified foods.

The European Pollutant Emission Register

Another example of an EU-wide database established by the European Commission is the European Pollutant Emission Register (EPER), developed in 2004 as the first European-wide register of industrial emissions into air and water. Member States have to produce a triennial report on the emissions of industrial facilities into the air and waters, and the report covers 50 pollutants. EPER gives access to information on the annual emissions of thousands of industrial facilities in the Member States as well as Norway. It lets users group information easily, by pollutant, activity (sector), air and water (direct or via a sewerage system), or by country. In addition, it is possible to see detailed data on individual facilities by searching by name or by clicking on a map. Users can also look for the sources of a particular pollutant. The European Commission has made these data publicly accessible on a website hosted by the European Environment Agency (EEA).

Support of Research

The EU has been increasingly active in helping European research to achieve scientific excellence. In a variety of sectors covering the whole spectrum of modern technology, the EU finances projects undertaken by research centers, universities, and industry. The emphasis is on putting research and innovation to work for precise socioeconomic objectives, such as job creation and improved quality of life. Current research priorities include, among others, life sciences, nanotechnology, space, food quality, sustainable development, and the knowledge-based society. The European Commission has multi-year Framework Programmes for Research, for example, the 6th Framework Programme (2003–2007), which supports research in toxicology, risk assessment, and numerous other areas.

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http://europa.eu.int – European Commission, See index pages of The European Union at a Glance, EU News, Environment Directorate-General, Food Safety, Health and Consumer Protection Directorate-General, Health and Consumer Protection Directorate-General, Calls for Research Tenders, Risk Assessment Activities.

http://ecb.jrc.it – European Commission, Technical Guidance Document on Risk Assessment in Support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances. Commission Regulation (EC) No 1488/94 on Risk Assessment for existing substances, and Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market. Part 1 (2003).

http://ihcp.jrc.it – European Commission, Institute for Health and Consumer Protection (IHCP) website.

http://www.eper.cec.eu.int – European Pollutant Emission Register (EPER).

Flavor and Extract Manufacturers Association

Gwendolyn L Ball

The Flavor and Extract Manufacturers Association (FEMA), founded in 1909, represents the interests of its members in the US flavor industry including flavor manufacturers, users, ingredient suppliers, and other interested parties. FEMA maintains a strong scientific program to evaluate the safety of flavor ingredients.

Objectives

- To support the FEMA Expert Panel process for the independent evaluation of the safe use of flavor ingredients, using panel members with recognized expertise in areas such as toxicology, pharmacology, pathology, and medicine.
- To serve as an effective advocate for FEMA members in regulatory affairs and in collaboration with other organizations with related interests.
- To effectively communicate with members regarding current issues.
- To provide education and training services.
- To protect the intellectual property of FEMA members by addressing current regulatory issues.

Activities

- Convene the FEMA Expert Panel.
- Interact with national and international groups involved in the evaluation and/or regulation of flavor ingredients, including the Joint FAO/WHO Expert Committee on Food Additives (JECFA).
- Provide guidance to members on technical and regulatory issues.

Publications

- Twenty-one FEMA GRAS (generally recognized as safe) publications, the latest in 2003, with tables of use levels on which the FEMA Expert Panel based its GRAS determinations.
- FEMA GRAS Assessments of individual flavor ingredients made by the Expert Panel published in scientific journals.
- Flavor & Fragrance Ingredient Data Sheets.

Meetings

FEMA holds an Annual Convention in the spring and a Fall Symposium. Workshops and seminars on regulatory compliance and special issues are also held.

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Acknowledgment

Adapted from the FEMA website at http://www.femaflavor.org

Food and Agriculture Organization of the United Nations

Manfred Luetzow*

The Food and Agriculture Organization of the United Nations (FAO) was established on October 16, 1945, with the goal to promote global sustainable development of agriculture, fisheries, forestry, and food production and security, quality, and safety as well as the related socioeconomic issues in the member countries. There are 187 member countries plus the European Community in the organization (as of 2003). The headquarters of FAO is located in Rome and is organizationally divided into eight departments, with five regional, five subregional, five liaison, and more than 100 country offices with functions dealing with regional and in-country

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activities and projects. Five specialized offices within FAO headquarters assist the director-general in directing and managing this, the largest specialized agency of the United Nations system.

Within the FAO headquarters there are two departments with responsibilities that include toxicology-related activities. They are the Economic and Social Department (ES) and the Agriculture Department.

The ES Department, within its Food and Nutrition Division (ESN), houses the Secretariat of the Joint FAO/WHO Food Standards Program established in 1962 and implemented through the Codex Alimentarius Commission (CAC). CAC is an intergovernmental body that meets alternately in Rome and Geneva at the headquarters of the two parent organizations, FAO and World Health Organization (WHO), annually. The aims of CAC are to protect the health of the consumer and facilitate international trade through the harmonization of national legislation and regulations through establishing international codes of practice, general standards for food additives and contaminants, food commodity standards, maximum limits for residues of pesticides and residues of veterinary drugs in foods, food labeling standards, methods of analysis, etc. The preparatory work for these activities is accomplished by the CAC subsidiary bodies, the Codex General Subject and Commodity Committees. Three of these Codex committees are especially important in this connection: the Codex Committee on Food Additives and Contaminants (CCFAC), the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF), and the Codex Committee on Pesticide Residues (CCPR).

The Food and Nutrition Division also provides the FAO Secretariat of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). This Committee was established in the mid-1950s by FAO and WHO to assess chemical additives in food on an international basis. The first meeting was held in 1956 in response to recommendations made in 1955 at a FAO/WHO Conference on Food Additives meeting in Geneva. JECFA is managed by a Joint FAO/WHO Secretariat.

When the CAC was formed it decided to utilize the expert scientific advice provided by JECFA on matters relating to the toxicological and specifications activities of food additives. A system was established whereby the Codex Committee on Food Additives and Contaminants, a general subject committee, identified food additives that should receive priority attention, which were then referred to JECFA for assessment before being considered for inclusion in Codex food standards.

Over the years, JECFA's responsibilities have been expanded to include evaluation/assessment of food contaminants, naturally occurring toxins, and residues of veterinary drugs in food. JECFA's advice and evaluations are used by several Codex committees (e.g., CCFAC, CCRVDF). JECFA also provides scientific advice directly to FAO, WHO, and their member states. JECFA cooperates very closely with Codex but is not a component of the CAC.

Specialists invited to serve as members of JECFA are independent scientists who serve in their individual capacities as experts and not as representatives of their governments or employers. They also understand that the discussions at the meetings are confidential. The goal is to establish acceptable daily intakes (ADIs) (or equivalent tolerable intakes) for food chemicals and to develop specifications for identity and purity for food additives or maximum residue limits (MRLs) when veterinary drugs are used in accordance with good practice in the use of veterinary drugs.

FAO and WHO have complementary functions in selecting members for JECFA. FAO is responsible for selecting members to deal with the development of specifications for the identity and purity of food additives and the assessment of residue levels of veterinary drugs in food. WHO is responsible for selecting members to deal with the toxicological evaluations of the substances under consideration. Both FAO and WHO invite members who are responsible for assessing intake.

As of 2004, a total of 63 meetings of JECFA have been held and over 2100 food additives including more than 1500 flavoring agents, ~40 contaminants, and 93 veterinary drugs evaluated. The reports are published in the WHO Technical Report Series. The comprehensive toxicological evaluations, which review the data that serve as the basis for the safety assessments, are published in the WHO Food Additives Series. The specifications for food additives and residue evaluations of veterinary drugs are published in the FAO Food and Nutrition Paper Series.

JECFA meetings are convened twice a year, with one session devoted to the evaluation of food additives and contaminants and the other to the evaluation of residues of veterinary drugs in foods. The meetings are open only to the invited experts and the Joint Secretariat. JECFA can hold hearings during the meeting in which those who have submitted data for evaluation are invited to answer specific questions by the committee to clarify the submission. The JECFA procedures do not permit the committee to discuss the substances under review when the nonmembers are present during these hearings.

JECFA is one body: the discussions are held and decisions made in plenary sessions. The drafting, however, is done in separate groups. In the case of food additives, the FAO experts are responsible for proposing specifications of identity and purity for food additives. The three main objectives of the specifications prepared by the committee are to identify the substance that has been subjected to biological testing, to ensure that the substance is of the quality required for safe use in foods, and to reflect and encourage good manufacturing practice.

Experts invited by FAO also prepare Chemical and Technical Assessments (CTA) for the substances on the agenda to provide the committee with the information on the physical and chemical characteristics of the additive, on the raw material(s) used in commercial production of the additive, and on methods of manufacture by which the raw material(s) is converted into a finished commercial food additive. It is acknowledged that some of these data may be trade secrets. Therefore, such data are held in strict confidence. Furthermore, the CTA includes information on impurities including intermediates, functional use(s) with the technological purpose for using the additive and the levels of use on a commodity basis, reactions and fate in food, and effects on nutrients. In the case of contaminants, FAO experts are responsible for gathering information on their occurrence in food and methods for their analysis.

ADIs for food additives and veterinary drugs and provisional tolerable weekly intakes (PTWIs) for contaminants are proposed by the WHO experts.

In JECFA meetings dedicated to the evaluation of veterinary drug residues, the FAO experts are responsible for proposing MRLs for foods of animal origin based on pharmacokinetic and metabolism studies in experimental animals, target animals, and in humans when available. The Committee will consider the following when proposing MRL:

- Radiolabeled residue depletion studies in target animals from zero withdrawal time to periods beyond the recommended withdrawal time (these studies should provide information on total residues, including free and bound residues, and major residue components in order to select a marker residue and target tissue).
- Unlabeled drug depletion studies for analysis of marker residue in target animals including muscle, liver, kidney, fat, eggs, milk, and honey as applicable (this should include studies with appropriate formulations, routes of application, and species using up to maximum recommended doses).
- Methods for routine analysis that may be used by authorities for the detection of residues in target tissue.
- The ADI proposed by the WHO experts.
- The standard daily food intake of 300 g of muscle, 100 g of liver, 50 g of kidney, 50 g of fat, 100 g of eggs, and 1.5 l of milk ('food basket').

Other assumptions and variables may also be involved in determining MRLs, including safety factors used in establishing ADIs, withdrawal times, the contribution of bound residues, and the bioavailability of residues.

A veterinary drug is any substance applied or administered to any food-producing animal, such as meat or milk-producing animals, poultry, fish, or bees, whether used for therapeutic, prophylactic, or diagnostic purposes or for modification of physiological functions or behavior.

The MRL is the maximum permissible concentration of residue resulting from the use of a veterinary drug, expressed in milligram per kilogram or microgram per kilogram on a fresh weight basis. It is based on the type and amount of residue considered to be without toxicological hazard for human health as expressed by the ADI. Consideration is also given to residues of the compound that occur in food or plant origin and/or environment (the same active ingredient may be used as a veterinary drug and pesticide).

Metabolic studies identify and quantify the residues. These studies should simulate the conditions of use of the drug in animal husbandry as closely as practicable. The pharmacokinetics (distribution and elimination) of the residues should be examined between the time of administration of the drug and the time the animals enter the human food supply.

The withdrawal time after administration of a drug is the time during which animals or animal products should not be harvested by fishing, milking, slaughtering, egg collection, etc., for human consumption.

The total residues of a drug in animal-derived food consist of the parent drug, together with all the metabolites and drug-based products that remain in the food after the administration of the drug to food-producing animals. The amount of total residues is generally determined by means of a study using the radiolabelled drug and is expressed as the parent drug equivalent in milligram per kilogram or microgram per kilogram of the food.

The use of veterinary drugs in food-producing animals can result in residues that are neither extractable from tissues nor readily characterized (bound residues). The extractable residues are the

residues extracted from tissues or biological fluids by means of aqueous acidic or basic media, organic solvents, and/or hydrolysis with enzymes to hydrolyze conjugates. The nonextractable residues are obtained by subtracting the extractable residues from the total residues and comprise residues of the drug incorporated through normal metabolic pathways into endogenous compounds (e.g., amino acids, proteins, and nucleic acids) or chemically bound residues derived by interaction of residues of the parent drug or its metabolites with macromolecules. The bioavailable residues are the residues that can be shown, by means of an appropriate method, to be absorbed when fed to experimental (laboratory) animals. In the absence of relevant residue data, it should be assumed that all of the residues are bioavailable and that its potency is equal to that of the most toxic component of the residue.

A marker residue is a residue whose level decreases in a known relationship to the level of total residues in tissues, eggs, or milk. In other words, a marker residue is, or is representative of, the residue of toxicological concern in the target tissue and/or milk/eggs. Identification of a marker residue is important because it is the substance determined for control purposes in the enforcement of MRLs by the national authorities and other parties concerned.

A target tissue is defined within JECFA as the edible animal tissue (muscle, liver kidney, or fat) for which the MRL is recommended and that may be analyzed for purposes of the enforcement of the MRL.

In assessing the safety of veterinary drug residues, the Expert Committee determines the MRL expressed in terms of a named marker residue for target tissues of interest of individually specified animal species. The committee identifies at least two target tissues whenever possible, with one being muscle or fat and the other liver or kidney. Selection of an appropriate target tissue permits regulation of the MRL in international trade in meat (liver and kidney not available) as well as in national control programs.

In summary, when an ADI is established, consideration of the estimated intakes of the relevant foods by human beings allows an assessment to be made of a safe and acceptable residue level for the relevant animal tissue(s). If the levels of residues estimated from supervised trials, when the drug is administered according to good practices in the use of veterinary drugs (only the amount which is necessary to obtain the desired effect is used), are below those considered toxicologically acceptable, then the levels determined by good practice will dictate the acceptable residue level, provided that practical analytical methods are available at that level for routine residue analysis. The committee is reluctant to establish MRLs lower than a level twice that of the limit of quantitation of the previous analytical method.

If the levels of residues found in practice exceed those determined to be acceptable from the toxicological evaluation and consumption data, then drug use in the food-producing animals may need to be modified to reduce residue concentrations in edible tissues to acceptable levels. Possible modifications include extending the withdrawal periods and changing the drug dosage, formulation, or method of application.

When it has been determined that an ADI is unnecessary because the compound of interest is produced endogenously in human beings and animals, then the establishment of an acceptable residue limit is also unnecessary. At the other extreme, when an ADI has not been allocated because, on toxicological grounds, the safety of the compound cannot be assured, then no acceptable residue limit should be established.

The principles outlined here apply to the evaluation of residues of all veterinary drugs. For the establishment of tolerance limits for residues of certain chemotherapeutic agents, however, the antimicrobial properties of the residues must also be taken into account. Antimicrobial properties will become the determining factor in safety evaluation when the toxicity of the substances to be considered is so low that their residues in food could, from the toxicological viewpoint, be tolerated even at the height of therapeutically effective tissue concentrations. At the microbial level, concern for food safety is centered on the question of whether or not residues of antimicrobial agents ingested via food of animal origin pose a danger to human health by exerting a selective pressure on the intestinal flora and thus favoring the growth of microorganisms with natural or acquired resistance.

The committee does not attempt to derive withdrawal times for veterinary drugs that are necessary to ensure that the concentration of residues in food will be below the established MRL. Residue kinetics and withdrawal times depend on various parameters strictly linked to a given veterinary drug including, but not limited to, the pharmaceutical formulation, the concentration of the active ingredient, the dosage, and the route of administration. The determination of the appropriate withdrawal time for a given veterinary drug in order to comply with an assigned MRL is the responsibility of the appropriate national authority. Nevertheless, when determining MRLs, the committee verifies that those that it recommends can be achieved through realistic

withdrawal times and established good practices in the use of veterinary drugs.

To demonstrate how the ADI and MRLs are linked together and how the maximum ingested residue of the parent drug and its equivalents is calculated, the following information is presented as extracted from the Food and Nutrition Paper No. 41/6 and the Technical Report Series No. 851.

Based on the ADI of 0–6 $\mu g\,kg^{-1}$ of body weight for levamisole (parent drug) established by the committee, the permitted daily intake of the parent drug and/or its equivalents is 360 μg for a 60 kg person.

The following factors were considered in estimating the MRLs: the ADI; the parent drug is a suitable residue marker and is 2.4% of the total residues; all the residues in muscle and fat are equivalent to the parent drug; 50% of the residues in liver are bound and 15% of these bound residues are bioavailable; the residues in kidney are qualitatively similar to those in liver; it is assumed that all bioavailable residues in liver and kidney are equivalent to the parent drug; and the residues are similar in cattle, sheep, and pigs.

The committee recommends MRLs of 10 $\mu g\,kg^{-1}$ for muscle, kidney, and fat and 100 $\mu g\,kg^{-1}$ for liver of cattle, sheep, poultry, and swine expressed as the parent drug. Because residues in eggs at recommended dose level, at 1 day withdrawal, are ~1000 $\mu g\,kg^{-1}$, the committee considered that levamisole should not be used in laying hens.

The previous assumptions can be used to calculate maximum theoretical daily intake of levamisole equivalents if a consumer ate the standard meat diet containing concentrations of levamisole at the proposed MRLs. The maximum ingested residue of the parent drug and its equivalents is 397 $\mu g\,day^{-1}$, which consists of 14 $\mu g\,day^{-1}$ of parent drug and 383 $\mu g\,day^{-1}$ of levamisole equivalents. The calculation is shown in **Table 1**.

Considering the inherent uncertainty of the total levamisole equivalents based on levamisole as the marker residue and considering that only a small proportion of the total residues are used to estimate the total levamisole equivalents (397 μg), the committee considered this value to be equivalent to the maximum ADI.

Depending on the subject of the meeting, the outcome of the JECFA meetings will be the ADIs for food additives or veterinary drug residues, PTWIs for food contaminants, MRLs for residues of veterinary drugs, or specifications for identity and purity of food additives. The MRLs and specifications are then discussed by the CCRVDF and CCFAC, respectively, and if found acceptable, forwarded to the CAC for adoption as Codex Maximum Residue Limits and Codex Specifications. The ADIs for food additives and PTWIs for contaminants are used by the CCFAC in the preparation of general standards for food additives and contaminants and in recommending of maximum-use levels of food additives or guideline levels of food contaminants in food. Aside from the Codex committees, the outcomes of the JECFA evaluations are freely available to all parties concerned.

The Agriculture Department, within its Plant Production and Protection Division, includes the FAO Secretariat of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Toxicological and Environmental Core Assessment Groups, otherwise known as the Joint FAO/WHO Meeting on Pesticide Residues (JMPR). JMPR was established following the resolution of the FAO Conference in 1962 by the Codex Alimentarius Commission to recommend MRLs for pesticide residues and environmental contaminants in specific food products, including methods of sampling and analysis to ensure safety of food containing residues.

The JMPR meetings are closed to nonmember participation and are held annually in September, alternately in Rome and Geneva. WHO-invited members are responsible for proposing ADIs for the substances on the agenda. FAO-invited members draft MRLs for substances under evaluation based on findings in supervised field trials conducted in various countries worldwide. The ADI and MRL

Table 1 Calculation of maximum ingested residue of levamisole (parent drug) and its equivalents

Tissue	*Standard intake (kg)*	*MRL ($\mu g/kg^{-1}$)*	*UD (μg)*[a]	*EQ (μg)*[a]		*Total (μg)*
				Free	*Bound*	
Muscle	0.300	10	3	125	0	125
Liver	0.100	100	10	208	31	239
Kidney	0.050	10	0.5	10	2	12
Fat	0.050	10	0.5	21	0	21
Total	0.500		14	364	33	397

[a] UD, unchanged drug; EQ, parent drug equivalents.

proposals are discussed, examined, and the decisions made in the plenary when all the committee members are present. The report of the meeting and the *Evaluations, Part I – Residues* are published in the FAO Plant Production and Protection Paper Series and the *Evaluations, Part II – Toxicology* as a WHO/IPCS publication.

The cooperation between the JMPR and the Codex Committee on Pesticide Residues is close. CCPR identifies those substances which require priority evaluation. After the JMPR evaluation, CCPR discusses the recommended MRLs and forwards them, if they are acceptable, to the Codex Alimentarius Commission for adoption as Codex Maximum Residue Limits.

Contact Details

Food and Agriculture Organization of the United Nations
Viale delle Terme di Caracalla
0189 Rome, Italy
Tel.: +39-06-570-55425
URL: http://www.fao.org

Relevant Website

www.fao.org – The specifications for food additives, and residue evaluations of veterinary drugs are published in the FAO Food and Nutrition Paper Series. The FAO Secretariat maintains a webpage that provides online access to these publications.

Food and Drug Administration, US

Ankur V Dnyanmote and Harihara M Mehendale

The US Food and Drug Administration (FDA) is the federal agency responsible for ensuring that foods are safe, wholesome, and sanitary; human and veterinary drugs, biological products, and medical devices are safe and effective; cosmetics are safe; and electronic products that emit radiation are safe. Its jurisdiction encompasses most food products (other than meat and poultry), human and animal drugs, therapeutic agents of biological origin, medical devices, radiation-emitting products for consumer, medical, and occupational use, cosmetics, and animal feed. The agency grew from a single chemist in the US Department of Agriculture in 1862 to a staff of ~9100 employees and a budget of $1.294 billion in 2001, comprising chemists, pharmacologists, physicians, microbiologists, veterinarians, pharmacists, lawyers, and many others.

The FDA is also responsible for advancing the public health by helping to speed innovations that make medicines and foods more effective, safer, and more affordable; and helping the public get the accurate, science-based information they need to use medicines and foods to improve their health. The FDA also ensures that these products are honestly, accurately, and informatively represented to the public. Some of the agency's specific responsibilities include:

1. *Biologics*
   - Product and manufacturing establishment licensing
   - Safety of the nation's blood supply
   - Research to establish product standards and develop improved testing methods
2. *Cosmetics*
   - Safety
   - Labeling
   - Drugs
3. *Product approvals*
   - Over-the-counter and prescription drug labeling
   - Drug manufacturing standards
   - Foods
4. *Labeling*
   - Safety of all food products (except meat and poultry)
   - Bottled water
   - Medical devices
5. *Premarket approval of new devices*
   - Manufacturing and performance standards
   - Tracking reports of device malfunctioning and serious adverse reactions
   - Radiation-emitting electronic products
6. *Radiation safety performance standards for microwave ovens, television receivers, diagnostic*
   - X-ray equipment, cabinet X-ray systems (such as baggage X-rays at airports), laser products
   - Ultrasonic therapy equipment, mercury vapor lamps, and sunlamps
   - Accrediting and inspecting mammography facilities
   - Veterinary products

7. *Livestock feeds*
   - Pet foods
   - Veterinary drugs and devices

The FDA is an agency within the Department of Health and Human Services and consists of eight centers/offices, whose webpages can be found in the FDA website:

- Center for Biologics Evaluation and Research (CBER);
- Center for Devices and Radiological Health (CDRH);
- Center for Drug Evaluation and Research (CDER);
- Center for Food Safety and Applied Nutrition (CFSAN);
- Center for Veterinary Medicine (CVM);
- National Center for Toxicological Research (NCTR);
- Office of the Commissioner (OC); and
- Office of Regulatory Affairs (ORA).

For general food safety questions, call the FDA Consumer Hotline at 888-INFO-FDA (888-463-6332). For questions about seafood, call the FDA Seafood Hotline at 800-FDA-4010. Questions involving meat or poultry products can be asked at the US Department of Agriculture's hotline at 800-535-4555. In case of emergencies, call 301-443-1240, which is staffed 24 hours a day.

Contact Details

US Food and Drug Administration
5600 Fishers Lane
Rockville, MD 20857-0001, USA
Tel: 1-888-463-6332 (1-888-INFO-FDA)
URL: http://www.fda.gov

Relevant Website

http://www.fda.gov – Website of the US Food and Drug Administration.

Intergovernmental Forum on Chemical Safety (IFCS)

Judy A Stober

History

Effective coordination of activities and strong cooperation among governments and organizations working in the field of chemical safety is key for achieving sound chemicals management. The 1992 United Nations Conference on Environment and Development recognized this need, and action was taken in 1994 at the International Conference on Chemical Safety, where the Intergovernmental Forum on Chemical Safety (IFCS) was formed.

Purpose and Structure

The IFCS was established to improve coordination and cooperation by identifying and building consensus on chemicals assessment and management priorities, coordinating, and monitoring international and regional action related to sound chemicals management.

The IFCS is an overarching, participatory forum where governments meet with intergovernmental and nongovernmental organizations to discuss chemical safety issues and provide policy guidance for the sound management of chemicals, to be implemented by national governments and organizations. All questions concerning chemical risks are within the purview of the Forum. The IFCS monitors performance against indicators, and sets the agenda for research regarding chemicals management and new and emerging environmental health issues. The World Health Organization is the administering agency for the IFCS and its Secretariat. IFCS expenses are covered by voluntary contributions from United Nations Member States and other IFCS participants.

The IFCS structure is unique. It places a strong emphasis on the full and open participation of all sectors relevant to the sound management of chemicals. It engages industry, public interest, science, labor, and academia, as well as governments in discussion and debate that is not constrained by the structures and process of formal negotiations. A key benefit of the Forum is that national concerns may be expressed directly by participants, rather than indirectly through governing bodies of major organizations. While decisions taken are not obligatory, they are taken as authoritative commitments by governments and organizations.

Strong regional participation is a focus for IFCS. Each government and sector is asked to have a single

National Focal Point to act as a conduit for communication on IFCS activities (currently, the forum has representatives for 152 countries). Between sessions of the Forum, the Forum Standing Committee (FSC), under the chairmanship of the President of the Forum, monitors the progress on the work of the IFCS and provides advice and assistance on preparations for the next forum session.

The FSC comprises 25 IFCS participants, including five regional Vice-Presidents (Africa, Asia-Pacific, Central and Eastern Europe, Latin America and Caribbean, and Western Europe and Other Groups), and is supported by an Executive Secretariat. *Ad hoc* working groups are established by the Forum or the FSC to undertake specific tasks between forum meetings, such as the preparation of documents for forum meetings.

Forum Meetings

Forum meetings are convened approximately every 3 years. Forum III was held in October 2000 in Salvador da Bahia, Brazil. Forum III conducted a full review of the IFCS. It established the 'Priorities for Action Beyond 2000', which outline realistic and measurable targets set for defined timeframes in the following priority areas: international assessment of chemical risks; harmonizing classification and labeling of chemicals; exchanging information on toxic chemicals and chemical risks; establishing risk reduction programs; strengthening national capabilities and capacities for the management of chemicals; and preventing illegal international traffic in toxic and dangerous products. Participants at Forum III also adopted the 'Bahia Declaration', a statement to reaffirm commitment to the goals for chemical safety set in 1992 at the United Nations Conference on Environment and Development held in Rio de Janeiro, Brazil.

The fourth session of the Forum (Forum IV) was held from 1 to 7 November 2003 in Bangkok, and hosted by the Royal Government of Thailand. At Forum IV, the IFCS 'took stock' of the progress achieved on the commitments and recommendations made in past Forum meetings and set an agenda for action on chemicals management over the next several years.

The theme of Forum IV was 'Chemical Safety in a Vulnerable World'. As such, the Forum focused on populations that are particularly susceptible to health risks due to chemical exposure, such as children. Governments and stakeholders, for example, were asked to prepare initial national assessments on children's health and chemical safety as a basic information tool to identify priority concerns. Improving the provision of information on chemical risks to workers and consumers was also high on the agenda. Forum IV called for new efforts by industry and governments to generate and make available practical information on hazardous chemicals. Furthermore, governments were provided tools and guidance to implement the Globally Harmonized System for the Classification and Labelling of Chemicals – an international hazard communication system with standardized chemical labels and safety information.

Forum IV also examined the problems associated with acutely toxic pesticides; the widening gap in the ability of developing countries to keep pace with developed countries in implementing chemical safety policies and conventions; capacity building for the sound management of chemicals; and the further development of a strategic approach to international chemicals management (SAICM). The SAICM will be founded on the 'Bahia Declaration' and the 'Priorities for Action Beyond 2000'. The SAICM process will identify gaps in chemicals management regimes, obstacles to achieving current targets, and will seek improvements in the current system of international chemicals management.

Forum V will be hosted by Hungary in 2005/2006.

The IFCS website has more information regarding the IFCS, its officers and participants, and Forum meetings.

Contact Details

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International Agency for Research on Cancer

Peter Boyle

Introduction

The mission of the International Agency for Research on Cancer (IARC) is to promote, by way of international collaborative research and other means, improvement of health through reduction of the incidence and mortality from cancer throughout the world by:

- Conducting research into the occurrence, impact, causes, early detection, treatment, and prevention of human cancer.
- Evaluating and disseminating the results of such research.
- Training personnel in relevant scientific and technical skills.

Its unique role in international cancer research depends on its international status, its independence of national political interests, its experience and acceptability as a coordinator of research in developing countries, and its capacity within its own structure to combine epidemiological and laboratory approaches to cancer research.

The agency's research concentrates on the occurrence, causes, treatment, and prevention of cancer. It does not develop cancer control policies or implement cancer control measures except where this is necessary to achieve its research objectives.

Selection of specific activities for the agency's scientific program is based on its mission, considerations of scientific quality, ethical issues, and the known or potential impact on public health of particular cancers or agents that may cause or prevent cancer. The agency's activities have concentrated on three main fields: (1) research into the prevention and early detection of cancer, particularly intervention studies in both developing and developed countries; (2) use of biomarkers of exposure, effects, and susceptibility in epidemiological studies; and (3) studies with potential cancer prevention applications, particularly in developing countries. For the next few years, there will be new research lines, focusing on molecular carcinogenesis, genetics and epidemiology, clinical epidemiology including translational research and treatment outcomes, and on tobacco control and prevention strategies.

In this short review, the general directions of the IARC are briefly presented.

Background

The IARC was established almost 40 years ago by the World Health Assembly on May 20, 1965. The agency was created in a spirit of altruism by several of the wealthier countries of the world to provide capacity for research on important cancer problems wherever they might occur. In the spirit of its creation, the public health-oriented mission of the IARC is to promote, by way of international collaborative research and other means, improvement of health through reduction of the incidence and mortality from cancer throughout the world by conducting research into the causes, early detection, and prevention of cancer; by evaluating and disseminating the results of such research; and by organizing training also for those parts of the world which otherwise would be somewhat deprived of the educational possibilities in cancer research and control. Research into treatment or other aspects of the care does not usually form part of the agency's mission.

Some of the agency's lines of activities are described in the following sections in general terms.

Identification, Elucidation, and Evaluation of Environmental Causes of Cancer

The IARC Monographs Programme on the Evaluation of Carcinogenic Risks to Humans is one of the cornerstones of the agency's activities. During the past few years, it has become increasingly evident that in addition to environmental chemical carcinogens, which have been the traditional target of the IARC monographs, infectious agents contribute significantly to the human cancer burden. In addition, chemical carcinogens and infectious agents may interact in producing their adverse effects on biological systems, leading to an overproportional increase in cancer risk, as exemplified by the combined effects on liver cancer risk of aflatoxin B1 exposure and hepatitis B virus (HBV) infection. Other biological agents that have been evaluated in the monographs series, and found to carry a cancer risk, include schistosomiasis (bladder cancer), liver flukes (cholanglocarcinoma of the liver), *Helicobacter pylori* (stomach cancer), and human papilloma viruses (HPV) (types 16 and 18) (cervix cancer).

The use of tobacco products remains a central focus of the agency's epidemiological work since it is

generally agreed that in industrialized countries, ~30–35% of all known cancers are related to tobacco consumption and, worldwide, one in seven cancers can be attributed to smoking. In addition, the program has now evaluated the carcinogenic hazards associated with 'involuntary smoking' and classified that exposure as carcinogenic to humans. There is no doubt that the high incidence of all cancers in the Indian subcontinent is related to the oral use of tobacco. Subject to the results of a feasibility study now in progress, the agency plans a prospective study of oral tobacco use and cancer in more than 100 000 men and women in Bombay, India.

In addition to environmental factors acting as cancer causing agents, carcinogens may also be formed endogenously; for example, in the context of chronic inflammatory states. One mechanism is the production of reactive oxygen and nitrogen species that may cause tissue and DNA damage. The Endogenous Risk Factors Group focuses on biochemical mechanisms by which oxidative stress produces DNA damage and protein oxidation/nitrosation. These studies will be conducted in a variety of conditions including inflammation and precancerous lesions.

The agency has a long and successful history of achievements in the field of DNA damage and its relationship to mutation and cancer. This has led to the development of highly sensitive methods to assess the extent of interaction between environmental agents and the human genome. Scientists at the agency have succeeded in assessing specific mutations in the tumor-suppressor gene p53 and the Molecular Carcinogenesis Group maintains a database of known p53 mutations.

It is now generally agreed that phenotypic changes occurring in cells during the process of malignant transformation reflect the sequential acquisition of genetic alterations. This applies to all tissues, but the type of oncogene and tumor-suppressor gene and the sequence in which they contribute to tumor progression show a remarkable degree of organ specificity.

IARC scientists have therefore focused their attention on some organ sites (e.g., esophagus, stomach, liver, and cervix) that contribute significantly to the overall human cancer burden. Analyses of genetic alterations associated with tumor progression not only help us to understand the evolution of human cancers but, in some cases, also provide a tool to identify the environmental agent responsible for the initiation of malignant transformation. This has been shown in tumors of the skin (ultraviolet irradiation), liver (aflatoxin B1), and hemangiosarcoma of the liver (vinyl chloride monomer). In the case of stomach cancer, basic laboratory research will be extended into preventive measures, particularly with respect to the role of *H. pylori* in the causation of human stomach cancer. This is similarly true for cancer of the cervix, which in many parts of Central America and Asia remains the most frequent cause of cancer mortality in women. The agency has in the past conducted extensive surveys in different world regions regarding the prevalence of certain types of HPV and their association with cancer of the cervix. It is now conducting a large study on screening modalities for cervical cancer in India and Africa, with a view to establishing the most cost-effective preventive measures in low resource settings.

Mechanisms of Carcinogenesis, Host Factors, and their Interaction with Environmental Agents

The cytochrome P450 enzyme system has been the focus of investigation in several laboratories worldwide since these enzymes participate in the bioactivation of many environmental carcinogens. IARC scientists focus their research on the role of individual patterns of cytochrome P450 isozymes as determinants of genetic susceptibility to environmental carcinogens, in particular tobacco smoke. Over the past few years, evidence has accumulated indicating that individual susceptibility may, at least in part, be related to the individual pattern expression of genes involved in the bioactivation of xenobiotics. This question will also be pursued with respect to the genetic polymorphism of enzymes involved in the detoxification of environmental carcinogens. Individual capacity for DNA repair also appears to play a role in genetic cancer susceptibility. Methodological progress will allow us to launch, in the future, epidemiological projects to analyze the complex relationship between the bioactivation and detoxification of environmental carcinogens, DNA repair, and the production of mutations in critical target cells and transformation-associated genes.

For some human cancers, including brain tumors, our understanding of the etiology is still incomplete. The fraction of cases attributable to radiation or environmental agents is very small. The possibility exists that genetic alterations observed in the evolution of gliomas may be due to endogenous DNA damage rather than interaction with environmental factors. Also, there is strong evidence that germline mutations may play a larger role in brain tumor development than previously anticipated. The Molecular Pathology Group focuses much of its work on the etiopathogenesis of human brain tumors, particularly in children.

A new promising line of research has evolved from the observation that in some human cancers genomic instability may be reflected in microsatellite DNA changes, which commonly originate from replication errors. Effective mismatch repair may be the underlying cause, but so far only a restricted number of fragile microsatellite foci have been identified.

Research on the Prevention of Cancer and Its Consequences

The Gambia Hepatitis Intervention Study is a major effort to determine the role of chronic HBV infection in the evolution of hepatocellular carcinomas. Vaccinated children are being followed-up for serological markers of HBV infection and, later in life, the occurrence of liver tumors. This is a long-term study, but the agency is committed to lead this important work to a successful conclusion.

It is now increasingly possible to identify individuals with a high risk to cancer development; for example, through genetic predisposition, high levels of exposure to environmental carcinogens, or the occurrence of a tumor at a site where second primary tumors are frequent. It is, therefore, necessary to offer these subjects advice and treatment. More targeted screening for early neoplastic lesions is advisable and, in addition, chemoprevention may be a tool to reduce the incidence of malignant transformation and to revert early stages of cancer development. Since cancer prevention is a key element of the agency's mission, IARC scientists would like to be involved in this important research area. This is planned to be done in two ways. Similar to, but distinct from, the monographs series on the *Carcinogenic Risks to Humans*, the *IARC Handbook of Cancer Prevention* series has published a number of volumes concentrating on primary prevention and screening strategies such as: the use of sunscreen, weight control and physical activity, breast cancer screening, and consumption of fruits and vegetables. In addition, a newly created tobacco group will focus on the etiology of tobacco-induced cancers, while producing a new publication series on tobacco control. The agency is intent on establishing itself as the world's leading agency in cancer prevention.

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International Conference on Harmonisation

Robin C Guy

The International Conference on Harmonisation (ICH, formally known as The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use) is a joint initiative involving both regulators and industry from Europe, Japan, and the United States as equal partners in the scientific and technical discussions of the testing procedures which are required for product registration and to ensure and assess the safety, quality, and efficacy of medicines. The focus of ICH has been on the technical requirements for medicinal products containing new drugs. The vast majority of those new drugs and medicines are developed in Western Europe, Japan, and the United States and therefore, when ICH was established, it was agreed that its scope would be confined to registration in those three regions.

History

The pharmaceutical industry has become very global, and while a particular pharmaceutical may be sold to worldwide markets, its registration in each country remained a nation-by-nation process. Individual nations' regulatory systems were based on the same fundamental obligations to evaluate the quality, safety and efficacy. However, the detailed technical requirements had diverged over time to such an extent that industry found it necessary to duplicate many time-consuming and expensive test procedures, in order to market new products internationally. Harmonization of regulatory requirements was pioneered by the European Community (EC), in the 1980s, as the EC (now the European Union) moved towards the development of a single market for pharmaceuticals. The success achieved in Europe demonstrated that harmonization was feasible. It was, however, at the WHO International Conference of Drug Regulatory Authorities (ICDRA), in Paris, in

1989, that specific plans for action began to harmonize procedures in Europe, Japan, and the United States. Soon afterwards, the authorities approached IFPMA to discuss a joint regulatory-industry initiative on international harmonization, and ICH was conceived. The founding of the ICH took place at a meeting in April 1990, hosted by the EFPIA in Brussels. Representatives of the regulatory agencies and industry associations of Europe, Japan, and the United States met, primarily to plan an international conference but the meeting also discussed the wider implications and terms of reference of ICH.

Mission, Purpose, Objectives, Goals

The purpose of ICH is to make recommendations on ways to achieve greater harmonization in the interpretation and application of technical guidelines and requirements for pharmaceutical registration in Europe, Japan, and the United States, in order to reduce or preclude the need to duplicate the testing during research and development. The objective of such harmonization is a more economical use of human, animal, and material resources, and the elimination of unnecessary delay in the global development and availability of new medicines whilst maintaining safeguards on quality, safety, and efficacy, and regulatory obligations to protect public health.

Membership Criteria

Six parties to ICH represent the regulatory bodies and research-based industry in the three regions, Europe, Japan, and the United States, where the vast majority of new medicines are currently developed. These Six Parties are directly involved in the decision making process and were selected as they represent the regulatory bodies and the research-based industry in the European Union, Japan, and the United States. However, the Conference, its preparations, and follow-up activities are conducted in an open and transparent manner and the presence of observers from other regulatory authorities and WHO is welcomed as a means of ensuring that the benefits of progress towards harmonization can be utilized worldwide. The Six Parties are as follows:

- *European Commission – European Union (EU)*: The European Commission represents the 15 members of the EU. The Commission works through harmonization of technical requirements and procedures, to achieve a single market in pharmaceuticals, which would allow free movement of products throughout the EU.
- *European Federation of Pharmaceutical Industries and Associations (EFPIA)*: EFPIA is situated in Brussels and has, as its members, Member Associations in 16 countries in Western Europe. Much of the Federation's work is concerned with the activities of the European Commission and the EMEA. Companies in membership of EFPIA are manufacturers of prescription medicines and include all of Europe's major research-based pharmaceutical companies.
- *Ministry of Health, Labor, and Welfare, Japan (MHLW)*: Affiliated institutions include the National Institute of Health Sciences and academia that carries out research and testing on drugs, vaccines, and biologicals.
- *Japan Pharmaceutical Manufacturers Association (JPMA)*: JPMA represents 90 member companies. Membership includes all the major research-based pharmaceutical manufacturers in Japan.
- *US Food and Drug Administration (FDA)*: The US FDA has a wide range of responsibilities for drugs, biologicals, medical devices, cosmetics, and radiological products. The largest of the world's drug regulatory agencies, FDA is responsible for the approval of all drug products used in the United States.
- *Pharmaceutical Research and Manufacturers of America (PhRMA)*: The PhRMA represents the research-based industry in the United States. The Association has 67 companies in membership, which are involved in the discovery, development and manufacture of prescription medicines. There are also 24 research affiliates that conduct biological research related to the development of drugs and vaccines.

Others also are part of the ICH process. They include Observers, the Secretariat, and Administration.

Observers act as links with non-ICH countries and regions. They nominate nonvoting participants to attend the ICH Steering Committee Meetings.

The Observers to ICH are:

- the World Health Organization (WHO);
- the European Free Trade Area (EFTA), represented at ICH by Switzerland; and
- Canada, represented at ICH by Health Canada

Secretariat: The International Federation of Pharmaceutical Manufacturers Association (IFPMA) is a Federation of Member Associations representing the research-based pharmaceutical industry and other manufacturers of prescription medicines in 56

countries throughout the world. IFPMA runs the ICH Secretariat.

Administration: ICH is administered by the ICH Steering Committee, which is supported by the ICH Secretariat. Each of the six sponsors has had two seats on the ICH Steering Committee (SQ, which oversees the harmonization activities.

Additionally, groups are broken down into the Steering Committee (SC) and Expert Working Groups. The SC determines the policies and procedures for ICH, selects topics for harmonization and monitors the progress of harmonization initiatives. The SC meets at least twice a year. The Expert Working Groups are assigned by each of the six parties to designate a Topic Leader for the new topic.

Key Activities

ICH provides an opportunity for regulators and industry worldwide to reach consensus on the steps needed to achieve harmonization of technical requirements and to set out practical and realistic targets for harmonizing requirements where significant obstacles to drug development and the regulatory process have been identified.

There is a five-step process for ICH activities:

1. Consensus building, where an initial draft of a guideline or recommendation, is prepared, then signed off by the Expert Working Group Members. This is then which is submitted to the SC.
2. Start of regulatory action, when the SC agrees that there is sufficient scientific consensus on the technical issues, for the draft guideline or recommendation to proceed to the next stage of regulatory consultation.
3. Regulatory consultation, where the guideline or recommendation leaves the ICH process and becomes the subject of normal wide-ranging regulatory consultation in the three regions. In the EU, it is published as a draft CPMP Guideline; in the United States, it is published as draft guidance in the Federal Register; and in Japan, it is translated and issued by MHLW, for internal and external consultation.
4. Adoption of a tripartite harmonized text occurs when the topic returns to the ICH forum where the SC receives a report regarding the regulatory and industry satisfaction that the consensus achieved at step 2 is not substantially altered as a result of the consultation, the text is adopted by the SC. This adoption takes place on the signatures from the three regulatory parties to ICH affirming that the Guideline is recommended for adoption by the regulatory bodies in the three regions.
5. Regulatory implementation is carried out according to the same national/regional procedures that apply to other regulatory guidelines and requirements, in the EU, Japan, and the United States.

Key Accomplishments

Many procedures, including technical guidelines and format and content of registration applications have been grouped and have successfully been harmonized. These are readily available to the scientific community.

The ICH Topics are divided into four major categories and ICH Topic Codes are assigned according to these categories. The ICH website has summary charts with the status of harmonization of the ICH Topics and Guidelines.

- Q = 'Quality' topics, that is, those relating to chemical and pharmaceutical Quality Assurance. Examples: Q I Stability Testing, Q3 Impurity Testing.
- S = 'Safety' topics, that is, those relating to *in vitro* and *in vivo* preclinical studies. Examples: S I Carcinogenicity Testing, S2 Genotoxicity Testing.
- E = 'Efficacy' topics, that is, those relating to clinical studies in human subject. Examples: E4 Dose–Response Studies, Carcinogenicity Testing, E6 Good Clinical Practices.
- M = Multidisciplinary topics, that is, topics which do not fit uniquely into one of the above categories.
- M1: Medical Terminology.
- M2: Electronic Standards for Transmission of Regulatory Information (ESTRI).
- M3: Timing of Preclinical Studies in Relation to Clinical Trials.
- M4: The Common Technical Document.

Meetings

ICH 1 (i.e., first Conference) was held in Brussels in 1991
ICH 2 was held in Orlando, Florida in 1993
ICH 3 was held in Yokohama, Japan in 1995
ICH 4 was held in Brussels in 1997
ICH 5 was held in San Diego, California in 2000
ICH 6 was held in Osaka, Japan in November, 2003.

Relevant Websites

http://www.ich.org – International Conference on Harmonization (ICH).

http://www.efpia.org – European Federation of Pharmaceutical Industries and Associations (EFPIA).
http://www.mhlw.go.jp – Ministry of Health, Labor and Welfare, Japan (MHLW).
http://www.jpma.go.jp – Japan Pharmaceutical Manufacturers Association (JPMA).
http://www.fda.gov – US Food and Drug Administration (FDA).
http://www.phrma.org – Pharmaceutical Research and Manufacturers of America (PhRMA).
http://www.ifpma.org – International Federation of Pharmaceutical Manufacturers Association (IFPMA).

International Fragrance Association (IFRA)

Audrey Martin

History and Organization

The International Fragrance Association (IFRA) was founded in 1973, in Geneva, to represent the collective interests of the fragrance industry worldwide. IFRA is a Swiss association with a Belgian branch.

IFRA is headed by an Executive Director and a Board of Directors. The General Assembly is the governing body of IFRA. Each national delegation/ordinary member has one vote. The General Assembly meets at least once a year.

The association has a Scientific Director who oversees the technical and scientific aspects of the association.

Mission and Activities

The primary focus of IFRA is the worldwide development and advancement of the fragrance industry. The activities of IFRA cover three major areas: science, regulatory affairs, and communication. This occurs through:

- Development and implementation of a Code of Practice and Safety Standards for the good manufacture and safe use of fragrances, based on broadly recognized scientific principles and utilized worldwide with the final objective to protect the consumer and the environment.
- Close association with RIFM (Research Institute for Fragrance Materials), an independent international nonprofit research institute founded in 1966 for the purpose of developing and maintaining ingredient safety information.[1]
- Promotion of the self-regulatory practices of the industry, in keeping with the idea that the adaptation of worldwide industry rules to new scientific findings can occur more quickly through self-regulation than a change of legislation in different countries on different continents.
- Analysis and review of pending regulations applicable to fragrances, as well as legislative trends in related areas such as cosmetics, intellectual property, chemicals, and occupational health and safety.
- Development and maintenance of open communication and cooperation with national and international government bodies, concerned members of the medical and scientific community, related industries (e.g., cosmetics), and other stakeholders.
- Dissemination of timely and comprehensive information to membership on matters of relevance to the industry.
- Promotion of the merits of fragrances and the role they play in enhancing the quality of life.

Membership

IFRA membership is open to all countries and currently comprises the national associations of fragrance manufacturers from Australia, Europe, the Far East, and North and South America. Since there is no company membership in IFRA, individual fragrance companies belong to IFRA through IFRA's member associations.

Ordinary member associations and their member companies benefit from IFRA membership as follows:

- participation in the work of the IFRA Board and of the IFRA Committees;
- receipt of all information disseminated by the IFRA headquarters, covering a range of critical subjects relevant to fragrance use, safety, and operations;

[1] The scientific foundation of RIFM is based on an international Panel of Experts, made up of toxicologists, pharmacologists, and dermatologists who have no commercial ties to the fragrance industry, and whose work involves the safety evaluation of fragrance materials under conditions of intended use. Their evaluations are (*footnote continued*) based on existing data or, where insufficient data exist, on testing performed by RIFM itself.

- contacts with experts and industry colleagues from all over the world; and
- assistance of IFRA, as an avenue of support to the resolution of complex issues faced by fragrance companies in international commerce.

Publications, Databases, and Services

Code of Practice for the Fragrance Industry: The main thrust of IFRA's scientific activities consists in developing, communicating, and implementing a Code of Practice for the international fragrance industry. The Code of Practice provides Standards of good operating practice and product safety. It was first issued in 1973 and has been followed by fragrance companies worldwide ever since. Amendments and updates are issued on a regular basis. Standards regarding use restrictions are based on safety assessments by the Panel of Experts of RIFM and are carefully reviewed by the IFRA Scientific Committee.

Meetings

Regular meetings of IFRA Committees and Working Groups. For example

- Scientific Committee;
- Environmental Task Force; and
- Communications Working Group.

Regular meetings of joint Committees. For example

- IFRA/Customer Industry Joint Advisory Group;
- IFRA/EFFA Analytical Working Group;
- EFFA/IFRA/IOFI Labeling Group; and
- IFRA/IOFI Committee on Health, the Environment and Workplace Safety.

Related Organizations

COLIPA	European Cosmetic, Toiletry and Perfumery Association
CTFA	US Cosmetics, Toiletry and Fragrance Association
EFFA	European Flavour and Fragrance Association
IOFI	International Organization of the Flavor Industry
RIFM	Research Institute for Fragrance Materials

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International Labour Organization (ILO)

Pertti J Hakkinen

History and Mandate

The International Labour Organization is the United Nations (UN) specialized agency that seeks the promotion of social justice and internationally recognized human and labor rights. It was founded in 1919 at the end of the First World War, during the Peace Conference convened first in Paris and then at Versailles. Two industrialists, Robert Owen of Wales and Daniel Legrand of France, had advocated the need for such an organization in the nineteenth century. After having been put to the test within the International Association for Labour Legislation, founded in Basel in 1901, their ideas were incorporated into the Constitution of the International Labour Organization, adopted by the Peace Conference. The ILO is the only surviving major creation of the Treaty of Versailles that brought the League of Nations into being, and it became the first specialized agency of the United Nations in 1946.

The ILO formulates international labor standards in the form of Conventions and Recommendations setting minimum standards of basic labor rights, that is, freedom of association, the right to organize, collective bargaining, abolition of forced labor, equality of opportunity and treatment, and other

standards regulating conditions across the entire spectrum of work-related issues. It provides technical assistance primarily in the fields of: vocational training and vocational rehabilitation, employment policy, labor administration, labor law and industrial relations, working conditions, management development, cooperatives, social security, labor statistics, and occupational safety and health.

The ILO promotes the development of independent employers' and workers' organizations and provides training and advisory services to those organizations. Within the UN system, the ILO has a unique tripartite structure with workers and employers participating as equal partners with governments in the work of its governing organs. The ILO accomplishes its work through these three main bodies, all of which encompass its tripartite structure:

1. *International Labour Conference*: The member States (currently 177 countries) of the ILO meet at the International Labour Conference in June of each year, in Geneva. Two government delegates, an employer delegate and a worker delegate, represent each Member State and technical advisors accompany them. The Conference establishes and adopts international labor standards, and acts as a forum where social and labor questions of importance to the entire world are discussed. The Conference also adopts the budget of the Organization and elects the Governing Body.
2. *Governing Body*: The Governing Body is the executive council of the ILO and meets three times a year in Geneva. It takes decisions on ILO's policy, and establishes the program and the budget that it then submits to the Conference for adoption. It also elects the Director-General. It is composed of 28 government members, 14 employer members, and 14 worker members.
3. *International Labour Office*: The International Labor Office is the permanent secretariat of the ILO and focal point for the overall activities that it prepares under the scrutiny of the Governing Body and under the leadership of a Director-General. The Office includes the Geneva headquarters and 40 field offices around the world. In addition, experts undertake missions in all regions of the world under the program of technical cooperation. The Office also constitutes a research and documentation center and a printing house issuing a broad range of specialized studies, reports, and periodicals.

Examples of ILO Resources

Books, journals, databases, CD-ROMs, and videos: The ILO is the world's major resource center for information, analysis, and guidance on the world of work. The ILO also is an international publishing house, including publications on social security, occupational safety and health, industrial relations, labor law, training, management development, and other aspects of the world of work. For example, the *ILO Encyclopaedia of Occupational Health and Safety* (the fourth edition is available in a four-volume print version and on CD-ROM) serves as a worldwide reference reflecting the state of the art in occupational health and safety.

In addition, the ILO publishes statistical, legal, and bibliographic materials in both printed and interactive electronic forms. *The Yearbook of Labour Statistics* also exists in the form of a database (LABORSTA). The ILO journal, *International Labour Review*, features current policy analysis on employment and labor worldwide, and the ILO also publishes the magazine *World of Work* and *The Bulletin of Labour Statistics*. As noted below, the ILO publishes other documents, databases, CD-ROMs, and videos.

The ILO Library: The ILO Library provides access to a multilingual collection of over 1.5 million books, reports, journals, national legislation texts and statistical publications, and electronic information sources on all aspects of the world of work. The Library produces LABORDOC, a unique bibliographic database providing international, multilingual coverage of social and labor affairs, available online, as a CD-ROM, and in print (*International Labour Documentation*). The library also publishes the *ILO Thesaurus* (fifth edition, 1998), and develops projects and training courses for labor librarians.

International Institute for Labour Studies: Established in 1960, the ILO International Institute for Labour Studies in Geneva promotes a wider study and public discussion of policy issues of concern to the ILO and its constituents, through systematic interaction between the Organization and the international academic community, and other policymakers. The central theme of its activities is the interaction between labor institutions, economic growth, and equity. The Institute's means of action include research networks, social policy forums, courses and seminars, visiting scholar and internship programs, and publications.

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International Life Sciences Institute – North America

Penny Fenner-Crisp

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History, Purpose, and Financial Support

The International Life Sciences Institute (ILSI) is a nonprofit, worldwide foundation established in 1978 to advance the understanding of scientific issues relating to nutrition, food safety, toxicology, risk assessment, and the environment. By bringing together scientists from academia, government, industry, and the public interest/public health sector, ILSI seeks a balanced approach to solving problems of common concern for the well-being of the general public. ILSI receives financial support from industry, governments, and foundations.

Affiliations

ILSI is affiliated with the World Health Organization as a nongovernmental organization, and has specialized consultative status with the Food and Agriculture Organization of the United Nations.

Locations

ILSI is headquartered in Washington, DC. ILSI branches include Argentina, Brazil, Europe, India, Japan, Korea, Mexico, North Africa and Gulf Region, North America, North Andean, South Africa, South Andean, Southeast Asia Region, the Focal Point in China, and the ILSI Health and Environmental Sciences Institute. ILSI also accomplishes its work through the ILSI Research Foundation (composed of the ILSI Human Nutrition Institute and the ILSI Risk Science Institute) and the ILSI Center for Health Promotion.

Publications

ILSI publishes two journals (*Nutrition Reviews* and *Nutrition in Clinical Care*) and a series of newsletters from the parent organization and its branches. All of these are available through the ILSI website. In addition, ILSI Press has published many books and monographs, reflecting the broad range of ILSI's areas of interests. Some recent publications include *Present Knowledge in Nutrition*; *Direct Dosing of Pre-weaning Mammals in Toxicity Testing; Microbial Pathogens and Disinfection By-products in Drinking Water: Health Effects and Management of Risk; Functional Foods – Scientific and Global Perspectives*; and *Similarities and Differences Between Children and Adults: Implications for Risk Assessment*. A catalog of all publications available from ILSI Press can be found on the ILSI website.

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International Organization of the Flavor Industry (IOFI)

Thierry Cachet

History and Organization

The International Organization of the Flavor Industry (IOFI) was founded in 1969 in Geneva to represent the collective interests of the flavor industry worldwide. IOFI is a Swiss association with a Belgian branch, and is headed by an Executive Director and a Board of Directors. The General Assembly is the governing body of IOFI. Each national delegation/ordinary member has one vote. The General Assembly meets at least once a year. The association has a Scientific Director who supervises the technical and scientific aspects of the association.

Mission and Activities

IOFI is the representative of the global flavor industry. Acting directly and through its members, IOFI provides the industry, its customers, government agencies, and consumers with sound scientific

information, education, and training in order to promote the benefits and safe use of flavors. The activities of IOFI cover Science, Advocacy, Communication, and Intellectual Property protection. This is done by (1) serving as an effective advocate for IOFI members by representing industry's interests before global legislative and regulatory bodies, (2) participating in the harmonization of global flavor legislation, (3) providing information and advice to emerging countries on the status of flavor regulation in the United States, Europe, and Japan, (4) collecting confidential information on a worldwide basis on the identity and use levels of flavoring substances, (5) collecting data on safety studies on flavoring ingredients, with the aim of facilitating their evaluation by scientific bodies, and (6) participating in international meetings, hearings, and conferences to help promote the interests of IOFI and the global harmonization of flavor legislation.

IOFI also functions by (1) maintaining good relationships with related associations (e.g., the International Federation of Essentials Oils and Aroma Trades (IFEAT), International Life Sciences Institute (ILSI), and regular communication with the Food and Agriculture Organization (FAO), the World Health Organization (WHO), Codex Alimentarius Commission, the Joint FAO/WHO Expert Committee on Food Additives (JECFA), and the Council of Europe), (2) preparing publications on safety and regulatory matters, (3) keeping IOFI's members fully informed on scientific, legal, regulatory, and other relevant matters, through information letters, circular letters, a Code of Practice, and other guidelines, and (4) keeping the Code of Practice and similar IOFI documents updated.

Membership

IOFI Membership is open to all countries and currently comprises the national associations of flavor manufacturers from Australia, Europe, the Far East, and North and South America, such as FEMA (United States), EFFA (European Union), and JFFMA (Japan). Since there is no company membership in IOFI, individual flavor companies belong to IOFI through IOFI's member associations.

Ordinary member associations and their member companies benefit from IOFI membership via (1) participation in the work of the IOFI Board and of the IOFI committees, (2) receiving information disseminated by IOFI, covering a wide range of critical subjects relevant to flavor use, safety, and operations, (3) contact with experts and industry colleagues from all over the world, and (4) assistance of IOFI as an avenue of support to the resolution of complex issues faced by flavor companies in international commerce.

Publications, Databases, Services

Code of Practice for the flavor industry: The main thrust of IOFI's scientific activities consists of developing, communicating, and implementing a Code of Practice for the international flavor industry. The Code of Practice provides Standards of good operating practice and product safety. It was first issued in 1969 and has been followed by flavor companies worldwide ever since. Amendments and updates are issued on a regular basis.

Meetings

Regular meetings of IOFI Committees and Working Groups, for example, the Global Safety Management Committee (GSMC), Technical Experts Committee (TEC), and the Working Group on Methods of Analysis (WGMA). In addition, there are regular meetings of joint Committees, for example, EFFA/IFRA/IOFI Labeling Group and the IFRA/IOFI Committee on Health, the Environment and Workplace Safety (CHEW).

Related Organizations

- European Flavour and Fragrance Association (EFFA).
- International Fragrance Association (IFRA).
- Flavor and Extract Manufacturers Association (FEMA).
- Japan Flavor and Fragrance Materials Association (JFFMA).

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International Programme on Chemical Safety

Lynne Haber

The International Programme on Chemical Safety (IPCS) is a cooperative program of the United Nations Environment Programme (UNEP), the International Labor Organization (ILO), and the World Health Organization (WHO). The central unit of IPCS is based at WHOs Programme for the Promotion of Chemical Safety. The 1992 United Nations Conference on Environment and Development (UNCED) stated that collaboration on chemical safety among UNEP, ILO, and WHO in the IPCS should be the nucleus for international cooperation on environmentally sound management of chemicals. The work of the IPCS is divided into four areas: evaluation of chemical risks to human health; poisons information, prevention and management; chemical incidents and emergencies including public health preparedness, response, prevention and surveillance; and capacity building. The IPCS also has an Interregional Research Unit (IRRU) located at the National Institute of Environmental Health Sciences (Research Triangle Park, NC, USA).

The Intersecretariat Coordinating Committee meets regularly to ensure participation of the three cooperating organizations (COs) in the programme. The Inter-Organization Programme for the Sound Management of Chemicals (IOMC) has been established to promote coordination of the relevant policies and activities of UNEP, ILO, UN Food and Agricultural Organization (FAO), WHO, UNIDO, UNITAR, and OECD.

Overall policy guidance for the work of IPCS is provided by the Programme Advisory Committee, which is composed of 20 external experts who serve as individuals, rather than representing their respective organizations. This committee provides advice on scientific, technical, ethical, administrative, and regulatory aspects of the activities of the IPCS.

Collaborative Structure of the IPCS with Other Organizations

Countries or national agencies wishing to participate actively in the work of the IPCS sign a Memorandum of Understanding (MOU) that specifies the scope and areas of collaboration. The IPCS collaborates closely with the European Commission (EC) and the OECD as well as with several nongovernmental organizations active in the field of chemical safety. These nongovernmental organizations include the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), the CropLife International, the International Life Sciences Institute, the International Organisation of Consumers Unions (Consumers International), the International Union of Pharmacology (IUPHAR), the International Union of Pure and Applied Chemistry (IUPAC), and the International Union of Toxicology (IUTOX).

Many IPCS activities are implemented through a network of governmental and nongovernmental institutions, which are designated as Participating Institutions. These institutions include various types of centers of excellence, usually conducting scientific activities concerning effects of chemicals. A number of specific IPCS projects have their own network of participating centers covering particular project activities. For example, the International Agency for Research on Cancer (IARC) participates in the work of the IPCS in the field of chemical carcinogenicity.

Task Groups and Working Groups function as informal advisory mechanisms for IPCS. *Ad hoc* groups provide advice on specific technical and scientific topics concerning the program's work. Such groups are convened as required by the Coordinator of the IPCS.

Roles and Activity Areas of the IPCS

IPCS Objectives

The two main objectives of the IPCS are to establish the scientific health and environmental risk assessment basis for safe use of chemicals (normative functions) and to strengthen national capabilities for chemical safety (technical cooperation). As a result of a redesign of its program of activities in 2002–03, IPCS is emphasizing the collection of evidence for chemical-related adverse effects and determining the quality of the evidence used in risk assessment, including improved use of observational data from human exposures. IPCS is also emphasizing the use of risk assessment products by individual countries for the support of chemical management activities. Support of WHO normative activities is also being emphasized, including development of WHO drinking water and air-quality guidelines.

IPCS Areas of Activities

In fulfilling its roles, IPCS conducts activities in the following areas:

1. Carries out and disseminates evaluations of the risk to human health and the environment from

exposure to chemicals and produces health- or environment-based guideline values for exposure to the agents evaluated.
2. Promotes the development, improvement, validation, harmonization and use of methods for laboratory testing and ecological and epidemiological studies and other methods suitable for the evaluation of health and environmental risks and hazards from chemicals.
3. Promotes research to improve the scientific basis for health and environmental risk assessment to ensure a sound management of chemicals.
4. Promotes technical cooperation with Member States, in particular developing countries, to strengthen their capabilities and capacities in the area of chemical safety.
5. Promotes effective international cooperation with respect to emergencies and accidents involving chemicals.
6. Supports national programs for prevention and treatment of poisonings.
7. Contributes to the harmonization of classification and labeling of chemicals.
8. Promotes development of the human resources required in the areas above.

IPCS Achievements

Within its eight activity areas, IPCS conducts or supports a broad range of activities, with a variety of target audiences.

Evaluation of Chemical Risks to Human Health and the Environment Since its conception, the IPCS has evaluated an impressive list of commonly used and internationally traded agricultural and industrial chemicals. IPCS has also evaluated radioisotopes, chemicals frequently found as dangerous air and water pollutants, chemicals associated with global atmospheric changes, such as the greenhouse gases and the chlorofluorocarbons, as well as certain natural toxins and certain physical factors, such as noise and low-frequency radiation.

Chemical evaluations published by the IPCS include:

- *Environmental Health Criteria* (EHC) documents are detailed chemical evaluations designed for scientific experts responsible for evaluation of the risk posed by chemicals to human health and the environment, enabling relevant authorities to establish policies for the safe use of these chemicals.
- *Health and Safety Guides* are designed for a wide range of administrators, managers, and decision-makers in various ministries and governmental agencies, as well as in commerce, industry, and trade unions, who are involved in various aspects of using chemicals safely and avoiding environmental health hazards. They are short documents summarizing toxicity information in nontechnical language, and provide practical advice on matters such as safe storage, handling and disposal of the chemicals, accident prevention and health protection measures, first aid and medical treatment in cases of exposure leading to acute effects, and clean-up procedures.
- *International Chemical Safety Cards* (ICSCs) summarize essential data using standard phrases on a single sheet, and are intended for use in the workplace and field. These cards have no legally binding status and are not intended to be used in the regulatory process in any specific country.
- *Concise International Chemical Assessment Documents* (CICADS) are concise documents that provide summaries of the relevant scientific information concerning the potential effects of chemicals upon human health and/or the environment. They are based on selected national or regional evaluation documents or on existing EHCs. Before acceptance for publication as CICADs by IPCS, these documents have undergone extensive peer review by internationally selected experts to ensure their completeness, their accuracy in the presentation of original data, and the validity of the conclusions drawn.

Toxicological evaluations of chemicals associated with food are made jointly with the FAO, through the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR). The IPCS also collaborates with the FAO and WHO in the preparation of Pesticide Data Sheets (PDSs). These data sheets contain not only toxicological and risk information but also guidance on first aid and laboratory analysis as well as recommendations to regulatory authorities. IPCS also contributes to development of WHO Guidelines for Drinking-Water Quality. These guidelines are the international reference point for standard setting and drinking-water safety. The guidelines are supported by other publications that explain how they were derived and that assist in implementing safe water activities.

Methodologies for Evaluation of Hazards and Risks IPCS aims at promoting the development, improvement, validation, harmonization, and use of generally

acceptable, scientifically sound methodologies for the evaluation of risks to human health and the environment from exposure to chemicals. IPCS aims to promote global harmonization of approaches to risk assessment through increased understanding, focusing on specific issues, and striving for agreement on basic principles. IPCS notes that such harmonization should not be perceived as standardization but rather as an understanding of the methods and practices used by various countries and organizations so as to develop confidence in an acceptance of assessments that use different approaches. It further involves a willingness to work toward convergence of these approaches or methods as a long-term goal. Progress through all stages of this project will result in efficient use of resources and consistency among assessments, avoiding unnecessary duplication of efforts, and increasing the volume of information made available to countries.

The development and improvement of methods will provide the scientific basis for safe use of chemicals and therefore enhance the capabilities of countries in risk assessment and management. IPCS has published a number of EHC documents on risk methods, including monographs on general principles, evaluation of toxic effects in specific organs, systems, or end points, and consideration of susceptible populations. Other monographs address principles of environmental epidemiology and toxicokinetic studies, and the principles and concepts of using biomarkers in risk assessment.

Emerging Issues in Chemical Safety The work of IPCS on endocrine disruptors is an example of work on emerging issues and falls into two main areas. The Global Inventory of Ongoing Research on Endocrine Disruptors is an Internet-accessible repository of ongoing global research activities on the health and ecological effects of endocrine disruptors, and is designed to facilitate communication and minimize duplication of effort. IPCS also published in 2002 a Global Assessment of the State-of-the-Science of Endocrine disruptors.

Other emerging areas include risk assessment of vulnerable population groups, toxicogenomics, and the integration of human and ecological risk assessment.

Prevention and Management of Toxic Exposures and Chemical Emergencies IPCS develops and supports a number of information resources to aid in this area. The IPCS INCHEM database (see 'Relevant Websites') contains many of the publications of the IPCS, including Pesticide Data Sheets (PDSs), Poisons Information Monographs (PIMs), the full text of Environmental Health Criteria documents, CICADs, Health and Safety Guides, carcinogenicity summaries and evaluations from the International Agency for Research on Cancer (IARC), International Chemical Safety Cards, monographs from JECFA and JMPR, and Screening Information Data Sets (SIDs) for High Production Volume Chemicals. Many of these publications are also available on compact disk (CD-ROM).

IPCS INTOX is an essential tool for poison centers and other organizations concerned with preventing, recording, evaluating, diagnosing, treating, and reporting on chemical emergencies. It includes a databank of consolidated, authoritative information on toxic agents and the management of toxic exposures; an information management tool for poison centers and others dealing with toxic exposures; the gateway to a global electronic network of poison centers and other users of the package; and it provides a forum for collaboration between experts and those responding to emergencies concerning toxic exposures.

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International Society for the Study of Xenobiotics

Ankur V Dnyanmote and Harihara M Mehendale

Objectives

The purpose of the International Society for the Study of Xenobiotics (ISSX) is

- to facilitate the association of scientists engaged in xenobiotic research and in other related disciplines;
- to disseminate, discuss, and publish results of research and related matters of interest in this field;
- to promote public awareness of the field and its social and environmental implications; and
- to promote education and training in this field.

History

Man's use of xenobiotics dates from antiquity but interest in foreign compound metabolism dates from only the mid-nineteenth century, when the knowledge and techniques of organic chemistry were first applied to its study. For nearly a century thereafter biotransformation was generally equated with 'detoxication' or the elimination of a compound's biological activity.

This view changed in the late 1930s with the discovery that the synthetic azo-dye prontosil owed its life-saving antibacterial activity to its metabolite, sulfanilamide. Since the 1950s, the biological effects of numerous xenobiotic substances have been shown to be due to biotransformation products rather than to effects of the parent compound. The importance of biotransformation and other aspects of the interactions of xenobiotics with biological systems has been continuously reinforced by regulatory agencies worldwide. Their need for scientific knowledge on which to base regulations and safety evaluations for chemicals and drugs provides one important motivation for the study of xenobiotics.

Scientists working in such diverse fields as clinical and basic pharmacology, biochemistry, toxicology, and oncology were drawn into metabolism studies, both in universities and research institutes, and in the pharmaceutical, chemical, agrochemical, food processing, tobacco, and cosmetic industries. In 1981, a small group of scientists, brought together during the 1970s under the aegis of the Gordon Research Conferences on Drug Metabolism, took the bold step of suggesting the organization of an international society to promote the interaction of scientists dedicated broadly to the study of xenobiotics in living systems. Thus, the International Society for the Study of Xenobiotics was formed.

Membership

Currently, ISSX has more than 2700 members in 57 countries. The majority of members work in the areas of pharmacology, toxicology, biochemistry, analytical chemistry, etc. ISSX offers two types of memberships: full member and Graduate Student/Postdoctoral Researcher member. In addition, the Council may award Honorary memberships to persons who are members or nonmembers of the Society in recognition of outstanding and sustained achievement in the field of xenobiotics.

Publications

The Society publishes the quarterly ISSX Newsletter that is distributed to members. The newsletter includes announcements of ISSX meetings, meeting reports, and book reviews, as well as a calendar of future meetings in the field and other items of relevance to the membership. The journal *Drug Metabolism Reviews* has been adopted as the official journal of the Society, and this journal publishes abstracts of all ISSX meetings and full manuscripts from selected speakers. ISSX members may subscribe to several journals offered at reduced subscription rates.

Meetings

The Society organizes an international meeting every 3 years in locations that rotate from North America, to Asia/Pacific, to Europe. During years when an international meeting is not scheduled, each of these three regions of the Society may organize a regional meeting. North American regional meetings are presented every October during years without an international meeting. The schedule for the next several years is as follows:

August 29–September 2, 2004	*International* meeting in Vancouver, BC, Canada
October 23–27, 2005	*Regional North American* meeting in Maui, Hawaii Cosponsored with Japanese Society for the Study of Xenobiotics (JSSX)
June, 2006	*Regional European* meeting in the UK
October, 2006	*Regional North American* meeting in Puerto Rico
Summer 2007	*International* meeting in Sendai, Japan

Awards and Grants

ISSX has a Society awards system that follows the schedule of its meetings. The R.T. Williams Distinguished Scientific Achievement Award and the Frederick J. DiCarlo Distinguished Service Award, which recognize outstanding individuals in each category worldwide, are presented during the Society's international meetings every 3 years. A regional Scientific Achievement Award and regional New Investigator Award are presented at each of the Society's regional meetings.

In addition, Best Poster Awards are presented to recognize superior research in both the predoctoral and postdoctoral research categories at Society meetings.

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International Society of Exposure Analysis

Pertti J Hakkinen

History, Purpose, and Objectives

The International Society of Exposure Analysis (ISEA) was established in 1989 to foster and advance the science of exposure analysis related to environmental contaminants, both for human populations and ecosystems. The membership promotes communication among –all disciplines involved in exposure analysis, recommends exposure analysis approaches to address substantive or methodological concerns, and works to strengthen the impact of exposure assessment on environmental policy.

Membership Criteria

Any individual with a professional interest in exposure analysis is invited to join. The Society seeks broad participation from various disciplines such as exposure assessment, chemistry, biochemistry, risk assessment, biostatistics, physiology, toxicology, epidemiology, ecology, environmental engineering, and others. There are currently several hundred members of ISEA, with a US focus but international representation. Students and international professionals with an interest in exposure assessment are especially encouraged to join.

Membership Benefits

Members get voting privileges in the election of officers and councilors, and Web-based access to the membership directory. In addition, members get a subscription to the *Journal of Exposure Analysis and Environmental Epidemiology* (JEAEE), and Web-based access to current and recent issues of this journal. The JEAEE focuses on manuscripts dealing with measurements, modeling, instrumentation, questionnaires, studies on chemical, biological, and physical principles required to analyze human exposure from single and multiple media and routes, and epidemiological investigations. The ISEA website provides online access to additional information, for example, funding opportunities for research.

Meetings

An annual meeting is held, sometimes in conjunction with the International Society for Environmental Epidemiology.

Awards

Various awards are announced at the annual meeting, including the Jerome J Wesolowski Award ('in recognition of outstanding contributions to the knowledge and practice of human exposure assessment'), the Joan M Daisey Outstanding Young Scientist Award ('to recognize outstanding contributions to the science of human exposure analysis by a young scientist'), and the Constance L Mehlman Award ('in recognition of outstanding contributions in exposure analysis research that helped shape a National or State policy' or 'that provided new approaches for reduction or prevention of exposures').

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International Union of Pure and Applied Chemistry

John H Duffus

The International Union of Pure and Applied Chemistry (IUPAC) aims to advance the worldwide aspects of the chemical sciences and to contribute to the application of chemistry in the service of mankind. As a scientific, international, nongovernmental, and objective body, IUPAC addresses many global issues involving the chemical sciences.

IUPAC was formed in 1919 by chemists from industry and academia. Over nearly eight decades, the Union has fostered worldwide communications in the chemical sciences and has united academic, industrial, and public sector chemistry in a common language. IUPAC is recognized as the world authority on chemical nomenclature, terminology, standardized methods for measurement, atomic weights, and many other critically evaluated data. The Union sponsors major international meetings ranging from specialized scientific symposia to CHEMRAWN meetings with broad societal impact.

IUPAC is an association of various bodies, National Adhering Organizations, which represent the chemists of the member countries. There are 44 National Adhering Organizations, and 20 other countries are linked to IUPAC as Associate National Adhering Organizations. Almost 1000 chemists throughout the world are engaged on a voluntary basis in the scientific work of IUPAC, primarily through projects, which are components of eight Divisions and several Committees. The Divisions of IUPAC are as follows:

I Physical and biophysical chemistry

II Inorganic chemistry

III Organic and biomolecular chemistry

IV Macromolecular

V Analytical chemistry

VI Chemistry and the environment

VII Chemistry and human health

VIII Chemical nomenclature and structure representation

IUPAC and Nomenclature

IUPAC's nomenclature books are used by professional chemists in academia, government, and chemical industry throughout the world. They are commonly identified by the colors of their covers:

- *Gold*: Chemical terminology.
- *Green*: Quantities, units, and symbols in physical chemistry.
- *Red*: Nomenclature of inorganic chemistry.
- *Blue*: Nomenclature of organic compounds.
- *Purple*: Macromolecular nomenclature.
- *Orange*: Analytical nomenclature.
- *Silver*: Nomenclature and symbols in clinical chemistry.

Standards

IUPAC publishes definitive and up-to-date data on atomic weights and isotopic abundances. It also publishes a wide variety of other chemical data of immense value to chemists and chemical engineers:

- International thermodynamic tables of the fluid state.
- Solubility data series – over 70 volumes of data in this series have already been published.
- Stability constants – this database of metal–complex stability constants available on disk contains ~25 000 pieces of data.
- Enthalpies of vaporization of organic compounds.
- Thermodynamic and transport properties of alkali metals.
- Recommended reference materials for achievement of specific physicochemical properties.
- Evaluated kinetic and photochemical data for atmospheric chemistry.

IUPAC is widely involved in establishing standard methods for use in analytical, clinical, quality control, and research laboratories. Some examples are given below:

- Standard methods for the analysis of oils, fats, and derivatives.
- Harmonization of international quality assurance schemes for analytical laboratories.
- Protocol for self-auditing of analytical laboratories for ISO 9000 certification.
- Quality assurance and sampling.
- Standardization of immunoassay determinations.
- Standard methods for the determination of trace elements in body fluids.
- JCAMP-DX, a standard format for the exchange of spectra in computer readable form.
- Experimental thermodynamics: measurement of the transport properties of fluids; solution calorimetry.

Environment

The various Commissions and Committees of IUPAC have undertaken an extensive array of environmental projects. Some examples are given below:

- environmental analytical chemistry;
- environmental particles;
- polymer recycling;
- determination of trace elements in the environment;
- gas kinetic data for atmospheric chemistry;
- glossary of atmospheric chemistry terms; and
- pesticides in surface water.

IUPAC Congress and Other Meetings

IUPAC organizes a biennial Congress, and each year IUPAC sponsors a large number of independently organized symposia that cover a wide range of specialized topics in chemistry. Sponsorship by IUPAC attests to the quality of the scientific program and indicates the host country's assurance that scientists from all countries may participate. IUPAC sponsors a continuing series of conferences on CHEMical Research Applied to World Needs (CHEMRAWN). These meetings focus on topics in chemistry that have sociopolitical impact, such as availability of raw materials, food chemistry, and environmental matters.

IUPAC Division VII – Chemistry and Human Health

Toxicology is one of the concerns of this division. The main activities of the Division covered by three Subcommittees are:

- Nomenclature, Properties, and Units in Laboratory Medicine.
- Medicinal Chemistry and Drug Development.
- Toxicology and Risk Assessment.

All of these contribute to relevant areas of chemical toxicology.

Subcommittee on Nomenclature, Properties, and Units in Laboratory Medicine

In 1995, the predecessor of the present Subcommittee, the Commission on Nomenclature, Properties and Units (C-NPU of IFCC and IUPAC) started publishing a series of papers on a coding system (i.e., a structure or a framework for the pairs of codes and meaning) and a coding scheme (i.e., the pairs of codes and their meaning), for Properties in Laboratory Medicine. 'Meanings' can be descriptions of properties that are measured or observed in laboratory medicine. The codes offer unique and sufficient information about properties and are designed to facilitate the transfer of information between laboratories and the end users of laboratory information. The codes make it possible to translate the data to any language automatically. So far the meanings have been tested for translation into 18 languages, including many of the European languages, Arabic, and Cantonese.

In order to test functionality, the coding scheme has been successfully mapped to the various codes that are presently used in more than 50 medical laboratories in Denmark and Sweden. To accommodate national or local needs special codes can be used. The coding scheme is accessible on the IFCC website. The coding scheme now includes clinical pharmacology and toxicology, and environmental toxicology.

Subcommittee on Medicinal Chemistry and Drug Development

This subcommittee arranges two meetings a year, publishes books, most recently the *IUPAC Handbook of Pharmaceutically Acceptable Salts*, and prepares glossaries to aid communication between chemists working in this area. These glossaries include a 'Glossary of Terms Used in Medicinal Chemistry', which is available on the IUPAC website. The glossary was published in the *Annual Report on Medicinal Chemistry*, which is distributed by the American Chemical Society to over 10 000 medicinal chemists, and also made available on the Internet. This led further to production and publication of a 'Glossary of Terms Used in Computational Drug Design'.

To assist medicinal chemists in their understanding of combinatorial chemistry and to help with the acceptance of a universally understood language, a 'Glossary of Combinatorial Chemistry Terms' was published in *Pure and Applied Chemistry* and subsequently, in the *Journal of Combinatorial Chemistry*. This ensures its use within the American Chemical Society as a standard glossary of terms. Further work is focused on producing an opinion document on the legal implications of patenting virtual libraries. This is a very important issue which has profound implications for research and development in the pharmaceutical industry.

Other glossaries of terms are being prepared, including a Glossary of Drug Metabolism Terms, Glossary of Terms in Pharmaceutical Process Chemistry, and Glossary of Terms in Pharmaceutical Technology.

Training of Medicinal Chemists

A series of papers has been published on training. A syllabus for a short course on medicinal chemistry has also been published, and courses have been initiated in some Latin American countries.

Guidelines for Natural Product Collaborations

To facilitate collaborations, a document of guidelines for this was published in 1996 as IUPAC Recommendations entitled 'Preservation and utilization of natural biodiversity in context of the search for economically valuable medicinal biota'. Two other documents have been published on the subject: a technical report intended to help with drawing up contracts and an article titled 'Medicinal Chemistry in the Development of Societies'.

IUPAC Subcommittee on Toxicology and Risk Assessment

IUPAC Subcommittee on Toxicology and Risk Assessment is open to all those members of Division VII and other chemists (who may be co-opted) who are interested in toxicology.

Its terms of reference are as follows:

(i) To coordinate projects that have been approved by the Division VII Committee and which relate to toxicology and risk assessment.

(ii) To provide a forum for discussing the information content and progress of projects identified under (i) above.

(iii) To provide a forum for initiating new project submissions in the subject area of toxicology and risk assessment that are considered to be suitable activities for Division VII.

(iv) To provide the opportunity for coordination in both experimental and computational approaches to toxicology and risk assessment methods.

(v) To report to the Division VII President and the Division Committee on items (i) to (iii) above.

(vi) To provide a connection with other organizations concerned with toxicology such as the International Union of Toxicology (IUTOX), the International Programme for Chemical Safety (IPCS), the World Health Organization (WHO), the Organization for Economic Cooperation and Development (OECD), the International Labour Organization (ILO), the International Union of Biochemistry and Molecular Biology (IUBMB), the International Union of Immunological Societies (IUIS), the International Union of Pharmacology (IUPHAR), the International Federation of Clinical Chemistry (IFCC), other national and international toxicology and clinical chemistry societies, and chemical industry health and safety groups.

(vii) To provide an opportunity for IUPAC interaction with chemists in the Chemical Industry worldwide in the field of toxicology and risk assessment.

(viii) To broaden the activities of the Division by providing opportunities for other organizations involved in toxicology and risk assessment to work together with Division VII members.

(ix) To offer advice to the Division President and the Committee on matters concerning toxicology and risk assessment in all aspects from the purely chemical to the protection of human health and the natural environment.

One of the main concerns of the Subcommittee on Toxicology and Risk Assessment and its predecessor the Commission on Toxicology has been education of chemists in fundamental principles of toxicology. Activities here have involved the compilation of glossaries, educational modules, and reviews of matters of current concern. These can be found on the IUPAC website at http://www.iupac.org/divisions/VII/VII.C.2/index.html.

The Future of IUPAC

Chemistry historically emerged and developed as an interdisciplinary scientific field, with a broad definition of its borders. Paraphrasing Linus Pauling's definition of the chemical bond "whatever is convenient to the chemist to define as a bond", chemistry can be defined as a discipline encompassing all areas which are of interest to chemists and where molecular science makes significant contributions. The rich and diverse world of modern chemistry encompasses remarkable intellectual accomplishments, scientific creativity and originality and the generation of new knowledge. IUPAC serves international scientific endeavor in the dual function of a basic science and a mission-oriented Union. The Union is in a unique position to contribute to the central interdisciplinary chemical sciences. Strengthening international chemistry, striving towards inspiring high standards of excellence and relevance in academic and industrial research and promoting the service of chemistry to society and to global issues are the visions that shape IUPAC's activities toward the twenty-first century.

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International Union of Toxicology

Paolo Preziosi

The International Union of Toxicology (IUTOX) is an international body consisting of 44 national/supernational toxicological societies from all parts of the world. Its creation reflects the growing awareness of the wide range of toxic threats throughout the world and the need for concerted and coordinated efforts to resolve these problems. This awareness led initially to the creation of special toxicological sections or groups within international or national associations of scientists from various fields. Although the specific toxicological concerns of these groups were different, the basic principles and approaches used to investigate them are strikingly similar, and independent bodies dedicated specifically to the discipline of toxicology were soon established in many countries. The IUTOX was founded on July 6, 1980, in Brussels, Belgium, during the Second International Congress of Toxicology. Its purposes are to promote a full and uniform development of toxicology within and across various scientific disciplines and to provide an international platform for scientists active in toxicological research.

As a result of a recent (2001) change in its By-laws, the IUTOX is now directed by an 11-member executive committee composed of the President Elect, the Vice-President, the Secretary General, the treasurer, the Past President, and five directors. A new executive committee, reflecting the international membership of the Union, is elected every 3 years by a general assembly of the member societies. Each member society is allowed one vote for every 200 registered members, up to a maximum of five votes for those with more than 800 individual members. Standing committees include the Nominating Committee (standing Committee of the Council) and the Membership Committee (standing Committee of the Executive Committee).

The IUTOX has been an associate member of the International Council of Scientific Unions since 1987 and became a full member in 1996. In 1993, it was recognized as an official nongovernmental organization of the World Health Organization (WHO) and, as such, it contributes to the activities of the WHO's International Programme on Chemical Safety (WHO-IPCS) and sends delegates to the recently established International Forum on Chemical Safety.

Every 3 years, the IUTOX organizes an International Congress of Toxicology (ICT) lasting 4 or 5 days with an average attendance of from 800 to 1500 persons. During these congresses, joint symposia on specific questions are organized with other international scientific bodies, such as the International Union of Pharmacology, the International Union of Pure and Applied Chemistry (IUPAC), the WHO-IPCS, the International Agency for Research on Cancer, and the International Council for Laboratory Animal Sciences. Past congresses have been held in Brussels (1980), San Diego (1983), Tokyo (1986), Brighton (1989), Rome (1992), Seattle (1995), Paris (1998), and Brisbane (2001).

The 10th ICT was held in Tampere, Finland, in 2004 and the 11th ICT in Montreal, Canada, has been scheduled for 2007. A Congress of Toxicology in Developing Countries was held in 2003 in China and another is scheduled in 2006 in Croatia.

The IUTOX also sponsors numerous other international meetings (e.g., Joint Meeting of the Italian and French Societies of Toxicology in 1991, the Convention of the International Neurotoxicology Association in 1991, and the Second Nordic Toxicology Congress in 1992). Activities organized in developing countries include the Workshop on Prevention and Management of Poisonings in South America (Montevideo, Uruguay) in 1991, the Workshop on Development of Poison Control Programmes in South America (Montevideo, Uruguay) in 1992 (both were organized in conjunction with the WHO-IPCS), the Seminar and Training Course on Diagnosis, Management and Prevention of Poisoning for Francophone, Sub-Saharan Countries (Dakar, Senegal) in 1995, and the Second and Third Congresses of Toxicology in Developing Countries, held, respectively, in New Delhi, India, in 1991 and Cairo, Egypt, in 1995. A symposium on Inhalation Toxicology in Pilsen was co-organized by the IUTOX and EUROTOX as a satellite meeting to the 1995 EUROTOX Congress held in Prague.

One of the Union's most successful activities has been the development of a program for continuing education (CE) risk assessment. As part of this program, the IUTOX organizes Risk Assessment Summer Schools (RASS), which have been held every 2 years since 1984. The ninth RASS was held in Gozo (Malta) in 2002. These 1 week courses provide training for young toxicologists in strategies and skills associated with chemical risk assessment. The RASS project will be converted into a long-range education program of the IUTOX. IUTOX arranged for two CE courses in 2000, one in Poland and one in Hungary. A number of courses and lectures were held

in recent years including two CE courses in Mexico (1999), a workshop lecture in Egypt (2000), a congress lecture in Brazil (2000), workshop lectures in South Africa (2001), CE courses in Venezuela (2001) and Slovenia (2002), CE course and lectures in China (2001), and a workshop in Chile (2002).

As a member of the International Council for Science (ICSU), the IUTOX organized workshops on environmental estrogens as part of a program to expand upon information contained in the Book *Natural and Anthropogenic Environmental Oestrogens: The Scientific Basis for Risk Assessment*. These workshops were held in Canberra, Australia (1998); New Orleans, USA (1999); Keele, UK (1999); Oslo, Norway (1999); Antalya, Turkey (1999); and Seoul, Korea (2000). Their aim was to increase participants' awareness of controversial issues related to the impact of environmental estrogens on human health and the environment.

In 2001, thanks to a grant received from the ICSU, the IUTOX and the International Union of Nutritional Sciences published a monograph on genetically modified (GM) foods. The objective was to provide the global community with an informative, nonbiased review of the benefits and risks associated with GM foods. To prepare the monograph, a planning workshop was organized with representatives of five other International Unions, several ICSU committees, and various experts on issues surrounding the use of GM foods in developing countries and countries in transition.

IUTOX Member Societies

- *Founding members*: European Society of Toxicology, the British Toxicological Society, the Finnish Society of Toxicology, the French Society of Toxicology, the Italian Society of Toxicology, the Japanese Society of Toxicology, the Netherlands Society of Toxicology, the Norwegian Society of Pharmacology and Toxicology, the Society of Toxicology of Canada, the Indian Society of Toxicology, the Society of Toxicology, USA, the Swedish Society of Toxicology, the Swiss Society of Pharmacology and Toxicology, the Yugoslavian Society of Toxicology (now the Croatian and Slovenian Societies of Toxicology), and the Society of Toxicology of the Democratic Republic of Germany (now the German Society for Experimental and Clinical Pharmacology).
- *Other members*: The American Academy of Clinical Toxicology, the American College of Toxicology, the Argentine Toxicological Association, Asiatox, the Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists, the Brazilian Society of Toxicology, the Chinese Society of Toxicology, the Croatian Toxicology Society, the Danish Society of Pharmacology and Toxicology, the Egyptian Society of Toxicology, the European Association of Poison Centres and Clinical Toxicologists, EUROTOX, the French Society of Clinical Toxicology, the Hellenic Society of Toxicology, the International Neurotoxicology Association, the Hungarian Pharmacological Society, the Irish Society of Toxicology, the Israeli Society of Toxicology, the Japanese Society for Clinical Toxicology, the Korean Society of Toxicology, the Latin American Association of Toxicology, the Latvian Society of Toxicology, The Mexican Society of Toxicology, the Polish Society of Toxicology, the Russian Society of Toxicology, the Slovenian Society of Toxicology, the Toxicological Society of Thailand, the Spanish Association of Toxicology, the Spanish Society of Toxicology, the Toxicology Society of Taiwan, the Turkish Society of Toxicology, and the Union of Hungarian Toxicologists

The IUTOX has been particularly successful in developing new societies in areas of the world where toxicology has been underrepresented.

It has also played a significant role in the development of the International Assembly for the Recognition of Toxicologists (IART). The IART is a forum whose aims are (1) the development of criteria for recognizing qualified experts in toxicology; (2) assisting toxicology organizations in establishing and implementing these criteria; and (3) promotion of efforts to identify toxicological education and training needs.

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Inter-Organization Programme for the Sound Management of Chemicals

Pertti J Hakkinen

The Inter-Organization Programme for the Sound Management of Chemicals (IOMC) was established in 1995 by a Memorandum of Understanding among International Labour Organization (ILO), Organisation for Economic Co-operation and Development (OECD), Food and Agriculture Organization of the United Nations (FAO), United Nations Environment Programme (UNEP), United Nations Industrial Development Organization (UNIDO), and World Health Organization (WHO). This followed recommendations made by the 1992 UN Conference on Environment and Development in Rio de Janeiro, and in particular its Chapter 19, Agenda 21.

The United Nations Institute for Training and Research (UNITAR) joined the IOMC in 1997. The IOMC vision is to be the preeminent mechanism for initiating, facilitating, and coordinating international action to achieve the World Summit on Sustainable Development (WSSD) 2020 goal for round management of chemicals.

The ILO, OECD, FAO, UNEP, UNIDO, WHO, and UNITAR are the seven Participating Organizations (POs) which contribute to the work of the IOMC as the Inter-Organization Coordinating Committee (IOCC). The areas in which coordination is sought include the international assessment of chemical risks; harmonization of classification and labeling of chemicals, information exchange on chemicals and chemical risks; establishment of risk reduction programs; strengthening of national capabilities and capacities for management of chemicals, prevention of illegal international traffic in toxic and dangerous products; and other areas as agreed by all POs. Planning, programming, implementation, and monitoring of activities undertaken jointly or individually by the POs is carried out by the IOCC. This ensures full consultation among all those involved, with the aim to ensure effective implementation without duplication. The WHO is the administering organization for the IOMC and provides secretariat services to the IOCC.

Technical coordinating groups: Specific coordinating groups have been used to progress IOMC activities, including Harmonization of Chemical Classification Systems, Persistent Organic Pollutants (POP), Stocks of Obsolete Pesticides and Industrial Chemicals, Assessment of Existing Industrial Chemicals and Pollutants, Pollutant Release and Transfer Registers (PRTR), and Chemical Accident Prevention, Preparedness and Response. These groups provide a means for all interested bodies working in the respective areas to consult with each other on program plans and activities, and to discuss ways and means of ensuring that the activities are mutually supportive. Membership in the Coordinating Groups is not limited to intergovernmental bodies, and may involve nongovernmental organizations and appropriate national institutions. The IOMC website provides the terms of reference, memberships, and meeting reports for the Coordinating Groups.

Inventory of activities: The IOCC has established an Inventory of Activities database hosted by OECD. This provides a calender of events, and details of relevant activities of each PO, including a short description of the activities undertaken, with an indication of the relevant program areas of Chapter 19, Agenda 2, to which the work contributes. The title of each activity, the responsible IOMC PO for implementation, any partners involved, the objectives of the work, outputs, geographical coverage, and relevant contact point are also provided.

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National Center for Environmental Health

Sachin S Devi and Harihara M Mehendale

Agency for Toxic Substances and Disease Registry (ATSDR), located in Atlanta, Georgia, is a federal agency created in 1980 by the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), or what is more commonly known as Superfund legislation. Congress enacted Superfund as part of its response to two highly publicized and catastrophic events: discovery of the Love Canal hazardous waste site in Niagara Falls, New York, and an industrial fire in Elizabethtown, New Jersey, which set off the release of highly toxic fumes into the air in a densely populated area. Congress also created ATSDR to implement the health-related sections of laws that protect the public from hazardous wastes and environmental spills of hazardous substances.

In 1983, the secretary of the Department of Health and Human Services by administrative order established ATSDR as a separate agency of the Public Health Service. In 1984, amendments to the Resource Conservation and Recovery Act (RCRA) authorized ATSDR to conduct public health assessments at RCRA sites when requested by the US Environmental Protection Agency (EPA), states, or individuals, and to help EPA decide which substances should be regulated and at what levels those substances threaten human health.

In June 1983, ATSDR was formally organized to begin in concert with US EPA, the Centers for Disease Control (now the Centers for Disease Control and Prevention), and the National Institute of Environmental Health Sciences to address CERCLA, one of the most challenging and innovative environmental laws relating to public health.

Following the reauthorization of Superfund in 1986 under the Superfund Amendments and Reauthorization Act (SARA), the agency received major new mandates. SARA broadened ATSDR's responsibilities in the areas of public health assessments, establishment and maintenance of toxicological databases information dissemination, and medical education; new groups within ATSDR were organized to carry out the new tasks. By August 1989, the agency had assumed its current structure.

In October of 2003, The ASTSDR was consolidated with the National Center for Environmental Health (NCEH) of the CDC to form the NCEH/ATSDR.

Agency Mission

The mission of ATSDR is to prevent exposure and adverse human health effects and diminished quality of life associated with exposure to hazardous substances from waste sites, unplanned releases, and other sources of pollution in the environment. ATSDR works closely with state, local, and other federal agencies to reduce or eliminate illness, disability, and death that result from exposure of the public and workers to toxic substances at waste disposal and spill sites.

As the lead agency within the Public Health Service responsible for implementing the health-related provisions of CERCLA, ATSDR was charged with assessing the presence and nature of health hazards at specific Superfund sites, helping to prevent or reduce further exposure and the illnesses that result and expanding what is known about the health effects of exposure to hazardous substances. The newly consolidated NCEH/ATSDR has as its mission to serve the public by using the best science, taking responsive public health actions and providing trusted health information to prevent harmful exposures and disease related to toxic substances.

Range of Agency Activities

The following is a summary of the activities assigned to ATSDR in 1980 under the original Superfund statute:

- Determine the extent of danger to public health from a release or threatened release of a hazardous substance. (This mandate covers the range of public health assessment and other support activities provided to US EPA, states, and other federal agencies at emergency, immediate-removal, and remedial Superfund sites.)
- Conduct periodic surveys and screening programs to determine the relationships between exposure to hazardous substances and illness. (This mandate includes *in vivo* and *in vitro* toxicologic testing, human epidemiologic studies, and establishment of surveillance systems.)
- Establish and maintain a registry of serious diseases and illnesses and registries of all persons environmentally exposed to hazardous substances whenever inclusion of such persons in registries would be scientifically appropriate or valuable for long-term follow-up or specific scientific studies.
- Establish and maintain a comprehensive and publicly accessible inventory of literature on the health effects of hazardous substances.

- When public health emergencies are caused or are believed to be caused by exposure to hazardous substances, assist, consult, and coordinate with private or public health care providers in providing medical care and testing exposed individuals, including collecting and analyzing laboratory specimens as may be indicated by specific exposures.
- Establish and maintain a complete list of areas closed to the public or otherwise restricted in use because of hazardous substance contamination.

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National Center for Toxicological Research

Ankur V Dnyanmote and Harihara M Mehendale

The National Center for Toxicological Research (NCTR), a component of the Jefferson Laboratories of the Food and Drug Administration (FDA), is located in Jefferson, Arkansas. Its mission is to conduct innovative peer-reviewed scientific research focused on FDA regulatory needs. Research findings provide the basis for FDA to make sound science-based risk management decisions that promote the health of the American people.

The NCTR conducts a variety of research, including basic (scientific discovery and knowledge driven), translational (interpretation and/or revision of basic scientific concepts), and applied (developing and applying standards). This research is aimed at studying the biological effects of potentially toxic chemicals or microorganisms, defining the complex mechanisms that govern their toxicity, understanding critical biological events in the expression of toxicity, and developing methods to improve assessment of human exposure, susceptibility, and risk. Customized bioassessment of chemicals of vital interest to the FDA involves the coordination of expertise in the areas of biochemical and molecular markers of carcinogenicity, quantitative risk assessment, transgenics (mimicking responses in animal modes by insertion of human genes into a test animal or tissue culture), neurotoxicology, microbiology, chemistry, and genetic or reproductive/developmental toxicology.

Using its existing strengths in methods development, statistics, analytical chemistry, and spectroscopy, NCTR is developing and standardizing new technologies, such as genomics, proteomics, hepatotoxicology, metabonomics, phototoxicology, and nanotechnology. NCTR's Center for Toxicoinformatics uses software systems and analysis capability to manage and integrate the data from these new technologies with traditional toxicological data.

Perhaps of greater importance to its research accomplishments is the benefit gained by sharing knowledge through collaborations with scientific staff. A major emphasis for NCTR is to conduct research on compounds nominated by the FDA for evaluation by the National Institute of Environmental Health Sciences/National Toxicology Program (NIEHS/NTP). In addition to these collaborations with the other FDA centers and NTP, NCTR partners with 10 other government agencies, over 25 universities and medical centers (including local, national, and foreign), other research facilities around the world, and several industries to investigate predictive toxicology issues.

The NCTR organizational structure consists of the Office of the Director, encompassing staff offices to implement safety and security functions; the Office of Planning and Resource Management, which coordinates strategic and logistical planning, financial management, as well as administrative services and management; and the Office of Research, which includes eight divisions to conduct mission research.

NCTR employs ~230 full-time federal employees, supplemented by ~250 contractor employees providing animal diet, maintenance, and pathology; computer and information management services; facilities maintenance; onsite occupational health care; and administrative services (e.g., supplies receiving and warehousing, mail delivery, and document reproduction).

NCTR aggressively provides scientific training opportunities in its state-of-the-art research facilities to help increase the limited pool of qualified scientists. NCTR provides coordination and support of an

interdisciplinary toxicological program and regulatory science curriculum at two Arkansas universities and maintains a commitment to science education initiatives, which provide a 'pipeline' from high school to postgraduate training. In 2003, 79 people, 41 of whom represented 21 foreign countries, participated in independent or collaborative research supported by these initiatives.

The NCTR is an FDA-owned facility that houses more than 30 buildings containing over a million square feet of floor space on 496 acres and has $40 million worth of advanced research equipment. Laboratory space consists of 132 general or special purpose research labs, 82 breeding or conventional animal rooms, a nonhuman primate research facility, and 23 specialized laboratories for pathological processing and evaluation. A BioSafety Level 3 laboratory contains seven suites to support animal and microbial bioterrorism research. An onsite housing unit exists to support visiting scientists.

In calendar year 2003, NCTR staff participated in more than 310 scientific protocols and published 234 manuscripts, book chapters, and books (plus abstracts) and 34 final technical reports.

NCTR provides an online scientific journal entitled *Regulatory Research Perspectives*, which highlights some of the latest research topics in the scientific regulatory arena. Another online publication, *NCTR Quarter Page*, highlights special events, Center research activities and staff, and publications in scientific journals. These publications, as well as additional information about the NCTR, are available from the FDA website.

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National Institute for Occupational Safety and Health

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The National Institute for Occupational Safety and Health (NIOSH) is the federal agency responsible for conducting research and making recommendations for the prevention of work-related injury and illness. NIOSH is part of the Centers for Disease Control and Prevention (CDC) in the US Department of Health and Human Services. NIOSH provides national and world leadership to prevent work-related illness, injury, disability, and death by gathering information, conducting scientific research, and translating the knowledge gained into products and services.

The objectives of NIOSH include:

- Conducting research to reduce work-related illnesses and injuries.
- Promoting safe and healthy workplaces through interventions, recommendations, and capacity building.
- Enhancing global workplace safety and health through international collaborations.

NIOSH scientists work in multidisciplinary teams and carry out a focused program of intramural and extramural research to prevent or reduce work-related injury and illness. In 1996, NIOSH and over 500 partners established the National Occupational Research Agenda (NORA), a framework to guide the efforts of the occupational safety and health community in 21 priority research areas. NORA encompasses research areas such as traumatic injury, asthma and chronic obstructive pulmonary disease, hearing loss, and control technologies. These priority areas were identified through extensive input from NIOSH's federal and nonfederal partners. Since 1996, NIOSH has aligned its intramural and extramural research to increase its investment in NORA priority areas.

The Occupational Safety and Health Act of 1970 created both NIOSH and the Occupational Safety and Health Administration (OSHA). OSHA is in the US Department of Labor and is responsible for developing and enforcing workplace safety and health regulations. NIOSH is in the US Department of Health and Human Services and is an agency established to help assure safe and healthful working conditions for working men and women by providing research, information, education, and training in the field of occupational safety and health.

Toll-Free Technical Information Service

The NIOSH 800 number (+1-800-356-4674) is a toll-free technical information service that provides convenient public access to NIOSH and its information resources. The service is available to anyone in the continental United States, Alaska, Hawaii, Puerto Rico, or the Virgin Islands.

Callers may request information about NIOSH activities, order NIOSH publications, or request information about any aspect of occupational safety and health. However, this toll-free number is NOT a hotline for medical emergencies.

The 800-number combines an automated voicemail system with direct access to NIOSH technical information staff and the NIOSH Publications Office. The automated system operates 24 h a day. It provides recorded information on a variety of topics, including directions for ordering NIOSH publications. In addition, callers may speak directly with a technical information specialist or to a publications representative from 9:00 a.m. until 4:00 p.m. (EST). All information is provided free of charge within 10 working days.

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Relevant Website

http://www.cdc.gov – The NIOSH home page is located on this URL. The home page provides access to information about NIOSH and related activities, including NIOSH documents, databases, and other resources. The eNews weblink is a monthly electronic update that highlights the latest news at NIOSH.

National Institute of Environmental Health Sciences

Harihara M Mehendale

The National Institute of Environmental Health Sciences (NIEHS) is located in Research Triangle Park, North Carolina, and is part of the federal National Institutes of Health. Since its creation in 1966, the NIEHS has been the primary source of federal efforts for studying how environmental factors affect human health. The mission of the NIEHS is to define how exposures to environmental agents affect our health, how individuals differ in their susceptibility to these effects, and how these susceptibilities affect over time. Diseases and dysfunctions with an environmental component include cancer, birth defects, infertility, neurological impairments, immune disorders, and lung dysfunctions. Because of the broad scope of its mission, NIEHS research relies on essentially every discipline in the biological, chemical, and physical sciences. It maintains a multidisciplinary intramural research program at its Research Triangle Park facility and supports university-based research and training through a variety of grant mechanisms. Through its participation in the National Toxicology Program, NIEHS has made significant contributions in providing state-of-the-art toxicological characterization for a host of environmentally and commercially important agents. Because of the high quality of these studies, they serve to guide risk assessments both in the United States and abroad.

Basic science supported by NIEHS attempts to identify the environmental components of human disease and to understand the basic molecular mechanisms leading to these disease states. Environmentally related diseases of special interest to the institute are those dealing with women's health, children's health, minorities' health, aging, respiratory disorders, neurological disorders, immune system disruption, and cancer. Cellular processes that hold promise for explaining environmentally related diseases include regulatory genes that serve as targets for environmentally induced effects, cellular communication pathways, the integration of biological processes across organ systems, and the genetic basis of individual susceptibility to environmental agents and the diseases and disorders they cause. The NIEHS is also expanding its clinical research programs to enable it to more readily translate laboratory-based findings into human therapies.

Prevention and intervention efforts are a major focus of NIEHS activities. These efforts include hazard identification and characterization, both through

traditional animal testing and epidemiological studies and through incorporation of mechanistic considerations to arrive at new insights into the molecular basis of toxic effects. The improved understanding of the molecular foundation of environmentally associated effects will enable the institute to strengthen the validity of risk assessment schemes as a means of deciding regulatory policy. An improved understanding of the molecular basis of toxicant action could also lead to innovative molecular prevention and intervention therapies to circumvent clinical manifestations of environmentally caused diseases.

Finally, the NIEHS has devised a communication strategy, which ensures that the findings generated by its basic and applied research reach the groups that need the information. These groups include the lay public and the institute's partners in research, governmental agencies, advocacy groups, and the international community.

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National Institutes of Health

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The National Institutes of Health (NIH) is the principal biomedical research agency of the US Federal Government. Its mission is to employ science in the pursuit of knowledge to improve human health conditions. To accomplish this goal, the Institute seeks to expand fundamental knowledge about the nature and behavior of living systems, to apply that knowledge to extend the health of human lives, and to reduce the burdens resulting from disease and disability. In the quest of this mission, NIH supports biomedical and behavioral research domestically and abroad, conducts research in its own laboratories and clinics, trains promising young researchers, and promotes acquiring and distributing medical knowledge. Focal points have been established to assist in developing NIH-wide goals for health research and training programs related to women and minorities, coordinating program direction, and ensuring that research pertaining to women's and minority health is identified and addressed through research activities conducted and supported by NIH. Research activities conducted by NIH will determine much of the quality of health care for the future and reinforce the quality of health care currently available.

The NIH comprises the Office of the Director and 27 Institutes and Centers. The Office of the Director is responsible for setting policy for NIH and for planning, managing, and coordinating the programs and activities of all NIH components.

The major components of the NIH are

1. National Cancer Institute;
2. National Eye Institute;
3. National Heart, Lung, and Blood Institute;
4. National Human Genome Research Institute;
5. National Institute on Aging;
6. National Institute on Alcohol Abuse and Alcoholism;
7. National Institute of Allergy and Infectious Diseases;
8. National Institute of Arthritis and Musculoskeletal and Skin Diseases;
9. National Institute of Biomedical Imaging and Bioengineering;
10. National Institute of Child Health and Human Development;
11. National Institute on Deafness and Other Communication Disorders;
12. National Institute of Dental and Craniofacial Research;
13. National Institute of Diabetes and Digestive and Kidney Diseases;
14. National Institute of Diabetes and Digestive and Kidney Diseases;
15. National Institute of Environmental Health Sciences;
16. National Institute of General Medical Sciences;
17. National Institute of Mental Health;
18. National Institute of Neurological Disorders and Stroke;
19. National Institute of Nursing Research;
20. National Library of Medicine;
21. Center for Information Technology;
22. Center for Scientific Review;
23. John E. Fogarty International Center;

24. National Center for Complementary and Alternative Medicine;
25. National Center on Minority Health and Health Disparities;
26. National Center for Research Resources; and
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National Library of Medicine/TEHIP

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The National Library of Medicine (NLM), part of the National Institutes of Health (NIH) and located in Bethesda, Maryland, is by far the largest medical library in the world. It provides toxicological information through its substantial holdings of books and journals and, more specifically, through its Toxicology and Environmental Health Information Program (TEHIP). TEHIP, which resides in NLM's Division of Specialized Information Services (SIS) and whose origin dates back to 1967, offers a broad array of web-based, freely available databases and other electronic information resources in toxicology and environmental health; furthermore, it answers queries, sponsors publications, and responds to the information needs of other federal agencies. TEHIP is considered one of the world's major providers of toxicology and environmental health information resources.

TEHIP's primary information resource repository is the TOXNET system of databases. TOXNET comprises databases of summary toxicological information, technical literature, references, chemical nomenclature, and toxic releases. It also provides links to additional sources of information on toxicology, environmental health, and hazardous chemicals. The contents of the TOXNET databases are derived from federal agencies such as the US Environmental Protection Agency, the National Cancer Institute, and NLM itself.

The following paragraphs will summarize TEHIP's free, web-based toxicological information resources. The individual databases of the TOXNET system will be described first. Descriptions of miscellaneous additional electronic resources available through TEHIP will follow.

The TOXNET databases contain various types of information. One group of these databases, sometimes referred to as the 'factual databanks', contain toxicologically oriented records organized by chemical. They are the Chemical Carcinogenesis Research Information System (CCRIS), GENE-TOX, the Hazardous Substances Data Bank (HSDB), the Integrated Risk Information System (IRIS), and the International Toxicity Estimates for Risk (ITER). Following their descriptions, information is given about ChemIDplus (an online chemical dictionary), the Toxics Release Inventory (TRI), TOXNET's bibliographic databases, and other resources.

Factual Databanks

CCRIS

CCRIS is a scientifically evaluated and fully referenced data bank, developed and maintained by the National Cancer Institute (NCI). It contains over 8000 chemical records with carcinogenicity, mutagenicity, tumor promotion, and tumor inhibition test results. Data are derived from studies cited in primary journals, current awareness tools, NCI reports, and other special sources. Test results have been reviewed by experts in carcinogenesis and mutagenesis.

GENE-TOX

GENE-TOX is a toxicology data file created by the US Environmental Protection Agency (EPA) and contains genetic toxicology (mutagenicity) test data, resulting from expert peer review of the open scientific literature, on over 3000 chemicals. The GENE-TOX program was established to select assay systems for evaluation, review data in the scientific literature, and recommend proper testing protocols and evaluation procedures for these systems.

HSDB

HSDB is a toxicology data file that focuses on the toxicology of potentially hazardous chemicals. It covers information on human exposure, industrial hygiene, emergency handling procedures, environmental fate, regulatory requirements, and related

areas. All data are referenced and derived from a core set of books, government documents, technical reports, and selected primary journal literature. HSDB is peer reviewed by the Scientific Review Panel (SRP), a committee of experts in the major subject areas within the data bank's scope. HSDB contains over 4700 extensive and highly structured individual chemical records.

IRIS

IRIS is a toxicology data file that contains data in support of human health risk assessment. It is compiled by the US EPA and contains over 500 chemical records. IRIS data, focusing on hazard identification and dose–response assessment, are reviewed by work groups of EPA scientists and represents EPA consensus. Among the key data provided in IRIS are EPA carcinogen classifications, unit risks, slope factors, oral reference doses, and inhalation reference concentrations.

ITER

ITER is a toxicology data file that contains data in support of human health risk assessments. It is compiled by Toxicology Excellence for Risk Assessment (TERA) and contains over 600 chemical records with key data from the Agency for Toxic Substances and Disease Registry (ATSDR), Health Canada, the Dutch National Institute of Public Health and the Environment (RIVM), the US EPA, and independent parties whose risk values have undergone peer review. ITER provides a comparison of international risk assessment information in a side-by-side format and explains differences in risk values derived by different organizations. ITER data, focusing on hazard identification and dose–response assessment, are extracted from each agency's assessment and contain links to the source documentation.

Chemical Dictionary

ChemID*plus*

ChemID*plus* is a search system that provides access to structure and nomenclature authority files used for the identification of chemical substances cited in NLM databases. ChemID*plus* also provides structure searching and direct links to many biomedical resources at NLM and on the Internet for chemicals of interest. The database contains over 368 000 chemical records, of which over 206 000 include chemical structures, and is searchable by Name, Synonym, CAS Registry Number, Molecular Formula, Classification Code, Locator Code, and Structure.

Toxics Release Inventory

TRI

TRI is an annually compiled series of databases spanning the reporting years 1987–2001, and which contains information on the annual estimated releases of toxic chemicals to the environment. It is based upon data collected by the US EPA. Mandated by the Superfund legislation, TRI's data cover air, water, land, and underground injection releases, as well as transfers to waste sites, and waste treatment methods and efficiency, as reported by industrial facilities in the United States. TRI also includes data related to source reduction and recycling.

Bibliographic Databases

ALTBIB

Technically not part of TOXNET, but supplemental to it, ALTBIB is a bibliographic database on alternatives to the use of live vertebrates in biomedical research and testing. Its intent is to assist in identifying methods and procedures helpful in supporting the development, testing, application, and validation of alternatives to the use of vertebrates in biomedical research and toxicology testing. This bibliographic database, covering the literature for the time period 1992–2001, is produced from MEDLARS database searches, performed and analyzed by TEHIP subject experts.

DART (Developmental and Reproductive Toxicology)

DART is a bibliographic database that covers teratology and other aspects of developmental and reproductive toxicology. It contains over 100 000 references to literature published since 1965. DART/ETIC is funded by the US EPA, the National Institute of Environmental Health Sciences, the National Center for Toxicological Research of the Food and Drug Administration, and NLM.

TOXLINE

TOXLINE is a bibliographic database covering the multidisciplinary literature of toxicology. Its records provide bibliographic information on the biochemical, pharmacological, physiological, and toxicological effects of drugs and other chemicals. TOXLINE contains over 3 million bibliographic citations, most with abstracts and/or indexing terms and CAS Registry Numbers. TOXLINE references are drawn from various sources and are grouped into two parts: TOXLINE Core and TOXLINE Special. TOXLINE Core covers much of the standard journal literature

in toxicology. A search link to TOXLINE Core is available from the TOXLINE interface. It is also directly available via the PubMed system by selecting 'toxicology' as a subset limit. TOXLINE Special complements TOXLINE Core with references from an assortment of more specialized journals, technical reports, and other sources.

Health and Biomedicine Directory

DIRLINE

DIRLINE is a database containing location and descriptive information about a wide variety of information resources including organizations, research resources, projects, and databases concerned with health and biomedicine. This information may not be readily available in bibliographic databases. DIRLINE contains over 17 000 records and focuses primarily on health and biomedicine, although it also provides limited coverage of some other special interests. DIRLINE includes records pertinent to the areas of toxicology, chemical safety, and environmental health. Each record may contain information on the publications, holdings, and services provided.

Additional Specialized Resources

Haz-Map

Haz-Map is an occupational health database designed for health and safety professionals and for consumers seeking information about the health effects of exposure to chemicals at work. Haz-Map links jobs and hazardous tasks with occupational diseases and their symptoms. Chemicals and biological agents in Haz-Map are linked to industrial processes and other activities such as hobbies. Occupational diseases and their symptoms are associated with hazardous job tasks and possible exposure to hazardous agents. Information from textbooks, journal articles, and electronic databases such as HSDB (described above) is classified and summarized to create this database.

Household Products Database

The Household Products Database provides information on the potential health effects of chemicals contained in over 5000 common household products. This database allows scientists and consumers to find out about ingredients in brand-name products. It is designed to help answer the following questions: What chemicals are contained in specific brands and in what percentage? Which products contain specific chemicals? Who manufactures a specific brand? How can the manufacturer be contacted? What are the potential adverse health effects (acute and chronic) of the ingredients in a specific brand? What other information is available about such chemicals in toxicology-related NLM databases? The database allows browsing of product categories and searching of products by type, manufacturer, product ingredient/chemical name, and health effects. The record for each product shows the ingredients as reported in the manufacturer's Material Safety Data Sheet (MSDS) and includes other information such as handling, disposal, and health effects.

Internet Resources in Toxicology and Environmental Health

The Internet Resources section of the TEHIP Website covers topics such as Arsenic and Human Health, Biological Warfare, Chemical Warfare, Children's Environmental Health, Environmental Justice, September 11th World Trade Center Disaster Lingering Airborne Hazards, and Pesticides Used for West Nile Virus Control. For each topic, TEHIP provides numerous links to a variety of related Websites and other electronic resources.

NLM-Tox-Enviro-Health-L Listserv

The NLM-Tox-Enviro-Health-L listserv is an email announcement list whose purpose is to broadcast updates on SIS's resources, services, and outreach in toxicology and environmental health. The NLM-Tox-Enviro-Health-L Archives allow users to search list postings and to modify subscription options. Anyone interested in subscribing to this listserv should send an email to listserv@list.nih.gov.

Review of PDA Applications in Toxicology and Environmental Health

The Review of PDA Applications in Toxicology and Environmental Health was undertaken by TEHIP staff in light of the increasing use of personal digital assistants (PDAs) and specialized PDA software applications in the fields of toxicology and environmental health. The purpose of this Web resource is to make available descriptive reviews of the main technical and content features of selected PDA software applications. Individual reports in the review series are based on free, downloadable demo versions of the software and cover the following topics: General Information, Intended Users, Authorship/Data Source, Contents, Navigation, Requirements, Application Type/Price, Availability, Useful Web Links, and Updates.

Toxicology Tutorials

The Toxicology Tutorials are written at the introductory college student level and are intended to provide a basic understanding of toxicology as an aid for users of the toxicology literature, such as that found in the TOXNET databases described above. Toxicology Tutor I is the first in a set of three tutorials and covers basic principles of toxicology. Toxicology Tutor II covers toxicokinetics, while Toxicology Tutor III deals with the basic toxic mechanisms that operate at the cellular level, including those that interfere with normal biochemical functions. The Toxicology Tutorials are scheduled to be updated and expanded, and will be positioned as a highlight within a new page more broadly concerned with toxicology education.

TOXMAP

TOXMAP is a Web resource that uses maps of the United States to show the amount and location of toxic chemicals released into the environment. Data are derived from the TRI database (described above), which provides information on toxic releases into the environment as reported by US industry. TOXMAP helps users create nationwide or local area maps showing where chemicals are released into the air, water, and ground. It also identifies the releasing facilities, color-codes release amounts for a single year, and provides multi-year chemical release trends, starting with 1987. Users can search the system by chemical name, chemical name fragment, and/or location (such as city, state, or zip code). TOXMAP also overlays map data such as US Census population data.

Tox Town

Tox Town, an interactive guide to commonly encountered toxic substances, is designed to provide the following: information on common locations where one might find toxic chemicals; nontechnical descriptions of chemicals; links to selected, authoritative information about chemicals on the Internet; information concerning environmental impacts on human health; and Internet resources on environmental health topics. Tox Town uses color, graphics, sounds, and animation to add interest to learning about connections between chemicals, the environment, and public health. Tox Town's target audience comprises students above elementary-school level, educators, and the general public. Tox Town also provides some resources in Spanish and has a text version. Tox Town currently offers a 'Town' scene and a 'City' scene; a 'US–Mexico Border Community' scene is under development.

WISER

Wireless Information System for Emergency Responders (WISER) is a system designed to assist first responders in hazardous material incidents. The application provides a wide range of information on hazardous substances, including substance identification support, physical characteristics, human health information, and containment and suppression guidance. WISER features mobile support, comprehensive decision support (including assistance in identifying unknown substances and guidance on immediate actions required to save lives and protect the environment), access to 390 substances derived from HSDB (described above), rapid access to crucial information about them, and an intuitive, simple, and logical user interface.

TEHIP's New Initiatives

Drugs and Lactation Database

The Drugs and Lactation database will be a searchable database of records on drugs and their use during lactation. This database, which is intended to support medical decision-making by healthcare professionals, is envisioned as a comprehensive, evidence-based resource covering possible adverse effects of prescription and nonprescription drugs and diagnostic agents in breast-feeding infants. The Drugs and Lactation database will complement the suite of TOXNET databases and will be linked, where appropriate, to other TOXNET and NLM database records.

ToxSeek

ToxSeek is an experimental toxicology and environmental health meta-search engine that allows users to search diverse Web-accessible databases simultaneously. ToxSeek automatically identifies key concepts in search results and uses these 'Related Concepts' and other information to merge, rank, and intelligently cluster the items retrieved from heterogeneous information sources. ToxSeek also supports a focused drill-down in its 'Concept Clusters', as well as dynamic query modification and multiple spell-checkers for general English, medical terminology, and chemical names.

World Library of Toxicology, Chemical Safety, and Environmental Health

The World Library is envisioned as a Web portal to international information resources in the areas of toxicology, chemical safety, and environmental health. It will be hosted by TEHIP and maintained

in collaboration with national representatives (in-country experts who have volunteered their time and expertise). The World Library will link to credible sources of scientific and consumer information on chemical, biological, and physical (including radiation) hazards, and their effects on human, animal, and ecosystem health. The audience will include many sectors of the international community – research, academic, government, corporate, and non-profit.

More Toxicology Information from other Divisions of the National Library of Medicine

Finally, in addition to the many information resources provided by TEHIP, there are a few relevant toxicological information resources provided by other entities within NLM. One such information resource is PubMed, the world's largest medical database, which contains a great deal of toxicological information accessible via its 'toxicology' subset. As mentioned earlier, this information is equivalent to that accessible via a TOXLINE Core search. MEDLINEplus provides a wide range of consumer health information and thus represents a significant source of toxicological and environmental health information of interest to the general public. ClinicalTrials.gov (http://www.clinicaltrials.gov) provides regularly updated information about federally and privately supported clinical research in human volunteers. More specifically, ClinicalTrials.gov provides information about a trial's purpose, who may participate, locations, and phone numbers for further details. This database, which allows its users to access significant toxicological information, was developed in collaboration with the US Food and Drug Administration.

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Further Reading

Wexler P (2004) The US National Library of Medicine's Toxicology and Environmental Health Information Program. *Toxicology* 198(1–3).

Relevant Website

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

National Toxicology Program

Harihara M Mehendale

The National Toxicology Program (NTP), an interagency program, was established in 1978 as a cooperative effort within the US Public Health Service of the Department of Human Services. The four primary objectives of the NTP are to (1) coordinate toxicology research and testing activities within the department, (2) provide information about potentially toxic chemicals to health regulatory and research agencies, scientific and medical communities, and the public, (3) strengthen the science base in toxicology, and (4) develop and validate improved testing methods. In its 25 years, the NTP has become a world leader in designing, conducting, and interpreting various types of assays for toxicity. Through its activities, the NTP provides, directly or indirectly, a large component of the basic scientific data that other federal and state scientific and regulatory agencies, as well as private-sector organizations, use in responding to issues relevant to the effects of chemical and physical agents on human health and the environment. All of the NTP's activities are open to public scrutiny, including communication with all interested parties. The NTP draws strength and direction from the commitment of its scientists to exchanging information openly, maintaining impartiality, and applying rigorous scientific peer review.

The charter agencies of the NTP were the National Institute of Environmental Health Sciences (NIEHS) of the National Institutes of Health (NIH), the National Cancer Institute (NCI) of the NIH, the National Institute of Occupational Safety and Health of the Centers for Disease Control and Prevention, and the Food and Drug Administration's National Center for Toxicological Research. In 1981, the

carcinogenesis bioassay program of the NCI was transferred to the NIEHS. Although no longer a core NTP agency, the NCI remains active in the NTP and serves on the NTP Executive Committee. The director of the NIEHS also serves as director of the NTP and the administrative staff for the NTP is located at the NIEHS.

The NTP relies upon advice on its activities and priorities from its advisory committees. The NTP Executive Committee, composed of the heads (or their designees) of federal health regulatory and research agencies, provides primary policy oversight to the NTP. The NTP Board of Scientific Counselors and its two standing subcommittees provide primary scientific oversight for the NTP's intramural and collaborative activities. The Board is a federally chartered advisory committee composed primarily of nonfederal scientists comprising a broad spectrum of expertise and affiliations, including academia, industry, labor, public health, and state and federal governments. The Technical Reports Review Subcommittee of the Board provides peer review for NTP long-term toxicology and carcinogenicity studies and short-term toxicity reports. The Report on Carcinogens Subcommittee of the Board provides external scientific evaluation of substances nominated for listing in or delisting from the Report on Carcinogens, a Congressionally mandated document that lists substances known or reasonably anticipated to be human carcinogens, and to which a significant number of persons in the United States are exposed. The Scientific Advisory Committee on Alternative Toxicological Methods provides external scientific input on priorities and directives related to the development, validation, scientific review, and regulatory acceptance of new or revised toxicological test methods and on ways to foster communication and partnerships with all interested parties.

The NTP's mission is to evaluate agents of public health concern by developing and applying tools of modern toxicology and molecular biology. The program maintains a number of complex, interrelated research and testing programs that provide unique and critical information needed by health regulatory and research agencies to protect human health. The NTP's research and testing program includes chronic bioassays, short-term toxicity studies, mechanistic studies, model development, alternative models, and human studies.

The NTP maintains a balanced research and testing program that provides data on a wide variety of issues important to human health. The NTP continually solicits nominations of substances for study and invites nominations from all interested parties and groups. In particular, the NTP seeks nominations of studies that enhance the predictive ability of future NTP studies, address mechanisms of toxicity, or fill significant gaps in the knowledge of the toxicity of chemicals or classes of chemicals. Nominations undergo several levels of review that include the opportunity for public comment. The NTP strives to balance the selection of substances for study (e.g., occupational exposures, environmental pollutants, food additives, consumer products, and pharmaceuticals). The NTP evaluates selected substances for a variety of health-related effects, among them, general toxicity, reproductive and developmental toxicity, genotoxicity, immunotoxicity, neurotoxicity, metabolism, disposition, and carcinogenicity. In addition, there are special projects focused on AIDS therapeutics and toxicity of superfund chemicals.

As the NTP moves into the twenty-first century, the program is evaluating its key activities and in a focused and concerted effort determining how best to incorporate new scientific technologies of molecular biology, computer science, and genomics into its research and testing strategies and broaden scientific knowledge on the linkage between mechanism and disease. The NTP's vision is to move toxicology from a predominantly observational science at the level of disease-specific models to a predominantly predictive science focused upon a broad inclusion of target-specific, mechanism-based, biological observations. The NTP is inviting input on how best to achieve this vision from its federal partners, advisory committees, academia, industry, and the public. The NTP will use this input to develop a framework targeted toward achieving the vision and including the necessary components for implementation, management, and communication of changes in NTP activities. It is envisioned that the acceptance and implementation of this vision in addressing public health priorities will result in better science and ultimately better decisions that protect human health and the environment.

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Occupational Safety and Health Administration

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With the passage of the Occupational Safety and Health Act of 1970 (OSH Act, P.L. 91-596), the US Congress created the Occupational Safety and Health Administration (OSHA) to "assure so far as possible every working man and woman in the Nation safe and healthful working conditions."

OSHA provides leadership and encouragement to US employers and employees to help them recognize and realize the value of safety and health on the job. The agency's goal is to save lives, prevent injuries and illnesses, and protect the safety and health of American workers.

Since its inception in 1971, OSHA has helped to cut workplace fatalities by more than 60% and occupational injury and illness rates by 40%. At the same time, US employment has doubled from 56 million workers at 3.5 million worksites to more than 115 million workers at 7.1 million sites.

The Labor Department agency employs three strategies to promote safety and health in American workplaces: strong, fair, and effective enforcement; outreach, education, and compliance assistance; and cooperative and voluntary programs.

The OSH Act also encourages states to develop and operate their own safety and health plans. Approved under Section 18 (b) of the Act, these plans must adopt and enforce standards at least as effective as federal requirements.

OSHA offers safety and health training through the OSHA Training Institute in Des Plaines, Illinois, and through 20 Education Centers at 35 sites across the country. Training schedules are available on the agency's website along with interactive software called eTools, which offer step-by-step guidance on many safety and health issues. More than 65 compliance assistance specialists in local OSHA offices are also available to speak to groups, teach workshops, and present seminars on safety and health topics. Consultation programs in each state offer small businesses onsite safety and health guidance from experts.

Cooperative and voluntary programs sponsored by OSHA include Voluntary Protection Programs, the agency's premier partnership; Strategic Partnerships, which emphasizes effective safety and health management systems; SHARP, a recognition program for excellence for small businesses; and Alliances, which promotes safety and health training and sharing of best practices.

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Organisation for Economic Cooperation and Development

Robert Visser*

The Organisation for Economic Cooperation and Development (OECD) is an intergovernmental organization. Its aims and responsibilities are achieving the highest sustainable economic growth and employment; promoting economic and social welfare throughout the OECD region by coordinating policies of its member countries; and stimulating and harmonizing the efforts of member countries in favor of developing countries. At the time of writing, there are 30 member countries: Australia, Austria, Belgium, Canada, the Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Japan, Luxembourg, Mexico, The Netherlands, New Zealand, Norway, Poland, Portugal, Republic of Korea, the Slovak Republic, Spain, Sweden, Switzerland, Turkey, the United Kingdom, and the United States. The European Commission also participates in the Organization's work.

*Any opinions expressed in this article do not necessarily represent the opinions of the Organisation for Economic Cooperation and Development (OECD) or its member countries and should therefore be viewed as solely those of the author.

Within the OECD, the governments of these industrialized countries compare and, if they so decide, coordinate their domestic policies. Monitoring international economic trends is one of the Organization's best known activities. However, since the OECD was established in 1960 the number of policy areas in which it functions as a center for cooperation and exchange of views has steadily increased. Since 1971, work in the OECD related to chemical safety has been organized under the Chemicals Programme. The policy direction and priorities of the Chemicals Programme are determined by member country representatives, nominated by each country's government, who take part in the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology. The Chemicals Programme is supported by the Environment, Health and Safety Division of the OECD. Technical work on chemicals is carried out for the most part by experts from government, industry, nongovernmental interest groups, and academic institutions in member countries, who attend workshops and other meetings.

The main objectives of the Chemicals Programme are to assist member countries in identifying, preventing, and managing the risks of chemicals; promote the public's right to know about the hazards, exposures, and potential risks of chemicals; prevent unnecessary nontariff distortions in the trade of chemicals; facilitate the optimal use of resources available in governments and in industry for chemicals management; assist member countries in achieving the objectives of the United Nations Conference on Environment and Development's (UNCED's) Agenda 21, Chapter 19, and in the application of the criteria for environmental sustainability, as agreed upon by OECD Environment Ministers in the OECD Environment Strategy for the first decade of the twenty-first century; work with OECD countries and specific nonmembers to prevent globalization of the chemicals industry that would lead to negative impacts on human health and the environment throughout the world; assist member countries in the development of approaches to improve the use of voluntary actions for chemicals management by the industry; and promote the development and implementation in member countries of new and innovative instruments for more holistic approaches to management of the risks of chemicals throughout their life cycle, including those related to the risks of their use in products.

Since the 1970s, the OECD has been in the forefront in developing policies and instruments to control chemicals and chemical products. Work in the Chemicals Programme on the development, updating, and expansion of scientifically valid harmonized methods for testing and assessment helps OECD countries (and, increasingly, non-OECD countries that use these methods) to prevent new chemicals that might present unacceptable risks from entering the market and to assess the safety of chemicals already in use.

Many OECD chemical safety activities are carried out in cooperation with other relevant organizations (Food and Agriculture Organization of the United Nations, International Labour Organization, United Nations Environment Programme (UNEP), United Nations Industrial Development Organization, United Nations Institute for Training and Research, and World Health Organization) through the Inter Organization Programme on the Sound Management of Chemicals and with the United Nations Development Programme and the World Bank.

Mutual Acceptance of Data

One of the most significant achievements of cooperative work in the OECD aimed at the harmonization of national chemical control procedures and policies is the 1981 Council Act on the Mutual Acceptance of Data (MAD) in the Assessment of Chemicals. This Act contains a Decision that "data generated in the testing of chemicals in an OECD member country in accordance with OECD Test Guidelines and OECD Principles of Good Laboratory Practice (GLP) shall be accepted by other member countries for purposes of assessment and other uses relating to the protection of man and the environment." The Council is the OECD's highest authority, in which the governments of all member countries participate. Once member countries have adopted a Council Decision, they are under a legal obligation to implement it. Consequently, when data developed in an OECD country under the conditions set out in this Decision are submitted in other OECD countries to fulfill regulatory requirements, the data cannot be refused and so do not have to be developed over again for notification in each country. (The OECD Test Guidelines and OECD Principles of GLP are described below.)

The intention of this Council Act is to ensure that data generated in the safety testing of chemicals are of high quality and are based on internationally harmonized methods. Such data can then be used to assess chemical hazards and to make decisions on appropriate activities to prevent or reduce risk to human health or the environment. Where the need for duplicative testing is minimized through MAD, testing costs can

be reduced for both governments and industry (it has been calculated that the annual saving resulting from MAD in this respect amounts to ~US$60 million). Test facilities and specialist personnel can be utilized more efficiently, and fewer animals will be used in testing (an important consideration in OECD countries, where animal welfare is an issue of concern). Harmonized safety testing can also help prevent non-tariff barriers which could arise in the trade of chemicals and chemical products as a result of differences in national chemical control regulations.

Since 1997 a procedure through which non-OECD countries can adhere to the MAD system has been embodied in a Council Decision (Council Decision on the Adherence of Non-Member Countries to the Council Acts Related to the Mutual Acceptance of Data in the Assessment of Chemicals C(97)114/FINAL). South Africa is now a full participant. India, Israel, and Slovenia participate on a provisional basis and several important chemicals-producing nonmembers are at the point of also doing this.

The OECD Test Guidelines

The *OECD Guidelines for the Testing of Chemicals* is a collection of standard methods used by professionals in governments, industry, academic institutions, and independent laboratories for safety testing of chemical substances. They cover tests for physical–chemical properties, effects on biotic systems (ecotoxicity), environmental fate (degradation/accumulation), and health effects (toxicity) (see **Table 1**). The Test Guidelines are systematically updated to respond to scientific progress or to address new needs identified by member countries. They are published in two loose-leaf binders, and are also available in a CD-ROM version.

The OECD Test Guidelines began to be developed in the late 1970s, by several expert groups, with the goal of enhancing the validity and international acceptability of test data. They have become recognized in both OECD and non-OECD countries as the authoritative reference tool for testing chemicals in a regulatory context. Over the years, many new Test Guidelines have been developed to address new data requirements in the notification and registration of chemicals and pesticides. Furthermore, OECD Test Guidelines are continuously updated to bring them in line with the state of the art of science. A network of more than 1000 experts has been involved in the OECD work on Test Guidelines.

The Test Guidelines Programme is overseen by National Coordinators, who work to achieve consensus on draft versions of new and revised Test Guidelines. Proposals for new or updated Guidelines can be made by a National Coordinator, the international scientific community, and by the OECD Secretariat. To become effective, any new or updated Guideline must be adopted by member countries as part of the Council Decision on MAD.

Special attention is paid within the Test Guideline Programme to animal welfare issues, in particular the reduction, refinement, and replacement of animal use in the OECD Test Guidelines. In updating and developing Test Guidelines, wherever scientifically justified, test methods that do not require the use of animals or that require fewer test animals than existing methods are incorporated. A number of Test Guidelines have been developed or revised with a view to reducing the number of animals used and introducing a framework of testing that allows alternative methods to be applied first.

In 1996, the OECD established a Special Project on Endocrine Disrupter Testing and Assessment with the objectives of providing information on and coordinating the activities of member countries; developing new and revising existing Test Guidelines to detect endocrine disrupters; and harmonizing hazard and risk characterization approaches.

This activity was launched at the request of the member countries and the Business and Industry Advisory Committee to the OECD to ensure that testing and assessment approaches for endocrine disrupters would not substantially differ among countries. In addition to developing tests for endocrine disrupters in the human health and environmental fields, OECD has also developed a conceptual framework for endocrine disrupter testing, outlining consecutive steps that could be followed.

The OECD Principles of GLP

The OECD Principles of GLP provide quality assurance concepts concerning the organization of test laboratories and the conditions under which laboratory studies are planned, performed, monitored, and reported. The purpose of the GLP Principles is to make certain that test data are reliable. Like the Test Guidelines, the Principles of GLP began to be developed at the end of the 1970s and were established in the 1981 Council Decision on MAD.

In 1989, the OECD Council adopted an Act on Compliance with Principles of GLP. This Act contains a Decision that member countries shall (1) establish national procedures for monitoring compliance with GLP Principles, based on laboratory inspections and study audits; (2) designate national compliance monitoring authorities ('GLP inspectors'); and (3) require the management of test facilities to issue a

declaration, where applicable, that a study was carried out according to GLP Principles. It also contains a Decision that member countries shall, under specific conditions, recognize assurance from other member countries that test data have been generated in accordance with GLP Principles, and that they shall designate authorities for international liaison, exchange relevant information on compliance monitoring procedures, and implement procedures whereby, if good reason exists, information on GLP compliance by a test facility in one member country can be sought by another member country. Annexed to this Act are *Guides for Compliance Monitoring Procedures for GLP*, *Guidance for the Conduct of Laboratory Inspections and Study Audits*, and *Guidance for the Exchange of Information Concerning National Procedures for Monitoring Compliance*.

Information exchange takes place within the OECD on technical and administrative matters related to the application of the GLP Principles and the implementation of the compliance-monitoring procedures. The Working Group on GLP, made up of representatives of national GLP inspectors, oversees the Programme on GLP and develops common positions on the administration of compliance monitoring. One of the Working Group's responsibilities is to find solutions to problems involving the acceptance of compliance monitoring. OECD training courses are held for GLP inspectors, who perform laboratory inspections on behalf of national GLP-monitoring authorities. Several OECD expert groups have met to produce Consensus Documents on the harmonized application and interpretation of the GLP Principles in specific areas, or in relation to specific points.

The *OECD Series on Principles of GLP and Compliance Monitoring*, published in the form of short free-on-demand booklets, includes the GLP Principles, the 1981 and 1989 Council Acts, and the Consensus Documents. At the time of writing, there were 13 booklets in the series.

Within OECD, the inspectors are also undertaking mutual joint visits to review all the national GLP-monitoring programs. Each country is visited by a team, comprising inspectors of three other countries, and the program, inspections, and on-site study audits done by the country which is visited are evaluated and discussed among the inspectors.

Cooperative Investigation of High-Production Volume Chemicals

Much of the work in the Chemicals Programme in the 1970s and early 1980s involved the development of anticipatory policies to prevent chemicals that could present unacceptable risks to human health or the environment from reaching the market. In 1987, however, the OECD Council adopted an Act on the Systematic Investigation of Existing Chemicals. Existing chemicals are the many thousands of industrial chemicals already in use. Adequate safety data or hazard assessments for these chemicals (some of which have been in use for a long time) are often unavailable. This Council Act contains a Decision that member countries "shall establish or strengthen national programmes to systematically investigate existing chemicals, in order to identify those which need to be managed and/or controlled." For the purposes of the Act, systematic investigation could include the following steps: identification of relevant chemicals; priority setting, including collection or estimation of information needed for setting priorities; generation of necessary further information, including testing; and performance of hazard assessments.

The 1987 Council Act was intended to strengthen and harmonize member countries' policies with regard to existing chemicals. To avoid duplication of efforts, and to facilitate sharing the financial and administrative burden (for governments and chemical companies) of investigating these chemicals, the Council decided in a 1990 follow-up Act that member countries shall cooperatively investigate high-production volume (HPV) chemicals in order to identify those which are potentially hazardous; cooperatively select the HPV chemicals to be investigated; agree on a set of data needed to make an informed judgment concerning the potential hazards of each chemical, through collection of available data or by ensuring that testing is undertaken; and cooperatively make an initial assessment of the potential hazards of each chemical using that basic data set. These Decisions are contained in the Council Act on the Cooperative Investigation and Risk Reduction of Existing Chemicals.

Following the adoption of this 1990 Council Act, HPV chemicals are defined as those produced or imported in volumes of at least 1000 tons year^{-1} in at least one OECD country. Governments, in consultation with the chemical industry provide the OECD with information on the chemicals produced in these volumes in their countries. A consolidated OECD Representative List of more than 5000 HPV chemicals is regularly prepared and updated. These chemicals represent an estimated 90–95% of the total volume of chemicals produced in member countries. While there was already adequate information concerning the health and environmental effects of certain chemicals on this HPV list, little or no information of this type was available for many chemicals despite their HPV.

Table 1 OECD Guidelines for the Testing of Chemicals

Section 1: Physical–Chemical Properties
101 UV–VIS Absorption Spectra (original guideline, adopted May 12, 1981)
102 Melting Point/Melting Range (updated guideline, adopted July 27, 1995)
103 Boiling Point (updated guideline, adopted July 27, 1995)
104 Vapour Pressure (updated guideline, adopted July 27, 1995)
105 Water Solubility (updated guideline, adopted July 27, 1995)
106 Absorption–Desorption Using a Batch Equilibrium Method (updated guideline, adopted January 21, 2000)
107 Partition Coefficient (*n*-octanol/water): Shake Flask Method (updated guideline, adopted July 27, 1995)
108 Complex Formation Ability in Water (original guideline, adopted May 12, 1981)
109 Density of Liquids and Solids (updated guideline, adopted July 27, 1995)
110 Particle Size Distribution/Fibre Length and Diameter Distributions (original guideline, adopted May 12, 1981)
111 Hydrolysis as a Function of pH (original guideline, adopted May 12, 1981) (see draft guidelines below)
112 Dissociation Constants in Water (original guideline, adopted May 12, 1981)
113 Screening Test for Thermal Stability and Stability in Air (original guideline, adopted May 12, 1981)
114 Viscosity of Liquids (original guideline, adopted May 12, 1981)
115 Surface Tension of Aqueous Solutions (updated guideline, adopted July 27, 1995)
116 Fat Solubility of Solid and Liquid Substances (original guideline, adopted May 12, 1981)
117 Partition Coefficient (*n*-octanol/water), HPLC Method (original guideline, adopted March 30, 1989)
118 Determination of the Number-Average Molecular Weight and the Molecular Weight Distribution of Polymers Using Gel Permeation Chromatography (original guideline, adopted June 14, 1996)
119 Determination of the Low Molecular Weight Content of a Polymer Using Gel Permeation Chromatography (original guideline, adopted June 14, 1996)
120 Solution/Extraction Behaviour of Polymers in Water (updated guideline, adopted January 21, 2000)
121 Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge Using High Performance Liquid Chromatography (HPLC) (original guideline, adopted January 22, 2001)
Section 2: Effects on Biotic Systems
201 Alga, Growth Inhibition Test (updated guideline, adopted June 7, 1984)
202 *Daphnia* sp. Acute Immobilisation Test and Reproduction Test (updated guideline, adopted April 4, 1984)
203 Fish, Acute Toxicity Test (updated guideline, adopted July 17, 1992)
204 Fish, Prolonged Toxicity Test: 14 Day Study (original guideline, adopted April 4, 1984)
205 Avian Dietary Toxicity Test (original guideline, adopted April 4, 1984)
206 Avian Reproduction Test (original guideline, adopted April 4, 1984)
207 Earthworm, Acute Toxicity Tests (original guideline, adopted April 4, 1984)
208 Terrestrial Plants, Growth Test (original guideline, adopted April 4, 1984)
209 Activated Sludge, Respiration Inhibition Test (original guideline, adopted April 4, 1984)
210 Fish, Early-Life Stage Toxicity Test (original guideline, adopted July 17, 1992)
211 *Daphnia magna* Reproduction Test (original guideline, adopted September 21, 1998)
212 Fish, Short-Term Toxicity Test on Embryo and Sac-Fry Stages (original guideline, adopted September 21, 1998)
213 Honeybees, Acute Oral Toxicity Test (original guideline, adopted September 21, 1998)
214 Honeybees, Acute Contact Toxicity Test (original guideline, adopted September 21, 1998)
215 Fish, Juvenile Growth Test (original guideline, adopted January 21, 2000)
216 Soil Microorganisms, Nitrogen Transformation Test (original guideline, adopted January 21, 2000)
217 Soil Microorganisms, Carbon Transformation Test (original guideline, adopted January 21, 2000)
Section 3: Degradation and Accumulation
301 Ready Biodegradability
A: DOC Die-Away Test
B: CO2 Evolution Test
C: Modified MITI Test (I)
D: Closed Bottle Test
E: Modified OECD Screening Test
F: Manometric Respirometry Test (updated guideline, adopted July 17, 1992)
302A Inherent Biodegradability: Modified SCAS Test (original guideline, adopted May 12, 1981)
302B Inherent Biodegradability: Zahn-Wellens/EMPA Test (updated guideline, adopted July 17, 1992)
302C Inherent Biodegradability: Modified MITI Test (II) (original guideline, adopted May 12, 1981)
303 Simulation Test – Aerobic Sewage Treatment
303A Activated Sludge Units; 303B Biofilms (updated guidelines, adopted January 22, 2001)
304A Inherent Biodegradability in Soil (original guideline, adopted May 12, 1981)
305 Bioconcentration: Flow-Through Fish Test (updated guideline, adopted June 14, 1996)
306 Biodegradability in Seawater (original guideline, adopted July 17, 1992)
307 Aerobic and Anaerobic Transformation in Soil (original guideline, adopted April 24, 2002)
308 Aerobic and Anaerobic Transformation in Aquatic Sediment Systems (original guideline, adopted April 24, 2002)
Section 4: Health Effects
402 Acute Dermal Toxicity (updated guideline, adopted February 24, 1987)
403 Acute Inhalation Toxicity (original guideline, adopted May 12, 1981)

Table 1 Continued

404 Acute Dermal Irritation/Corrosion (updated guideline, adopted April 24, 2002)
405 Acute Eye Irritation/Corrosion (updated guideline, adopted April 24, 2002)
406 Skin Sensitisation (Updated Guideline, adopted 17th July 1992)
407 Repeated Dose 28-Day Oral Toxicity Study in Rodents (updated guideline, adopted July 27, 1995)
408 Repeated Dose 90-Day Oral Toxicity Study in Rodents (updated guideline, adopted September 21, 1998)
409 Repeated Dose 90-Day Oral Toxicity Study in Non-Rodents (updated guideline, adopted September 21, 1998)
410 Repeated Dose Dermal Toxicity: 21/28-Day Study (original guideline, adopted May 12, 1981)
411 Subchronic Dermal Toxicity: 90-Day Study (original guideline, adopted May 12, 1981)
412 Repeated Dose Inhalation Toxicity: 28-Day or 14-Day Study (original guideline, adopted May 12, 1981)
413 Subchronic Inhalation Toxicity: 90-Day Study (original guideline, adopted May 12, 1981)
414 Prenatal Developmental Toxicity Study (updated guideline, adopted January 22, 2001)
415 One-Generation Reproduction Toxicity Study (original guideline, adopted May 26, 1983)
416 Two-Generation Reproduction Toxicity Study (updated guideline, adopted January 22, 2001)
417 Toxicokinetics (updated guideline, adopted April 4, 1984)
418 Delayed Neurotoxicity of Organophosphorus Substances Following Acute Exposure (updated guideline, adopted July 27, 1995)
419 Delayed Neurotoxicity of Organophosphorus Substances: 28-Day Repeated Dose Study (updated guideline, adopted July 27, 1995)
420 Acute Oral Toxicity – Fixed Dose Method (updated guideline, adopted December 20, 2001)
421 Reproduction/Developmental Toxicity Screening Test (original guideline, adopted July 27, 1995)
422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (original guideline, adopted March 22, 1996)
423 Acute Oral Toxicity – Acute Toxic Class Method (updated guideline, adopted December 20, 2001)
424 Neurotoxicity Study in Rodents (original guideline, adopted July 21, 1997)
425 Acute Oral Toxicity: Up-and-Down Procedure (updated guideline, adopted December 20, 2001)
429 Skin Sensitisation: Local Lymph Node Assay (updated guideline, adopted April 24, 2002)
451 Carcinogenicity Studies (original guideline, adopted May 12, 1981)
452 Chronic Toxicity Studies (original guideline, adopted May 12, 1981)
453 Combined Chronic Toxicity/Carcinogenicity Studies (original guideline, adopted May 12, 1981)
471 Bacterial Reverse Mutation Test (updated guideline, adopted July 21, 1997)
473 *In Vitro* Mammalian Chromosomal Aberration Test (updated guideline, adopted July 21, 1997)
474 Mammalian Erythrocyte Micronucleus Test (updated guideline, adopted July 21, 1997)
475 Mammalian Bone Marrow Chromosomal Aberration Test (updated guideline, adopted July 21, 1997)
476 *In Vitro* Mammalian Cell Gene Mutation Test (updated guideline, adopted July 21, 1997)
477 Genetic Toxicology: Sex-Linked Recessive Lethal Test in *Drosophila melanogaster* (updated guideline, adopted April 4, 1984)
478 Genetic Toxicology: Rodent Dominant Lethal Test (updated guideline, adopted April 4, 1984)
479 Genetic Toxicology: *In Vitro* Sister Chromatid Exchange Assay in Mammalian Cells (original guideline, adopted October 23, 1986)
480 Genetic Toxicology: *Saccharomyces cerevisiae*, Gene Mutation Assay (original guideline, adopted October 23, 1986)
481 Genetic Toxicology: *Saacharomyces cerevisiae*, Miotic Recombination Assay (original guideline, adopted October 23, 1986)
482 Genetic Toxicology: DNA Damage and Repair, Unscheduled DNA Synthesis in Mammalian Cells *In Vitro* (original guideline, adopted October 23, 1986)
483 Mammalian Spermatogonial Chromosome Aberration Test (original guideline, adopted July 21, 1997)
484 Genetic Toxicology: Mouse Spot Test (original guideline, adopted October 23, 1986)
485 Genetic Toxicology: Mouse Heritable Translocation Assay (original guideline, adopted October 23, 1986)
486 Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells *In Vivo* (original guideline, adopted July 21, 1997)

The objective of the work in OECD is that through cooperative investigation by member countries, thereby efficiently sharing the burden of the enormous task of systematically investigating existing chemicals, adequate safety data for all HPV chemicals become available.

At a minimum, the OECD's Screening Information Data Set (SIDS) should be available. The SIDS is a list of data elements similar to those which governments in most OECD countries require from industry before a new chemical can be marketed. It includes information on the chemical's identity, its physical and chemical properties, the indications on exposure and use, its environmental fate and how the chemical might be disseminated in the environment, as well as toxicological data. All of these data elements are essential if an initial assessment is to be made of a chemical's hazards (see **Table 2**).

In order to fill identified data gaps for a specific chemical, or replace data whose quality is considered insufficient, a SIDS Testing Plan is prepared. The chemicals industry then undertakes the necessary testing on a voluntary basis, in consultation with a sponsor country, which overlooks and manages the process for the chemical under consideration, paying particular attention to the quality of the data. Any test is performed only once, by a single company. In accordance with the 1981 Council Decision on Mutual Acceptance of Data, all member countries will accept the results as long as testing has been done according to the OECD Test Guidelines and Principles of GLP.

Table 2 Screening OECD's Screening Information Data Set (SIDS)

Chemical identity
Physical–chemical data
Melting point
Boiling point
Vapor pressure
Water solubility
Dissociation constant
Exposure information
Sources
Users
Estimates of releases
Estimates of exposure to man and environment
Environmental fate and pathways
Biodegradation
Abiotic degradability
Distribution estimates
Ecotoxicological data
Acute fish toxicity
Prolonged *Daphnia* toxicity
Terrestrial toxicity (if significant terrestrial exposure)
Toxicological data
Acute toxicity
Repeated-dose toxicity
Genetic toxicity
Point mutation
Chromosomal aberration
Reproductive toxicity

When (through a combination of data collection and testing) a complete SIDS has been compiled, the sponsor country provides OECD with a SIDS Initial Assessment Report. The Initial Assessment Report is discussed, in the presence of experts from companies producing the chemical and from public interest groups and trade unions, at a SIDS Initial Assessment Meeting (SIAM). At this meeting a cooperative assessment of the chemical is made and conclusions are drawn on the potential hazard(s) posed by the chemical and recommendations are made on the need for further work. The conclusions present a summary of the hazards of the chemical, written with sufficient detail and clarity so as to be informative and to assist countries with classification work and other hazard-based national decision making; exposure information to put the hazard information into context (e.g., on use in the sponsor country) is also included. The recommendation, based on these conclusions, can be either that the chemical is currently of low priority for further work or that it is a candidate for further work to clarify its potential risk (e.g., that further information is required to clarify concerns identified in the SIDS process, and that post-SIDS testing is recommended).

In principle, follow-up work is left to the member countries and they will decide on what to do, depending on the national exposure situation of member countries in the policy bodies of OECD. Member countries discuss and confirm all conclusions and recommendations made on all chemicals which have undergone a SIDS and draft initial assessments. When full SIDS dossiers and initial assessment reports are finalized, the results are made available worldwide through UNEP Chemicals.

In 1998 the global chemical industry through the International Council of Chemical Associations (ICCA) announced its intention to work with OECD by using the OECD HPV Chemicals List to establish a working list of $\sim$1000 substances as priorities for investigation by industry itself (based on presumed wide dispersive use, production in two or more global regions, or similarity to another chemical meeting either of these criteria). The ICCA set the goal of completing SIDS and draft initial hazard assessments on these chemicals by the end of 2004. The draft initial assessments reviewed by sponsor countries are then considered in OECD by a SIAM. This initiative is an important source of assessments for consideration in the OECD Programme. Industry is encouraged to collaborate with member countries to ensure that the chemicals they select for investigation will be brought forward to the OECD Programme by a government (sponsor country). Member countries are encouraged to work with the chemical industry in order to make the most efficient use of the information compiled through the ICCA initiative in meeting their commitments to investigate a certain proportion of the chemicals on the OECD HPV Chemicals List.

At the time of writing, almost 1000 HPV chemicals had undergone or were undergoing systematic investigation of their potential health or environmental effects. Many more HPV chemicals will be assessed in the near future. The OECD Programme is closely coordinated with the work ongoing in the US Chemicals Right to Know Programme on HPV Chemicals. There is furthermore close cooperation with the European Union (EU); the work undertaken in the EU is fully integrated into that of the OECD.

All data that become available through the OECD Existing Chemicals Programme are made publicly available. It has been agreed that SIDS Initial Assessment Reports will be forwarded to UNEP for publication with the OECD.

The Hazard Assessment Programme

Recently, OECD work has focused on exposure assessment including estimation of emission, monitoring, and modeling. Important products are the OECD Emission Scenario Documents (ESDs).

An ESD is a document that describes the sources, production processes, pathways, and use patterns with the aim of providing a quantified scenario for emissions (or releases) of a chemical from production, formulation, use (industrial use, professional use, private use of chemical substances/preparations), service life (use in articles), and recovery/disposal into water, air, soil, or solid waste. An ESD should ideally include all the following stages: (1) production; (2) formulation; (3) industrial use; (4) professional use; (5) private and consumer use; (6) service life of product/article; (7) recovery; and (8) waste disposal (incineration, landfill). ESDs are used in risk assessment of chemicals to establish the conditions of use and releases of the chemicals, and are the basis for estimating the concentration of chemicals in the environment.

Risk Management Program

The reason for testing and assessing chemicals is ultimately to prevent or reduce their risks. OECD governments, academia, NGOs, and industry work together to identify best practices and new techniques for managing risks, and then develop methodologies that can be used by governments and industry. In addition, if governments agree on the risks posed by a particular chemical, they can work together to take concerted action across OECD countries.

Most of OECD's current work is focused on developing guidance for risk management that applies to the chemical industry as a whole. This includes guidance on conducting socioeconomic analysis; risk communication; tools to help companies screen potentially dangerous chemicals before they are manufactured; and development of environmentally benign chemicals.

Over the last few years, there has been a significant increase in the use of new and innovative approaches for managing risks posed by chemicals. OECD countries have found that traditional 'command and control' techniques are not always the most effective or efficient ways to control risk. One approach that has been of particular interest is the use of 'non-regulatory initiatives'. In order to increase awareness of the range of approaches that can be used, OECD has produced a report which identifies factors that can contribute to, or inhibit, the success of these approaches.

OECD work on facilitating the development of environmentally benign chemicals includes several elements. To start with, effective techniques and approaches in the field of sustainable chemistry were identified. This includes such aspects as recognizing and regarding sustainable chemistry accomplishments; disseminating technical information; promoting the incorporation of sustainable chemistry principles into various levels of chemical education; and promoting the research, discovery, and development of innovative sustainable chemistry technologies. Work is under way on each of these approaches.

OECD has also started a new project to help member countries and others assess and manage the impacts of chemicals throughout their life cycle (i.e., from production of a chemical substance, to distribution, use, recycling and/or recovery, and final disposal). To date, most methodologies for generating and collecting data, conducting risk assessments, and making risk management decisions have focused primarily on the production stage. In this new work also much attention is being given to chemicals in products. This new approach will build off existing methodologies and develop new ones to support a more holistic approach to chemicals management.

Other Activities Related to Environment, Health, and Safety

Other OECD activities in the environment, health, and safety areas are concerned with pesticides; chemical accident prevention, preparation, and response; pollutant release and transfer registers; harmonization of regulatory oversight in biotechnology, and the safety of novel foods and feeds. These activities are closely connected with the work in the Chemicals Programme, and are carried out in cooperation with other parts of the OECD and other international organizations.

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Appendix

ECD Council Acts Related to Chemicals

The OECD Council, under the chairmanship of the OECD Secretary-General, is the focal point of a continuing review by member governments of the work of the OECD. The Council also decides on the

Programme of Work of OECD and its Budget. When appropriate, the Council may also agree by consensus on Decisions which are legally binding under international law. Alternatively, member governments, through the Council, may agree on Recommendations, which are expressions of political will to follow certain policies. Council Decisions and Council Recommendations are known collectively as Council Acts. The following list of Council Acts is relevant to the work of the Chemicals Programme.

- Decision on the Mutual Acceptance of Data in the Assessment of Chemicals (C(81)30/Final).
- Decision of the Council Amending the Decision Concerning the Mutual Acceptance of Data in the Assessment of Chemicals (C(81)30/Final) (C(97) 186/Final).
- Decision on the Minimum Pre-marketing Set of Data in the Assessment of Chemicals (C(82)196/ Final).
- OECD Council Decision on the Minimum Premarketing Set of Data in the Assessment of Chemicals.
- OECD Council Recommendation on the Protection of Proprietary Rights to Data Submitted in Notifications of New Chemicals (C(83)96/Final).
- OECD Council Recommendation on the Exchange of Confidential Data on Chemicals (C(83)97/Final).
- Recommendation on the OECD List of Non-Confidential Data on Chemicals (C(83)98/Final).
- Decision–Recommendation on Further Measures for the Protection of the Environment by Control of Polychlorinated Biphenyls (C(87)2/Final).
- Decision–Recommendation on the Systematic Investigation of Existing Chemicals (C(87)90/Final).
- Decision-Recommendation on Compliance with Principles of Good Laboratory Practice (C(89)87/ Final).
- Decision of the Council on the Exchange of Information Concerning Accidents Capable of Causing Transfrontier Damage (C(88)84/Final).
- Decision–Recommendation Concerning Provision of Information to the Public and Public Participation in Decision-Making Processes Related to the Prevention of, and Response to, Accidents Involving Hazardous Substances (C(88)85/Final).
- Recommendation on the Application of the Polluter-Pays Principle to Accidental Pollution (C(89)88/Final).
- OECD Council Decision–Recommendation on Compliance with Principles of Good Laboratory Practice (C(89)87/Final).
- OECD Decision-Recommendation on the Co-operative Investigation and Risk Reduction of Existing Chemicals (Chemicals (1990) C(90)163/Final).
- Recommendation on Integrated Pollution Prevention and Control (C(90)164/Final).
- Recommendation Concerning Chemical Accident Prevention, Preparedness and Response (C(92)1/ Final).
- Decision of the Council Amending the Annexes to the Council Decision–Recommendation on Compliance with Principles of Good Laboratory Practice (C(89)87/Final) (C(95)8/Final).
- Recommendation of the Council on Implementing Pollutant Release and Transfer Registers (C(96)41/ Final).
- Council Decision on Adherence of Non-Member Countries to the Council Acts Related to the Mutual Acceptance of Data in the Assessment of Chemicals (C(97) 114/Final).

Selected Documents Related to Test Guidelines

- Guidance Document for the Conduct of Studies of Occupational Exposure to Pesticides during Agricultural Application (1996).
- Chemicals Testing Monographs No. 1 Guidance Document for the Development of OECD Guidelines for the Testing of Chemicals (1998).
- No 19: Testing and Assessment: Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Humane Endpoints (2000).
- The Work of OECD on Testing and Assessment of Endocrine Disrupters (2001).
- Chemicals Testing Monographs No. 33: Harmonized Integrated Classification System for Human Health and Environmental Hazards of Chemical Substances and Mixtures (2001).
- Final Report of the OECD Workshop on Harmonization of Validation and Acceptance Criteria for Alternative Toxicological Test Methods (2002).

Documents Published in the OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring (Also Available in French and German Translations; Russian Translations are Underway)

- The OECD Principles of Good Laboratory Practice (1998).
- Revised Guides for Compliance Monitoring Procedures for Good Laboratory Practice (1995).
- Revised Guidance for the Conduct of Laboratory Inspections and Study Audits (1995).
- Quality Assurance and GLP, Paris (1998).
- Compliance of Laboratory Suppliers with GLP Principles (2000).
- The Application of the GLP Principles to Field Studies, Paris (1999).
- The Application of the GLP Principles to Short-Term Studies (1999).

- The Role and Responsibilities of the Study Director in GLP Studies (1999).
- Guidance for the Preparation of GLP Inspection Reports (1995).
- The Application of the Principles of GLP to Computerised Systems (1995).
- The Role and Responsibilities of the Sponsor in the Application of the Principles of GLP (1998).
- Requesting and Carrying Out Inspections and Study Audits in Another Country (2000).
- The Application of the OECD Principles of GLP to the Organisation and Management of Multi-Site Studies (2002).

Selected Documents Related to Hazard/Risk Assessment

- Guidance Document on Emission Scenario Documents (2000).
- OECD/IPCS Database on Hazard-Risk Assessment Methodologies (2001).
- Database on Use and Releases of Chemicals (2001).
- Report of the OECD/UNEP Workshop on the Use of Multimedia Models for Estimating Overall Environmental Persistence and Long Range Transport in the Context of PBTS/POPS Assessment (2002).
- Emission Scenario Documents on Wood Preservatives, Part 1, Part 2, Part 3, Part 4 (2003).

Selected Documents Related to Pesticides

- OECD Guidance Documents for Pesticide Registration (2001).
- OECD Guidance for Industry Data Submissions on Plant Protection Products and Their Active Substances (2001).
- Monograph Guidance – OECD Guidance for Country Data Review Reports on Plant Protection Products and Their Active Substances (2001).
- Survey of Best Practices in the Regulation of Pesticides in Twelve OECD Countries (2001).
- Guidelines for the Collection of Pesticides Usage Statistics Within Agriculture and Horticulture (2002).
- Guidance for Registration Requirements for Pheromones and Other Semiochemicals Used for Arthropod Pest Control (2002).
- Report of the OECD Workshop on Electronic Tools for Data Submission (2003).
- Guidance for Registration Requirements for Microbial Pesticides (2003).

Selected Documents Related to Risk Management

- Proceedings of the OECD Workshop on Non-Regulatory Initiatives for Chemical Risk Management (1997).
- Proceedings for the OECD Workshop on Sustainable Chemistry (1998).
- Guidance for Conducting Retrospective Studies on Socio-economic Analysis (1999).
- Lead Risk Management Activities in OECD Member Countries (1993 to 1998) Part 1.
- OECD Guidance Document on Risk Communication for Chemical Risk Management (2002).
- Technical Guidance Document on the Use of Socio-Economic Analysis in Chemical Risk Management Decision Making (2002).
- Framework for Integrating Socio-Economic Analysis in Chemical Risk Management Decision Making (2002).
- Proceedings of the OECD Workshop on the Integration of Socio-Economic Analysis in Chemical Risk Management Decision Making (2002).

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Further Reading

Current OECD environment, health, and safety activities are described in greater detail in a brochure, *The OECD Environment, Health and Safety Programme*, which is available from the OECD, Environment, Health and Safety Division, 2 rue André-Pascal, 75775 Paris Cedex 16, France, E-mail: ehscont@oecd.org.

Public Health Service, US

C Charles Barton and Alan G Parham

Introduction

The main task of the Public Health Service (PHS) is protecting and advancing the health of our nation's people and contributing to the delivery of health care worldwide. The PHS is a principal part of the Department of Health and Human Services (DHHS) and the major health agency of the Federal Government.

The mission of the PHS is to provide highly trained and mobile health professionals who carry out programs to promote the health of the nation, understand and prevent disease and injury, assure safe and effective drugs and medical devices, deliver health services to Federal beneficiaries, and furnish health expertise in times of war or other national or international emergencies.

In order to fulfill its very broad mission of promoting health in our nation and the world, the PHS has designed programs and created agencies that help control and prevent diseases; conduct and fund biomedical research that will eventually lead to better treatment and prevention of diseases; protect us against unsafe food, drugs, and medical devices; improve mental health and deal with drug and alcohol abuse; expand health resources; and provide health care to people in medically under-served areas and to those with special needs.

The eight major agencies that make up the PHS and that do this work are the Centers for Disease Control and Prevention (CDC), the Agency for Toxic Substances and Disease Registry (ATSDR), the National Institutes of Health (NIH), the Food and Drug Administration (FDA), the Substance Abuse and Mental Health Services Administration (SAMHSA), the Health Resources and Services Administration (HRSA), the Agency for Health Care Policy and Research (AHCPR), and the Indian Health Service (IHS).

The Assistant Secretary for Health, with the assistance of the Surgeon General, heads the PHS, advises the DHHS Secretary on health and health-related matters, and directs the activities of the major PHS agencies. The PHS continues to fulfill its mission to protect and advance the public's health. It has grown from a small collection of marine hospitals to the largest public health program in the world. As part of the DHHS, the PHS consists of the Office of Public Health and Science (headed by the Assistant Secretary for Health and including the Surgeon General), ten Regional Health Administrators, and eight operating divisions.

History

The Public Health Service traces its origins to an Act of Congress signed by President John Adams on July 16, 1798, which provided for the care and relief of sick and injured merchant seamen. These seamen traveled widely, often became sick at sea, and then, away from their homes and families, could not find adequate health care in the port cities they visited or would overburden the meager public hospitals then in existence. Since they came from all the new states and former colonies, and could get sick anywhere, their health care became a national or Federal problem. The Marine Hospital Service (MHS), a loose network of marine hospitals located mainly in port cities, was established by Congress in 1798 to care for these sick and disabled seamen. The earliest marine hospitals created to care for the seamen were located along the East Coast, with Boston being the site of the first such facility; later they were also established along inland waterways, the Great Lakes, and the Gulf and Pacific Coasts.

The Federal Government had only three executive departments then to administer all Federal programs – State, Treasury, and War. The MHS was placed under the Revenue Marine Division of the Treasury Department. Funds to pay physicians and build marine hospitals were appropriated by taxing American seamen 20 cents a month. This was one of the first direct taxes enacted by the new republic and the first medical insurance program in the United States. The monies were collected from ship masters by the customs collectors in different US ports.

Lack of money and any supervisory authority were major problems for the MHS. The demand for medical services far exceeded the funds available. For that reason, sailors with chronic or incurable conditions were excluded from the hospitals and a 4-month limit was placed on hospital care for the rest. Additional funds had to be appropriated constantly from Congress in order to maintain the Service and to build the hospitals. Because of these problems, Congress was forced to act and in 1870 reorganized the MHS from a loose network of locally controlled hospitals to a centrally controlled national agency with its own administrative staff and headquarters in Washington, DC.

Through this reorganization, the MHS became a separate bureau of the Treasury Department under the direction of the Supervising Surgeon, who was

appointed by the Secretary of the Treasury. The title of the central administrator was changed to Supervising Surgeon General in 1875 and to Surgeon General in 1902. Additional money to fund the reorganized Service was appropriated by raising the hospital tax on seamen from 20 to 40 cents per month. The money collected was deposited in a separate MHS fund.

Taxing seamen to fund the MHS was abolished in 1884. From 1884 to 1906 the cost of maintaining the marine hospitals was paid from the proceeds of a tonnage tax on vessels entering the United States, and from 1906 to 1981, when the Public Health Service hospitals were closed, by direct appropriations from Congress.

The reorganization in 1870 created the position of Supervising Surgeon (later Surgeon General) to administer the Service, and John Maynard Woodworth was appointed as the first incumbent in 1871. He moved quickly to reform the system and adopted a military model for his medical staff, instituting examinations for applicants and putting his physicians in uniforms. Woodworth created a cadre of mobile, career service physicians who could be assigned as needed to the various marine hospitals.

The 1870 reorganization also changed the general character of the Service. It became national in scope and military in outlook and organization. Medical officers, called surgeons, were required to pass entrance examinations and wear uniforms. In 1889, the medical officers were given titles and pay corresponding to Army and Navy grades. Physicians who passed the examinations were appointed to the general service, rather than to a particular hospital, and were assigned wherever needed. The goal was to create a professional, mobile, health corps, free as far as possible from political favoritism and patronage, and able to deal with the new health needs of a rapidly growing and industrializing nation.

Beginning with the control of infectious diseases, the scope of activities of the MHS began to expand well beyond the care of merchant seamen in the closing decades of the nineteenth century. Responsibility for quarantine was originally a function of the states rather than the Federal Government, but the National Quarantine Act of 1878 conferred quarantine authority on the MHS. Over the course of the next half a century, the MHS increasingly took over quarantine functions from state authorities.

Beginning in 1891 as immigration increased dramatically in the late nineteenth century, the Federal Government also took over the processing of immigrants from the states. The MHS was assigned the responsibility for the medical inspection of immigrants arriving at sites such as Ellis Island in New York. Commissioned officers played a major role in fulfilling the Service's commitment to prevent disease from entering the country.

To help diagnose infectious diseases among passengers of incoming ships, the MHS established in 1887 a small bacteriology laboratory, called the Hygienic Laboratory, at the marine hospital on Staten Island, New York. That laboratory later moved to Washington, DC, and became the National Institutes of Health, the largest biomedical research organization in the world.

Because of the broadening responsibilities of the Service, its name was changed in 1902 to the Public Health and Marine Hospital Service, and again in 1912 to just the Public Health Service. The Service continued to expand its public health activities as the nation entered the twentieth century. As the century progressed, PHS officers served their country by controlling the spread of contagious diseases such as smallpox and yellow fever, conducting important biomedical research, regulating the food and drug supply, providing health care to underserved groups, supplying medical assistance in the aftermath of disasters, and in numerous other ways.

The PHS Today

Throughout all of the reorganizations which have shaped, defined, and established the PHS in its present place in the Federal Government, and which have spanned nearly two centuries, the PHS has never lost sight of its primary goal – providing health care for those with special needs. From the care of sick and disabled sailors the PHS has extended its activities to other groups with special needs (such as the American Indians, the Alaska Natives, migrant workers, Federal prisoners, and refugees), and to the nation as a whole.

The duties and functions of the PHS have expanded to include disease control and prevention, biomedical research, regulation of food and drugs, mental health and drug abuse, and health care delivery.

Today, the PHS is a part of the DHHS. It consists of the Office of Public Health and Science (headed by the Assistant Secretary for Health), ten Regional Health Administrators, and eight operating divisions. There are more than 6000 officers on active duty. Officers are assigned to all of the PHS Agencies and to a number of agencies outside of PHS, including the Bureau of Prisons, US Coast Guard, Environmental Protection Agency, Health Care Financing Administration, and the Commission on Mental Health of the District of Columbia.

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Further Reading

Bienia R, Stein E, and Bienia B (1983) United States Public Health Service hospitals (1798–1981) – The end of an era. *New England Journal of Medicine* 308: 166–168.

Harmon R (1996) Training and education for public health: The role of the US Public Health Service. *American Journal of Preventive Medicine* 12: 151–155.

Koop C and Ginzburg H (1989) The revitalization of the Public Health Service Commissioned Corps. *Public Health Reports* 104: 105–110.

Parascandola J (1999) From marine hospital to public health service. *Medicina nei Secoli* 1: 197–207.

Satcher D (1999) The history of the Public Health Service and the Surgeon General's priorities. *Food and Drug Law Journal* 54: 13–19.

Research Institute for Fragrance Materials (RIFM)

Anne Marie Api

History and Organization

The Research Institute for Fragrance Materials, Inc. (RIFM) was formally chartered as a nonprofit, international organization on April 12, 1966.

The headquarters is located in New Jersey, USA, and is directed by a President, who is appointed by the Board of Directors. Responsibilities of the corporation are carried out in close cooperation with the Scientific Director of the association through a scientific and support staff of 20 full- and part-time individuals.

RIFM is administered by a Board of Directors, which meets four times a year and is elected by the general membership during its annual meeting. Membership categories include Active, for those companies primarily engaged in the manufacture and/or sale or distribution of fragrance materials at other than the retail level; Supporting, for those companies at the retail level of consumer products using or consisting of fragrance or fragrance ingredients; and Associate, for those companies engaged as brokers or dealers in the fragrance industry.

All scientific efforts are reviewed by an independent experts panel of academic dermatologists, toxicologists, and environmental scientists. The experts panel uses a decision tree approach to assessing the dermal, systemic, and environmental endpoints. Conclusions of the expert panel on safe use, drawn from critical evaluation of all available hazard data, and exposure information provided by industry, form the basis for standards issued by the International Fragrance Association.

Fragrance materials are prioritized by human health and environmental endpoints and are evaluated by using a group approach. Chemical structure helps to predict transdermal absorption, metabolism and disposition, and functional groups that can influence toxicity. Using the group approach permits some generalizations; 88% are structurally simple, low molecular weight, predominantly semivolatile substances consisting of carbon, hydrogen, and oxygen. The majority of fragrance materials can be assigned to several homologous groups of structurally related materials in which one might reasonable predict some degree of consistency of metabolism and toxicity. These structural homologies allow safety issues to be considered within the context of the information that exists for the structural group as a whole. In many cases existing information for a structural group may obviate the need to submit a particular individual substance to full toxicological testing. In other cases, it may be necessary to test one or more particular members of a structural class to obtain more robust data to solidify assessment of the class as a whole.

Mission and Activities

The primary objectives of RIFM are to: gather and analyze scientific data from industry and open literature, engage in the evaluation and testing of fragrance ingredients, review and evaluate the standards and methods employed by industry for testing on a continuous basis. RIFM has a comprehensive research and testing program in the areas of fragrance allergy, respiratory safety, human health and environmental methodology, group safety evaluations, and user level support. Results of RIFM sponsored studies are published in open, peer-reviewed

scientific literature. In addition to sharing data with official international agencies, RIFM actively works with international industry associations. RIFM also maintains, for its members, the most extensive technical database of human health effects, environmental fate, and product regulations on fragrance and flavor ingredients.

RIFM's stated mission includes being the international scientific authority for the safe use of fragrance materials. The stated mission also includes engaging in research and evaluation of fragrance materials through an independent expert panel; determining safety in use; gathering, analyzing, and publishing scientific information; distributing scientific data and safety assessment judgments to RIFM members, industry association and other interested parties and maintaining an active dialog with official international agencies.

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Further Reading

Bickers DR, Calow P, Greim HA, *et al.* (2003) The safety assessment of fragrance materials. *Regulatory Toxicology and Pharmacology* 37: 218–273.

Cadby PA, Troy WR, and Vey MGH (2002) Consumer exposure to fragrance ingredients: Providing estimates for safety evaluation. *Regulatory Toxicology and Pharmacology* 36: 246–252.

Ford RA, Domeyer B, Easterday O, Maier K, and Middleton J (2000) Criteria for development of a database for safety evaluation of fragrance ingredients. *Regulatory Toxicology and Pharmacology* 31: 166–181.

Hanifin JM, Api AM, and Bickers DR (2003) Considerations for testing irritancy, allergy, and photoreactivity in fragrance safety evaluations. *American Journal of Contact Dermatitis* 14(2): 100–103.

Salvito DT, Senna RJ, and Federle TW (2002) A framework for prioritizing fragrance materials for aquatic risk assessment. *Environmental Toxicology and Chemistry* 21(6): 1301–1308.

Smith LW (2003) The scientific basis for sound decisions on fragrance material use. *Regulatory Toxicology and Pharmacology* 37: 171–172.

Society for Chemical Hazard Communication

Michele R Sullivan

History

The Society for Chemical Hazard Communication (SCHC), originally know as the American Conference on Chemical Labeling (ACCL), was incorporated in 1982 as a nonprofit professional organization, but it had actually started several years earlier. When the Chemical Manufacturers Association (CMA) discontinued the Labeling and Precautionary Information Committee, its members recognized the need for individuals responsible for labeling to have a forum to discuss ideas and keep up to date on new requirements. CMA initially sponsored ACCL and its first four meetings.

The ACCL membership grew from 40 people in 1979 to ~700 in 2003. Over time the 'labeling issues' grew into 'hazard communication issues'. In 1992, ACCL changed its name to SCHC to reflect the expanded issue of hazard communication and the needs of the expanded membership. As the society grew, the structure has become more formal. However, SCHC is essentially a volunteer organization. Today, there is an elected Board of Directors consisting of seven members, the past president, president, vice president, and secretary–treasurer. To accomplish the Society's goals, SCHC has various standing committees: Arrangements, Awards, Exhibit, HazCom Resources, Membership, Newsletter, Nominating, Professional Development, Program, Small Package, and Web. The Board of Directors manages the affairs of the Society and its committees.

The Society

Purpose

SCHC's purpose is to promote effective communication of chemical hazards. The Society is committed to sharing knowledge and resources to ensure a consistent and uniform approach to assessing and communicating chemical hazards on product labels, material safety data sheet (MSDS), and other product literature and documentation by

- Monitoring legislative and standards development.
- Broadening awareness of new developments in research and practice.

- Facilitating understanding and interpretation of regulatory requirements.
- Fostering professional development.
- Providing opportunities for professional networking and exchange of ideas.
- Serving as a primary source of information on international standards regarding hazard communication.

Membership

SCHC is a professional society of individuals who are engaged in the business of hazard communication. The members have a broad range of occupations – chemistry, industrial hygiene, and toxicology are a few examples. Their jobs are also diverse. Many prepare labels and MSDSs for their employers' products. Others train users of hazardous chemicals, act as expert witnesses, or implement government regulations. They work in industry, government, and academia.

SCHC welcomes members who are involved in the field of hazard communication.

Meetings

The Society holds meetings to provide up-to-date information on current developments and education and networking opportunities for its members. Meetings are regularly held in the Washington, DC, area and frequently feature regulatory updates from the federal agencies involved in hazard communication requirements. In addition, other meetings are held at selected cities across the country that will attract membership participation and attendance. As part of its meetings, SCHC allows time for members to network with each other, has a section of the program allotted to member updates, and holds new member luncheons.

The SCHC strives to keep its members aware of the latest developments concerning hazard communication. Topics at meetings include: Internet resources; American National Standards Institute (ANSI) Standards; Environmental Protection Agency, Department of Transportation (DOT), and Occupational Safety and Health Administration (OSHA) updates; and international information. A major topic has been the international harmonization of hazard communication, the Globally Harmonized System (GHS) of Classification and Labeling of Chemicals. The development of the system was completed in 2001, adopted/endorsed by the UN in 2002, and is expected to affect hazard communication globally.

Also of interest to SCHC members are issues that affect their industry and professions. Presentations on these topics include: managing hazard communication programs; and liability in writing MSDSs and labels. Continuing education/maintenance points can be obtained for SCHC meetings.

Members are given the opportunity of participating in a technical poster session at meetings where they can exchange ideas in an informal environment. Ideas are presented as posters in an atmosphere where authors and attendees can mingle. Any topic related to chemical hazard communication, in its broadest sense, may be presented at the poster session. Poster Abstracts are published, distributed at the meeting, and posted on the web. The poster session is an excellent opportunity to present ideas and to receive feedback from other hazard communication professionals.

A computer software and vendor exhibit is also held once a year. This exhibit features vendors of products that aid SCHC members in the creation and maintenance of information for hazard communication. Members can interact at the exhibit with commercial product and service providers who display the latest in hazard communication technology and resources.

Professional Development

The Society's purpose has always been to educate and provide information on hazard communication. Professional development courses were first offered by SCHC in 1990 with a single half-day course. Today, the society offerings have grown to over 25 professional development courses including basic, advanced, and in-depth multiday courses. Continuing education/maintenance points can be obtained for SCHC courses.

Basic professional development courses offered by SCHC are: MSDS and Label Preparation Workshops; Science, Toxicology and Industrial Hygiene for Hazard Communication; and Hazard Determination & Risk Assessment. Regulatory courses include: Canadian & Mexican Hazard Communication; Pesticide & Consumer Product Labeling; Component Disclosure Requirements; European Union Hazard Communication; Transportation Classification & Labeling; HMIS/NFPA Labeling; and International Chemical Control Laws.

Several advanced courses have been developed such as Reproductive & Developmental Toxicology; Endocrine Disrupters, Clinical & Occupational Toxicology; Occupational Exposure Limits; and Life Cycle Assessment.

New courses on topics of current interest include: Hazard Communication for Asia, Pacific Rim & Latin America; Ecotoxicology for Hazard Communication;

and the GHS of Classification and Labeling of Chemicals.

SCHC offers HAZCOM 101, a 2 day course. This course, the first of its kind in the United States, is designed for people who have little formal HazCom training and are recently assigned to hazard communication, MSDS, labeling, or regulatory compliance responsibilities. The curriculum presents basic information, provides reference material, and practical exercises.

The SCHC Professional Development Committee recognizes students who have accumulated 40, 80, and 120 or more hours of SCHC professional development training and instructors and/or course directors who have contributed to 15 or 30 SCHC courses.

Outreach

The Society has a history of collaboration and outreach. The OSHA Hazard Communication Standard (HCS), 29 CFR 1910.1200, was published in 1983. Shortly thereafter, SCHC and OSHA collaborated to educate stakeholders on the new HCS. Jointly sponsored seminars were held on a regional basis with both OSHA and SCHC participating to inform both members and stakeholders about the HCS. Recently, SCHC and OSHA have signed an alliance to provide information and training on hazard communication, MSDSs, and the new GHS of Classification and Labeling of Chemicals. This alliance is another step in the longstanding relationship between SCHC and OSHA to promote effective hazard communication.

Publications

The Society maintains a website. The site contains Society and membership information, SCHC presentations, SCHC newsletters, SCHC meeting material, and professional development courses. There are links to hazard communication and related websites and a list of hazard communication and translation resources. Updates on legislative and standards activity concerning hazard communication are posted.

The *SCHC News* is published for distribution to members. It includes pictures, meeting information, articles, and news of general interest to SCHC members and others interested in hazard communication issues.

SCHC runs an Internet discussion group, known as the Forum. Comments and questions related to hazard communication and SCHC's activities can be posted. Forum topics include: United States of America, Canadian WHMIS, European Union, and Pacific Rim HazCom; general HazCom topics, and SCHC meeting and activity topics. Each discussion forum has a moderator and both the moderator and members participate in the discussions.

SCHC Awards

To recognize contributions to both the Society and the field of hazard communication, SCHC offers three types of awards. Nomination for awards are solicited by the Awards Committee and reviewed by the Board. The *HazCom Lifetime Achievement Award* recognizes individuals who have contributed significantly to the field of hazard communication or to SCHC over an extended period of time. Recipients of the award must have achieved at least one of the following additional criteria: exceptional performance in the field of hazard communication; lasting impact on the practice of hazard communication; and/or broad benefits to hazard communication professionals or users of hazard communication information.

The *Award for Excellence in Hazard Communication* recognizes individuals or groups who have made significant contributions to the field of hazard communication. The individual or group need not be a member of SCHC. Examples of activities meriting this award include: developing 'systems' to address hazard communication matters; publications in journals or periodicals; and formation of panels or organizations addressing hazard communication.

The *SCHC Distinguished Service Award* recognizes individuals who have contributed outstanding services to SCHC beyond their function as committee members or chairs.

Related Organizations

As SCHC's interests broadened to global hazard communication, international speakers were invited to SCHC meetings. A speaker from the United Kingdom (UK) Health and Safety Executive believed that hazard communication in the UK could benefit from a similar organization. SCHC provided infromation about forming a society. In 1994 a group of hazard communication professionals in the UK formed the Chemical Hazards Communication Society (CHCS). (http://www.ches.org.uk).

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Society for Environmental Toxicology and Chemistry

Harihara M Mehendale

In the 1970s, no forum existed for interdisciplinary communication among environmental scientists, biologists, chemists, toxicologists, and others interested in environmental issues such as managers and engineers. The Society of Environmental Toxicology and Chemistry (SETAC) was founded in 1979 to fill this void. Based on growing membership, attendance in meetings, and publications, the forum was needed.

A unique strength of SETAC is its commitment to balance the interests of academia, business, and government. The Society by-laws mandate equal representation from these three sectors for World Council Officers, Board of Directors/Council Members, and Committee members. And although there is no control mechanism, the proportion of members from each of the three sectors has remained nearly equal over the past 24 years.

Like many other professional societies, SETAC publishes an esteemed scientific journal and convenes annual meetings replete with state-of-the-science poster and platform presentations. Because of its multidisciplinary approach, however, the scope of the science of SETAC is much broader in concept and application than that of most other societies.

SETAC is concerned about global environmental issues. Its members are committed to good science worldwide, to timely and effective communication of research, and to interactions among professionals so that enhanced knowledge and increased personal exchanges occur. SETAC was founded in North America but membership was open to environmental scientists worldwide. SETAC Europe was organized in 1989, SETAC Asia/Pacific in 1997, and SETAC Latin America in 1999. Members voted overwhelmingly in 2001 to combine these 'geographic units' into one global society to form the SETAC World Council. SETAC meets the professional needs of individuals at local and regional levels throughout all geographic units, throughout national branches and chapters (Argentina, Brazil, United Kingdom, and soon-to-be organized Japan), through regional chapters (16 in North America), and through national-language chapters (Germany, France, and Iberia). International acceptance of the SETAC model continues with widespread interest in Russia and Africa. It is now the job of the SETAC World Council to oversee the myriad SETAC activities around the world and to assure the integrity of the Society.

Membership has increased from 230 Charter Members in October 1980 to nearly 5000 members from 50 US states, 13 Canadian provinces, and more than 60 other countries worldwide. Participants and technical presentations at SETAC annual meetings in North America have increased from 470 delegates and 86 technical presentations in 1980, to 2200 delegates and 1600 presentations in 2003. Annual meetings in Europe began in 1991 with 500 delegates and more than 200 presentations. In 2003, there were 1400 delegates and 1100 presentations. Meetings are also held in Asia/Pacific and Latin America.

Environmental Toxicology and Chemistry, an internationally acclaimed scientific journal, has grown from a quarterly publication of fewer than 400 pages annually in 1982 to a monthly publication of 3094 pages in 2003. SETAC publishes the global newsletter, *SETAC Globe*, peer-reviewed workshop and symposia proceedings, and a variety of technical reports.

Purpose and Goals

SETAC is a nonprofit, professional society established to provide a forum for individuals and institutions engaged in the study of environmental issues, management and regulations of natural resources, education, and research and development.

Environmental toxicology and chemistry, in their broadest sense, embrace components of classical toxicology; physiology; genetics; biology; microbiology; ecology; anatomy; organic, environmental, and analytical chemistry; soil, water, and atmospheric sciences and engineering; and economics.

The purpose of the Society is to

- Promote research, education, training, and development in areas of environmental toxicology and chemistry, and promote the collective application of these sciences to risk assessment.
- Disseminate information on environmental toxicology and chemistry, and participate in the application of these sciences to issues concerned with the technology of risk assessment and risk management.
- Promote the study of concepts and the implementation of programs that can be used for the development of ecologically acceptable practices and principles.
- Provide a forum for communication among professionals in government, business, academia, and other segments of society involved in the use, protection, and management of the environment and in the protection and welfare of the general public.

The goals of the Society are to

- Represent toxicologists, chemists, engineers, and others interested in the environmental sciences at the local, regional, national, international, and global levels.
- Facilitate identification, evaluation, resolution, and communication of environmental problems and issues among SETAC members, and the global community of environmental scientists, engineers, and the general public.
- Organize and conduct local, regional, national, and international meetings, workshops, and symposia for SETAC members and others interested in the environmental sciences.
- Publish, disseminate, and archive a peer-reviewed journal (*Environmental Toxicology & Chemistry*), technical documents, and other materials concerning environmental issues and SETAC affairs.
- Provide scientific information to planners, legislators, managers, regulators, and others to influence the development and application of rational environmental policies, laws, and regulations.
- Assume an active leadership role in the development of environmental education programs and provide educational opportunities for SETAC members, the general public, and others interested in the environmental sciences.
- Provide efficient and effective management and service to SETAC members, and assure continuity of operations.
- Continue to strive for a membership balance among academia, government, and business.
- Encourage the integration of voluntary and professional services in the management operation of SETAC.
- Encourage participation of all members in Society affairs and continue to provide equal opportunity in the governance of SETAC.
- Obtain resources sufficient to accomplish goals.

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Society for Risk Analysis (SRA)

Mike Dourson and Pertti J Hakkinen

History, Purpose, and Objectives

The Society for Risk Analysis (SRA) was established in 1981 as a nonprofit organization to foster and promote: (1) knowledge and understanding of risk analysis techniques and their applications; (2) communication and interaction among individuals engaged in risk analysis; (3) application of risk analysis and risk management techniques to the hazards and risks to which individuals and populations are exposed; (4) dissemination of risk analysis information and concepts to all interested individuals; (5) advancement of the state-of-the-art techniques in all aspects of risk analysis; and (6) integration and interaction of the various disciplines involved in risk analysis.

Membership Criteria

There are currently ~2500 members of SRA, with international representation. Members of SRA include professionals from a wide range of institutions, including federal, state, and local governments, small and large industries, private and public academic institutions, not-for-profit organizations, law firms, and consulting groups. Those professionals include statisticians, engineers, safety officers, policy analysts, economists, lawyers, environmental and occupational health scientists, natural and physical scientists, environmental scientists, public administrators, and social, behavioral, and decision scientists.

There are five classes of members, including active members, student members, retired members, Fellows (see below), and sustaining members (organizations interested in risk analysis).

Membership Benefits and Website Contents

Members get voting privileges in the election of officers and councilors, and web-based access to the membership directory. In addition, members get a subscription to a bimonthly journal, *Risk Analysis*, and a quarterly newsletter, the *RISK Newsletter*. Members have web-based access to current and several recent years of issues of this journal and

newsletter. The SRA website provides online access to additional information, for example, funding opportunities for research, employment opportunities, links to sources of specific risk-related resources (data, models, technical reports, etc.), instructions for subscribing to an Internet (RISKANAL) mailing list, and a glossary of risk analysis terms.

Details about the Journal

The journal *Risk Analysis* provides a focal point for new developments in risk analysis covering a wide range of disciplines. It covers topics of interest to regulators, researchers, and scientific administrators, including research results on health risks, and the engineering, mathematical, and theoretical aspects of risks. This journal focuses on manuscripts dealing with measurements, modeling, instrumentation, questionnaires, and studies of chemicals. In addition, it covers the social and psychological aspects of risk such as risk perception, acceptability, economics, and ethics. All scientific articles in *Risk Analysis* are peer reviewed.

Meetings, Specialty Groups, Sections, and Chapters

Through its meetings and publications, SRA fosters a dialog on health, ecological, and engineering risks and natural hazards, and their socioeconomic dimensions. SRA has helped develop the field of risk analysis and has improved its credibility and viability. The society has a number of chapters and sections around the world and sponsors an annual meeting of the society, usually in December. The annual meeting includes meetings of specialty groups on dose response, economics and benefits analysis, ecological risk assessment, engineering, exposure assessment, food/water safety risk, risk communication, and risk science and law. The sections of SRA include SRA Europe and SRA Japan, and the chapters of SRA are located in various parts of the United States and Canada, and in other parts of the world. The sections and chapters conduct their own meetings at different locations and times; SRA's website contains contact information and summaries of the meetings.

Awards

Various awards are announced at the annual meeting, including the Distinguished Achievement Award (honors any person for extraordinary achievement in science or public policy relating to risk analysis), Outstanding Service Award (honors SRA members for extraordinary service to the Society), Outstanding Risk Practitioner Award (honors individuals who have made substantial contributions to the field of risk analysis through work in the public or private sectors), Chauncey Starr Award (honors individuals age 40 and under who have made exceptional contributions to the field of risk analysis), and the Fellow of the Society for Risk Analysis Award (recognizes and honors up to 1% of the Society's membership, selected based upon substantial achievement in science or public policy relating to risk analysis and substantial service to SRA. Fellows include all former SRA presidents).

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Society of Toxicology*

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Objectives

The Society of Toxicology (SOT) is a professional organization of scientists from academic institutions, government, and industry representing the great variety of scientists who practice toxicology in the United States and abroad. SOT promotes the development and integration of innovative basic and applied toxicology to enhance human, animal, and environmental health. Each member must commit to the SOT Code of Ethics. The Society facilitates the exchange of information among its members as well as among investigators in other scientific disciplines. SOT has a strong commitment to education in toxicology and to the recruitment of students and new members into the profession.

*Compiled from the information provided by Society of Toxicology.

History and Organization

SOT was founded in 1961 as a not-for-profit scientific society. The Society is governed by an 11 person elected Council and managed by an administrative office in the Washington, DC area.

The Society's activities are highly diverse and assisted by the efforts of 22 committees and task forces, such as Animals in Research Committee, Career Resource and Development Committee, Education Committee, Regulatory Affairs and Legislative Assistance Committee, and the Task Force for a Chemical/Biological Terrorism Resource Registry.

Membership

Currently, SOT has more than 5250 members in 44 countries. The majority of members are practicing toxicologists and scientists from allied disciplines. The Society offers three kinds of individual memberships: full, associate, and student/postdoctoral. There are 53 companies and other related organizations listed as SOT Affiliates. Undergraduate Affiliate status is available for pre-baccalaureate students. In addition, the Council may award honorary memberships to persons who are not members of the Society in recognition of outstanding and sustained achievement in the field of toxicology.

Specialty Sections

The Society has established 19 specialty sections that may propose sessions for the annual meeting, exchange information via newsletters, present awards, and participate in other scientific activities as subdisciplinary groups. The specialty sections of the Society are Biological Modeling, Carcinogenesis, Comparative and Veterinary, Dermal, Ethics, Legal & Social Issues, Epidemiology and Occupational Health, Food Safety, Immunotoxicology, *In Vitro*, Inhalation, Mechanisms, Metals, Molecular Biology, Neurotoxicology, Regulatory and Safety Evaluation, Reproductive and Developmental Toxicology, Risk Assessment, Toxicologic and Exploratory Pathology, and Women in Toxicology.

Regional Chapters

The SOT has 18 regional chapters that sponsor regular local meetings throughout the year. The purpose of the regional chapters is to foster scientific exchange at a local level, including regional meetings, poster awards for students, newsletters, as well as proposals for the annual meeting. Each regional chapter selects a student who serves on the Student Advisory Committee, the student voice in the SOT.

Publications

Toxicological Sciences, the official SOT journal, is distributed monthly in print and electronically. The journal publishes premier peer-reviewed, hypothesis-driven, original research articles that are broadly relevant to assessing the potentially adverse health effects resulting from exposure of humans or animals to chemicals, drugs, natural products, or synthetic materials. Studies may involve experimental animals or human subjects, or they may focus on *in vitro* methods or alternatives to the use of experimental animals. Sections include original research, reviews, forum articles on policy or research issues, editorials, letters to the editor, and supplementary data guidelines.

The Toxicologist, the abstract issue of *Toxicological Sciences*, is also an official publication of the SOT. Abstracts are from presentations for the symposium, platform, workshop, roundtable, poster, and continuing education sessions at the SOT Annual Meetings.

In striving to be the premier source of information in toxicology, SOT has gathered a diverse array of information for the public and for members on the Society webpage. The Society also publishes the *Communiqué*, the member newsletter, distributed electronically four times a year. The spring Special Edition is also printed.

Meetings

SOT conducts an annual meeting, the largest of its kind in the world. The meeting occurs in March of each year and over 2100 papers are presented on a variety of subjects. Sessions include platform sessions, workshops, and poster sessions. Continuing education courses and symposia sponsored by specialty sections of the Society are regular features at the annual meeting. The abstracts of all presented papers are published annually in *The Toxicologist*.

ToxExpo® provides the opportunity for attendees to see the latest in cutting-edge technology and services available on the market today and meet with vendors face-to-face. Online *ToxExpo*® provides access to products and services all year.

SOT offers occasional Contemporary Concepts in Toxicology workshops, and cosponsors a variety of meetings with other groups and governmental agencies.

Awards and Grants

The Society presents several awards annually that recognize outstanding achievement in the field of

toxicology. SOT also supports travel grants, fellowships, and other student awards. Special awards are presented at the discretion of the Council. SOT also presents a number of sponsored awards.

Related Societies

SOT maintains liaison with numerous affiliated societies, participates in the International Union of Toxicology, and supports intersociety activities and meetings.

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Trade Associations

Patricia M Nance

Trade associations are individuals and companies in a specific business or industry organized to promote common interests. Trade associations are associated with a type of business, type of product, or a specific product. The focus of this article is on trade associations in the United States, and non-US readers are urged to conduct Web searches for trade associations relevant to their chemicals, products, country, region, and continent. (A limited number of international trade associations appear as individual entries in this book, including the European Centre for Ecotoxicology and Toxicology (ECETOC), Flavor and Extract Manufacturers Association (FEMA), International Fragrance Association (IFRA), International Organization of the Flavor Industry (IOFI), and the Research Institute for Fragrance Materials (RIFM).)

The listing below includes various common trade associations related to the field of toxicology, focusing on those relevant to the United States.

American Chemistry Council

The American Chemistry Council represents the leading companies engaged in the business of chemistry. Council members apply the science of chemistry to make innovative products and services that make people's lives better, healthier, and safer. The Council is committed to improved environmental, health, and safety performance through Responsible Care®, common sense advocacy designed to address major public policy issues, and health and environmental research and chemical testing. The business of chemistry is a $450 billion enterprise in the United States and a key element of the nation's economy. It is the nation's largest exporter, accounting for 10 cents out of every dollar in US exports. Chemical companies invest more in research and development than any other business sector.

Chlorine Chemistry Council

The (US) Chlorine Chemistry Council® (CCC), a business council of the American Chemistry Council, is a national trade association based in Arlington, VA, representing the manufacturers and users of chlorine and chlorine-related products. CCC strives to achieve policies that promote the continuing, responsible uses of chlorine and chlorine-based products. Chlorine is widely used as a disease-fighting disinfection agent, as a basic component in pharmaceuticals and myriad other products that are essential to modern life.

Cosmetic, Toiletry, and Fragrance Association

The (US) Cosmetic, Toiletry, and Fragrance Association (CTFA) provides a complete range of services that support the personal care products industry's needs and interests in the scientific, legal, regulatory, legislative, and international fields. CTFA strives to ensure that the personal care products industry has the freedom to pursue creative product development and compete in a fair and responsible marketplace. CTFA represents the industry's interests at the local, state, national, and international levels, promoting voluntary industry self-regulation and reasonable governmental requirements that support the health and safety of consumers.

CTFA has ~600 member companies. Active members are manufacturers and distributors of finished products. Associate members are suppliers of

ingredients, raw materials, packaging, and other services used in the production and marketing of finished products, as well as consumer and trade publications. The association also coordinates educational activities and supports public service programs such as 'Work Your Image!', which in partnership with 'Women Work!' provides women reentering the work force with guidance on hygiene and the importance of a professional appearance in getting and keeping a job.

The CTFA Foundation works with the American Cancer Society and the National Cosmetology Association in implementing 'Look Good...Feel Better', a free, public service program that teaches makeup techniques to women undergoing cancer treatment, helping them to regain their self-confidence and to better cope with the appearance-related side effects of chemotherapy and radiation. CTFA also supports the Cosmetic Ingredient Review (CIR), a program it helped establish in 1976, which assesses the safety of ingredients used in cosmetics in an unbiased, independent forum with an expert panel comprised of world-renowned physicians and scientists.

Halogenated Solvents Industry Alliance, Inc.

The Halogenated Solvents Industry Alliance, Inc. (HSIA) was formed in 1980 by a group of executives in the chlorinated solvents industry to meet the growing challenges of government regulation. HSIA is dedicated to serving the interests of the halogenated solvents industry – interests that include solvent equipment manufacturers, and producers, distributors, and commercial users of halogenated solvents. By working together, the halogenated solvents industry and HSIA protect industry interests and promote the safe and responsible use of chlorinated solvents. From its office in Washington, DC, HSIA represents companies that manufacture, distribute, and use methylene chloride, perchloroethylene, trichloroethylene, and other halogenated compounds. HSIA places great emphasis on staying ahead of and actively participating in the decision-making process. The staff collects and analyzes information about the halogenated solvents and government plans and activities relating to them, and relays that information to HSIA board and committee members.

HSIA communicates with the European Chlorinated Solvent Association (ECSA) and the Japan Association for Hygiene of Chlorinated Solvents (JAHCS). The European Chlorinated Solvent Association was formed over 25 years ago by the leading chlorinated solvent manufacturers in Europe. Like HSIA, the goals of ECSA and JAHCS are to support safe use of chlorinated solvents and to encourage balanced regulation.

International Copper Association, Ltd.

The International Copper Association, Ltd. (ICA) is the leading organization for promoting the use of copper worldwide. ICA increases awareness and usage of copper by communicating the unique attributes that make this sustainable element an essential contributor to the formation of life, to advances in science and technology, and to a higher standard of living throughout the world. The Association's 35 member companies represent ~80% of the world's refined copper output and are among the largest copper producers, copper alloy fabricators, and wire and cable companies in the world. ICA is responsible for guiding policy, strategy, and funding international initiatives and promotional activities. Headquartered in New York, ICA has regional offices in Brussels, Santiago, Shanghai, and Singapore. ICA's programs and initiatives are executed in 24 countries through regional offices, 27 copper promotion centers, and copper fabricating companies. Programs to accomplish the goals of ICA's strategic plan are focused on copper's major end uses. These include wire and cable for the transmission of power and information, plumbing systems for potable water, products for architectural and industrial applications, scientific studies regarding copper's role in human health and the environment, and worldwide communications about the benefits of copper. ICA's mission is "to promote the use of copper by communicating the unique attributes that make this sustainable element an essential contributor to the formation of life, to advances in science and technology, and to a higher standard of living worldwide." The association was formed in 1989 by 24 of the world's leading primary copper producers to coordinate and improve the effectiveness of the international market development, research, and technology activities of the industry. The association evolved from the International Copper Research Association (INCRA), established in 1960.

Nickel Institute

The Nickel Institute, whose members represent over 70% of current world production, generates and communicates knowledge required to support safe and sustainable production, use and reuse of nickel. It was established on January 1, 2004.

For consumers, governments, regulators, and other stakeholders, the Nickel Institute is committed to responding effectively to the growing requests for

nickel-related information. For nickel producers and users it offers research-based, cutting-edge science and technical information.

The Institute provides a single membership and management structure for activities previously undertaken by the Nickel Development Institute (NiDI) and the Nickel Producers Environmental Research Association (NiPERA). NiPERA is an independently incorporated division of the Nickel Institute, continuing as a well-respected provider of peer-reviewed, published information on the human health and environmental science of nickel.

The Nickel Institute continues the use-related technical work of NiDI, but focuses more on nickel issues related to stewardship and sustainable development, especially the generation and use of knowledge about the full life cycle impacts of nickel.

The Institute develops partnerships with organizations representing the interests of the nickel-producing industry's downstream customers and other parts of the nickel life-cycle. The Institute also collaborates with regional and local metals industry organizations.

Soap and Detergent Association

Established in 1926, the (US) Soap and Detergent Association (SDA) is the national, nonprofit trade association representing ~135 manufacturers of household, industrial, and institutional cleaning products; the ingredients used in cleaning products; and finished packaging. SDA is dedicated to advancing public understanding of the safety and benefits of cleaning products, and protecting the ability of its members to formulate products that best meet consumer needs. SDA serves both its members and the public by developing and sharing information about industry products with the technical community, policy makers, child care and health professionals, educators, media, and consumers. SDA members produce more than 90% of the cleaning products marketed in the United States. Membership is open to US, Canadian, and Mexican companies.

Synthetic Organic Chemical Manufacturers Association

The (US) Synthetic Organic Chemical Manufacturers Association (SOCMA) is the leading trade association serving the specialty-batch and custom chemical industry since 1921. Its 300 member companies have more than 2000 manufacturing sites and 100 000 employees. SOCMA members encompass every segment of the industry, from small specialty producers to large multinational corporations, and manufacture 50 000 products annually valued at 60 billion dollars.

Batch chemical manufactures play a key role in the US chemical industry producing intermediates, specialty chemicals, and ingredients that are used to develop a wide range of commercial and consumer products. Thus, SOCMA's member companies manufacture products that are key building blocks and ingredients for a range of other production operations. Specialty chemicals made by many SOCMA members are formulated to meet the detailed specifications of various end users, and usually have unique purposes, such as making nylon fibers stronger or serving as the active ingredient in medicine. Therefore, specialty chemicals are often essential elements in the end-user's manufacturing process.

In batch manufacturing, the raw materials, processes, operating conditions, configuration of equipment, and end products change on a regular basis. Batch producers must respond quickly to new requests by customers, fill small market niches, and participate in the development of new products. The depth and expertise of this industry sector are vital components of the US chemical industry and contribute significantly to US global competitiveness.

Further Reading

Hakkinen PJ, Stoss FW, Behrendt B, and Wexler P (2000) Organizations. In: Wexler P, Hakkinen PJ, Kennedy GL Jr., and Stoss FW (eds.) *Information Resources in Toxicology*, 3rd edn., pp. 439–489. San Diego: Academic Press.

Wukovitz LD (2001) Using internet search engines and library catalogs to locate toxicology information. *Toxicology* 157: 121–139.

Relevant Websites

http://www.socma.com – Synthetic Organic Chemical Manufacturers Association.

http://www.americanchemistry.com – American Chemistry Council.

http://c3.org – Chlorine Chemistry Council.

http://www.ctfa.org – Cosmetic, Toiletry, and Fragrance Association.

http://www.eurocholor.org – European Chlorinated Solvent Association.

http://www.hsia.org – Halogenated Solvents Industry Alliance, Inc.

http://www.copperinfo.com – International Copper Association, Ltd.

http://www.nickelinstitute.org – Nickel Institute.

http://www.nipera.org – Nickel Institute (for health and environment issues only).

http://www.sdahq.org – Soap and Detergent Association.

Toxicology Excellence for Risk Assessment

Jacqueline Patterson

Toxicology Excellence for Risk Assessment (TERA), a nonprofit organization, has a mission to protect public health by developing and communicating human risk assessment values, improving risk methods through research, and educating the public on risk assessment issues. Dr. Michael Dourson, formerly of the United States (US) Environmental Protection Agency (EPA), established TERA in 1995. Project areas include: analyzing toxicity data and developing chemical risk assessments; compiling and distributing a comparative database of human health risk values from leading government agencies and organizations around the world; conducting cutting-edge research to improve risk assessment methods and approaches; convening expert peer review and consultations of risk documentation; and providing general assistance to US, other nonprofit organizations, and the public on human health risk assessment issues.

TERA is a small organization of ~12 scientists with expertise in toxicology, human health risk assessment, noncancer and cancer risk methods, pharmacology, risk communication, environmental science, technical writing, and other disciplines.

Chemical Risk Assessments

TERA scientists analyze available human and animal toxicity data to determine the potential for human health effects from exposure to chemicals. These assessments can include hazard assessments and determination/evaluations of mode of action and weight of evidence determinations for relevance of particular endpoints/effects to humans from environmental or occupational exposures. When adequate data are available, TERA derives noncancer and cancer risk estimates for various routes of exposure. TERA frequently publishes the results of the finalized assessment in peer reviewed journals and posts the assessments on its website.

Risk Assessment Database – International Toxicity Estimates for Risk

TERA created and manages the International Toxicity Estimates for Risk (ITER) database, a comparative database of human health risk values and cancer classifications from leading government agencies and organizations around the world. ITER is a free Internet database of human health risk values for over 600 chemicals. It can be accessed directly from the TERA website and from the (US) National Library of Medicine's TOXNET compilation of databases.

The database includes tabular summary information on the risk estimates and evaluations, along with information on peer review and links to source documents. The format allows for easy comparisons across organizations and, as appropriate, includes an explanation as to why the values may differ. ITER includes risk values and/or cancer classifications from the Agency for Toxic Substances and Disease Registry (ATSDR, United States), Health Canada (Canada), International Agency for Research on Cancer (IARC, World Health Organization member), National Institute of Public Health and the Environment (RIVM (see Relevant Websites section), The Netherlands), and the EPA (United States). In addition, risk values that have undergone independent peer review (see below) are included. Risk estimates and evaluations by additional groups will be added in the future.

Research into Risk Assessment Methods

TERA scientists conduct research to further develop and improve the scientific approaches used to evaluate human health risks from exposures to chemicals and other substances. TERA scientists have led efforts in the development of the reference dose, categorical regression, and use of mechanistic data in risk assessment. Areas being addressed include a chemical's mode of action for toxicity and evaluating the human health relevance of animal data, dose–response modeling to better estimate risk, considerations of special sensitivity of children and other subgroups, refining methods for estimating occupational exposure limits, and application of human health risk approaches to nonchemical exposures.

Peer Review and Consultation Meetings

TERA provides peer consultation and peer review services to meet the needs of public and private sponsors who have developed risk assessment documentation. Work products such as chemical assessments, methodologies, guidance documents, protocols for studies, or research plans are reviewed during expert panel meetings that are open to the public. TERA

works independently of the sponsors and authors of the documents to select an unbiased panel of experts. TERA scientists write reports of each peer review and consultation that summarize the discussions and conclusions of the panels. Risk values and cancer classifications that have been approved by an independent peer review panel may be included on the ITER database (see above).

Technical Assistance and Education

In keeping with its mission and nonprofit status, TERA provides a limited amount of free technical assistance to government and nonprofit organizations. This may include brief reviews of documents, guidance over the telephone, or a written review of an assessment done by the organization. TERA also develops educational materials on health risks of chemicals and risk assessment methods.

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Further Reading

Dourson ML and Patterson J (2003) A 20-year prospective on the development of non-cancer risk assessment methods. *Human and Ecological Risk Assessment* 9: 1239–1252.

Dourson ML, Anderson P, Cartledge D *et al.* (2002) Comparative dietary risk: Balance the risk and benefits of fish consumption. *Comments on Toxicology* (Special Issue) 8: 335–536.

Felter SP, Dourson M, and Patterson J (1997) Assessing risks to human health from chemicals in the environment. In: Calow P (ed.) *Handbook of Environmental Risk Assessment & Management*, ch. 2, pp. 9–23. Oxford: Blackwell Science.

Haber LT, Dollarhide JS, Maier A, and Dourson ML (2001) Noncancer risk assessment: Principles and practice in environmental and occupational settings. In: Bingham E, Cohrssen E, and Powell CH (eds.) *Patty's Toxicology*, 5th edn. New York: Wiley.

Relevant Websites

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

http://www.tera.org – the process of adding oral risk data to ITER from NSF International, an independent, not-for-profit global leader in providing public health and safety risk management solutions is in progress, in the meantime more information about NSF International can be obtained at http://www.nsf.org.

Toxicology Forum

Latrice Vincent

The Toxicology Forum is an international nonprofit organization devoted entirely to the organization of open dialogs among the various segments of society concerned with problems in toxicology. Meetings are organized at which experts from government regulatory agencies, international health agencies, industry, academia, politicians, and consumers exchange views. The scientific community is aware of the scrupulously balanced approach taken in presentation of the issues at the Forum; for each issue alternative positions are presented. The presentations and comments of all participants are recorded and a transcript is made available. The unique nonadversarial atmosphere of Forum meetings promotes uninhibited, productive discussions unencumbered by a need to arrive at a consensus.

Subjects chosen for particular sessions represent the interests of Forum members. This is an important advantage of membership. Every suggestion is given a thorough review by the program committee, which comprises academic, government, and industry representatives. Because emphasis is given to leading edge problems in toxicology as well as programming flexibility, it is common for topics to be addressed years in advance of other organizations. In an era of increasing international trade, another important feature of Toxicology Forum programs is that they are international in scope. Members include people from the public and private sectors in the United States, Canada, Japan, and several European countries.

A vital part of all Toxicology Forum meetings is a session on emerging issues, recent findings, or decisions of governments. These are an unparalleled source of up-to-date information. Numerous Forum meetings resulted in productive discussions of highly controversial subjects of great importance to industry and government. This kind of leadership promotes change in the directions of toxicological research and

permits debate on the public policy aspects of regulatory reform.

The Toxicology Forum holds two to three meetings per year. The Annual Winter Meeting takes place in Washington, DC, during the month of February. The Annual Summer Meeting is held in July in Aspen, Colorado. The European Meeting is usually held in spring or autumn.

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UNEP Chemicals

Pertti J Hakkinen*

The United Nations Environment Programme (UNEP) works to protect public health and the environment worldwide. UNEP Chemicals is the focus for all of UNEP's activities, and the main catalytic force in the UN system for concerted global action on the environmentally sound management of hazardous chemicals.

The main goals of UNEP Chemicals are to catalyze actions and to promote chemical safety by: providing countries with access to information about toxic chemicals; to assist countries in building their capacities to produce, use, and dispose of chemicals safely; and support global actions that are needed to reduce or eliminate chemical risks, such as the Stockholm and Rotterdam Conventions. To achieve these goals, UNEP Chemicals works closely with governments, UN agencies, intergovernmental organizations (IGOs), and nongovernmental organizations (NGOs). UNEP Chemicals concentrates its activities in several areas.

Rotterdam Convention

The Rotterdam Convention on the Prior Informed Consent (PIC) Procedure for Certain Hazardous Chemicals and Pesticides in International Trade was adopted on 10 September 1998. The Convention was ratified by 50 countries (Parties) and entered into force (e.i.f.) on February 24, 2004. Presently, it provides a legal basis for the implementing of the existing PIC procedure that was operated on voluntary basis since 1989. UNEP and the Food and Agriculture Organization of the United Nations (FAO) jointly serve as the secretariat for the Convention.

*The author would like to acknowledge Dr. Salem Milad, Scientfic Affairs Officer, UNEP Chemicals as a provider of much of the information used in this entry.

The objective of the Convention is to protect human health and the environment from certain hazardous chemicals by promoting shared responsibilities and cooperation among Parties with respect to their international trade and environmentally sound use, by facilitating relevant information exchange, and by providing an agreed process for making national decisions on the import and export of these chemicals, and for distribution of such decisions to Parties. At the present the Convention subjects to the PIC procedure 22 hazardous pesticides and five industrial chemicals. There are provisions for exchanging specified information between Parties, for labelling potentially hazardous chemicals that may be exported and imported, and for informing Parties of any national decision to ban or severely restrict a chemical. Other chemicals will be added to the Convention in the future through a specified process in which a Chemical Review Committee will evaluate candidates for addition, including pesticide formulations, nominated by developing countries or countries with economies in transition, and chemicals or pesticides that have been banned or severely restricted for health and environmental reasons by Parties in at least two geographic regions.

The UNEP/FAO joint Secretariat supports and promotes the Convention by:

- Continuing the implementation of the PIC procedure as outlined.
- Promoting understanding, training, ratification, and facilitating a smooth transition to the implementation of the Convention now that it is in force.
- Convening of the 11th session of the Intergovernmental Negotiating Committee that developed the Convention held in September 2004.
- Preparing for the meeting of the 9th Conference of the Parties (COP-1) of the Convention held in September 2004.

Stockholm Convention

The Stockholm Convention on Persistent Organic Pollutants (POPs) was adopted on May 22, 2001. It has been ratified by 50 Parties (countries) and entered into force (e.i.f.) on May 17, 2004.

The Convention was developed in response to the urgent need for global action to protect human health and the environment from 'POPs' through measures designated to reduce and eliminate their release. These are chemicals that are highly toxic, persistent, bioaccumulate, and move long distances in the environment.

Presently under the Convention, Parties are required to take action on an initial list of 12 specified chemicals, including intentionally produced pesticides and industrial chemicals, and unintentionally produced by-products of industrial and combustion processes. Specific goals are set for POPs, including POPs present in stockpiles and wastes.

UNEP Chemicals provides the Secretariat to the Convention. Its actions in support of the Convention include:

- Creating awareness of the POPs issue, the Convention, its provisions, and implementation actions.
- Assisting countries in developing their National Implementation Plans (NIPs).
- Regional, subregional, and national projects addressing specific issues such as dioxin/furans and PCBs.
- Strengthening institutional structures through Global Environment Facility (GEF) projects related to POPs on national and international levels.
- Preparing for the meeting of the 1st Conference of the Parties (COP-1) of the Convention held in early 2005.

Building National Capacities

The heart of UNEP Chemicals is its capacity building work that includes the following activities:

Capacity building. UNEP Chemicals is expanding and improving access to information and information tools to help countries develop the capability with which to assess and manage chemical risks. The UNEP Chemicals programme of support to governments in improving the management of chemicals has included over 75 workshops and conferences addressing priority issues, including:

- Implementing the Stockholm and Rotterdam Conventions.
- PCB identification and management.
- Dioxin and furan source identification and release estimation.
- Alternative to POPs pesticides.
- Establishing a chemicals information network.
- Best available techniques and best environmental practices.

In addition, a wide range of information products have been issued to assist countries and others responsible for chemical management in ensuring environmentally sound production, use and disposal practices.

National Implementation Plans for Stockholm Convention. The Stockholm Convention requires Parties to develop National Implementation Plans (NIPs) within 2 years of entry into force of the Convention. The NIPs outline the POPs', situation in the country, and the measures to be taken in implementing the Party's obligations under the Convention.

In 2003, 125 Countries had received funding from the Global Environment Facility (GEF) to develop their NIPs. Twelve of these countries are also participating in a pilot project aimed not only at NIPs, but also at the development of generic and technical guidelines for the development of NIPs and the adoption of POP management options. Over twenty-five national regional meetings, workshops, and/or training have been organized for the NIPs and pilot projects.

Strategic Approach to International Chemical Management (SAICM)

The development of a SAICM was mandated by UNEP's Governing Council in 2002, and subsequently endorsed by the World Summit on Sustainable Development. A consultative process engaging all stakeholders will culminate in an international conference around the end of 2005. UNEP Chemicals provides the SAICM secretariat and collaborates with a 10-organization steering committee comprising Inter-Organization Programme for the Sound Management of Chemicals (IOMC) partners, the Intergovernmental Forum on Chemical Safety (IFCS), United Nations Development Programme (UNDP), and the World Bank.

The first session of the Preparatory Committee for Development of a SAICM was held in Bangkok, from 9 to 13 November 2003 ('Prepcom1'). The second session of a SAICM was held in Nairobi, Kenya, from 4 to 8 October 2004 ('Prepcom2'). Background information and meeting documents for SAICM-Prepcom1 and Prepcom2 can be found at UNEP's website.

Mercury. In response to UNEP's Governing Council decision in 2001, UNEP Chemicals undertook an assessment of the risks from mercury in the environment. Following the publication of the 'Global Mercury Assessment' report in December 2002, the UNEP's Governing Council, in February 2003 found that there is evidence of 'significance global adverse impacts from mercury,' and called for further international action to reduce the risks to humans and wildlife. In response, a Mercury Program was established within UNEP Chemicals, which aims to promote national, regional, and global actions to reduce or eliminate as far as possible the use and release of mercury into the environment. As a first step during 2004, UNEP Chemicals began to develop plans to organize a series of workshops for developing countries to help them to identify and understand any mercury problems in their country and implement action to mitigate the problem.

Furthermore, a wide range of other information products has been issued by UNEP Chemicals, often with partner organizations like the International Programme on Chemical Safety (IPCS). For example, data sources about persistent organic pollutants include the UNEP Chemicals' extensive website home page on POPS, and reports on POPS workshops, global destruction capacity for polychlorinated biphenyls, and inventories of dioxins and furans. Other publications cover lead in gasoline, chemicals risk assessment, and the Organisation for Economic Co-operation and Development (OECD) Screening Information Data Sets (SIDS) for high protection volume (HPV) chemicals. The UNEP Chemicals Newsletter is published periodically to give readers an update of activities to promote the environmentally sound management of chemicals: A full listing of publications may be obtained and copies ordered free of charge by contacting UNEP Chemicals.

Contact Details

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United States Pharmacopoeia (USP)

Shayne C Gad

The United States Pharmacopoeia (USP) is a nongovernmental, standards-setting organization that advances public health by ensuring the quality and consistency of medicines, promoting the safe and proper use of medications, and verifying ingredients in dietary supplements. For historic reasons (plastics being used in containers for medicines), the USP also served to set the initial standards and test schemes for medical devices and device materials. USP standards are developed by a unique process of public involvement and are accepted worldwide. In addition to standards development, USP's other public health programs focus on promoting optimal health care delivery and are listed below. USP is a nonprofit organization that achieves its goals through the contributions of volunteers representing pharmacy, medicine, and other health care professionals, as well as science, academia, the US government, the pharmaceutical industry, and consumer organizations.

USP's activities and initiatives revolve around four public health programs: Standards, Dietary Supplement Verification Program, Health Care Information, and Patient Safety.

Standards

Establishing standards is a core USP activity. Currently, USP provides standards for more than 4000 prescription and nonprescription drugs, dietary supplements, veterinary drugs and health care products. These standards are published in the *United States Pharmacopoeia* and *National Formulary* (USP-NF), and are officially recognized in the Federal Food, Drug, and Cosmetic Act (21 U.S.C. ξ 321 *et seq*.). USP also produces Reference Standards, which are an integral part of USP's standards program. In addition, USP offers a Pharmacopoeial Education program that provides continuing educational courses for professionals working in the pharmaceutical industry – helping those who use the USP-NF better understand pharmacopoeial processes, standards, tests, and methods.

USP's standards-setting tradition began in 1817 when Lyman Spalding, a New York physician, responded to a growing desire among his physician

colleagues for standardization names and formulations, which, up to then, had differed from one region of the country to another. Between 1817 and 1820, working with physicians in medical schools and medical societies across the country, Spalding conducted a survey to determine those formulations physicians considered to be the most fully established and best understood. The survey asked what those medicines were called and how they were prepared. Later, Spalding and fellow physicians founded a group that met in the US Capitol's Senate chamber in Washington, DC. By the time the meeting adjourned, the groundwork was laid for the compilation of the Pharmacopoeia of the United States of America – a compendium that standardized the most fully established and best understood medicines of that era.

Today, the *United States Pharmacopoeia* and *National Formulary* contain specifications of strength, quality, purity, packaging, and labeling for more than 3800 prescription drugs, nonprescription drugs, dietary supplements, medical devices, excipients, botanicals, and other products. USP works closely with the Food and Drug Administration (FDA), the pharmaceutical industry, and the health professions to establish authoritative drug standards. These standards are enforceable by FDA and the governments of other countries and are recognized as the hallmark of quality.

Dietary Supplement Verification Program (DSVP)

USP developed this program in response to the increasing concerns expressed about dietary supplements in the marketplace. Through compliance testing and document review, adherence to good manufacturing practices (GMPs), and postmarketing surveillance, DSVP is designed to help ensure that dietary supplement products contain the declared ingredients in the declared quantities.

Health Care Information

USP provides health care professionals and patients with drug information about new and off-label uses of nearly all medicines in the United States and Canada. This drug information is contained in the *USP Drug Information* (DI) database and publications, and it is distributed in association with MICROMEDEX (a division of Thomson Publishing). USP provides oversight and approves drug information content in the USP DI. USP's Health Care Information program also consists of several global initiatives. USP was awarded several grants by the United States Agency for International Development's (USAID) Center for Population, Health and Nutrition (PHN). Currently, USAID is supporting the USP Drug Quality and Information (USP DQI) program, which funds programs in Nepal, Romania, Russia, Senegal, China, Kazakhstan, Mozambique, and the Mekong Delta region.

Patient Safety

The Center for the Advancement of Patient Safety (CAPS) was created in order to broaden USP's work within the patient safety arena. CAPS conducts data analysis and research, seeks grants, develops professional education programs, publishes articles on issues related to medication errors, participates in legislative activities, and proposes standard recommendations and guidelines for the goal of improving patient safety by preventing and reducing medication errors. In addition, USP operates two medication error reporting, tracking, and analysis programs: the Medication Errors Reporting (MER) Program (operated in collaboration with the Institute for Safe Medication Practices) and the MEDMARX^{SM} program. MEDMARX is an Internet-accessible database for hospitals to report and track medication errors anonymously (see Relevant Websites).

Collaborative Ventures

The United States Pharmacopoeia – a trusted leader in drug quality, standards, and information – in cooperation with the US Agency for International Development (USAID) manages the Drug Quality and Information (USP DQI) Program to support delivery of priority interventions for the USAID Center for Population, Health, and Nutrition. USP DQI focuses on ensuring the quality of pharmaceuticals and their informed and appropriate use worldwide.

Contact Details

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Further Reading

Anderson L and Higby GJ (1995) *The Spirit of Voluntarism, A Legacy of Commitment and Contribution: The United States Pharmacopoeia, 1820–1925*. Rockville, MD: USP.

Hicks RW, *et al.* (2004) Selected medication-error data from USP's MEDMARX program for 2002. *American Journal of Health-System Pharmacy* 61(10): 993–1000.

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APPENDIX 2
PUBLIC DOMAIN ONLINE CHEMICAL COMPENDIA – A BRIEF SELECTION

Chemical lists, compiled for regulatory, research, and other purposes, are widespread and may offer insight into potential hazards associated with chemicals. Many of these are subject to frequent change and update. A number of databases and peer-reviewed reports also contain important information on significant chemicals. Web URLs for a select number of these resources (all available at no charge) are presented below. In some cases the link is intended simply to a listing of chemicals, in others it is to peer-reviewed or otherwise substantive and reliable information on chemicals.

- Chemicals Known to the State to Cause Cancer or Reproductive Toxicity – Proposition 65 List (from the state of US California) – http://www.oehha.ca.gov

- CHEMIDplus (from the US National Library of Medicine) – a database with access to structural and nomenclature information for hundreds of thousands of chemicals, including links to toxicity and regulatory data – http://chem.sis.nlm.nih.gov

- Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) Priority List of Hazardous Substances – substances that are most commonly found at facilities on the National Priorities List (NPL) and which are determined to pose the most significant potential threat to human health due to their known or suspected toxicity and potential for human exposure – http://www.atsdr.cdc.gov

- Environmental Fate Data Base (EFDB) (from Syracuse Research Corporation) – data related to chemical environmental fate, microbial toxicity, biodegradation, etc. – http://www.syrres.com

- European Chemicals Bureau – extensive information on chemicals in Europe – http://ecb.jrc.it

- EXTOXNET – from a consortium of universities, offering a variety of information about pesticides – http://extoxnet.orst.edu

- Hazardous Air Pollutants (from the US Environmental Protection Agency (EPA)) – those pollutants that cause or may cause cancer or other serious health effects, such as reproductive effects or birth defects, or adverse environmental and ecological effects, and which the EPA is required to control – http://www.epa.gov

- INCHEM (from the International Programme on Chemical Safety) – internationally peer-reviewed information on chemicals from intergovernmental organizations – http://www.inchem.org

- Integrated Risk Information System (IRIS) (from the US EPA) – containing information on human health effects that may result from exposure to various chemicals in the environment – http://www.epa.gov (see link to IRIS page in this website) and also via the US National Library of Medicine's TOXNET system at http://toxnet.nlm.nih.gov

- International Agency for Research on Cancer (IARC) Monogrăphs Programme on the Evaluation of Carcinogenic Risks to Humans – http://monographs.iarc.fr

- Minimal Risk Levels (MRLs) for Hazardous Substances (from the US Agency for Toxic Substances and Disease Registry) – An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure – http://www.atsdr.cdc.gov

- Pesticides Database (from Pesticides Action Network (PAN)) – toxicity and regulatory information on an extensive array of pesticides – http://www.pesticideinfo.org

- Pocket Guide to Chemical Hazards (from the US National Institute for Occupational Safety and Health (NIOSH)) – a source of general industrial hygiene information on several hundred chemicals/classes for workers, employers, and occupational health professionals – http://www.cdc.gov

- List of Drinking Water Contaminants and their Maximum Contaminant Level (MCLs) (from the US EPA) – http://www.epa.gov

- National Toxicology Program, US (NTP) – extensive information on numerous chemicals studied by this US interagency group – http://ntp-server.niehs.nih.gov

- Report on Carcinogens (from the US NTP) – includes listings of both, known human carcinogens and reasonably anticipated to be human carcinogens – http://ntp.niehs.nih.gov (click on Report on Carcinogens)

- Right-to-Know Hazardous Substances Fact Sheets (from the New Jersey Department of Health and Senior Services) – information on hazardous substances in the workplace – http://www.state.nj.us

- Risk Assessment Information System (from the Oak Ridge National Laboratory and the University of Tennessee) – includes toxicity profiles on chemicals – http://risk.lsd.ornl.gov
- International Toxicity Estimates for Risk (ITER) (from Toxicology Excellence for Risk Assessment (TERA)) – provides tabular comparisons of risk values from an assortment of agencies and countries – http://www.tera.org and also from the US National Library of Medicine's TOXNET system at http://toxnet.nlm.nih.gov

- Toxicological Profiles (from the US Agency for Toxic Substances and Disease Registry) – detailed information on hazardous substances found at National Priorities List (NPL) sites – http://www.atsdr.cdc.gov

- Toxics Release Inventory (TRI) Chemical Lists (from the US EPA) – includes lists of chemicals subject to reporting requirements under Title III of the Superfund Amendments and Reauthorization Act of 1986 (SARA) – http://www.epa.gov and also from the US National Library of Medicine's TOXNET system at http://toxnet.nlm.nih.gov

- TOXNET (from the US National Library of Medicine) – an array of databases with copious information related to many aspects of toxicology, chemical safety, and environmental health – http://toxnet.nlm.nih.gov

Philip Wexler

Annexure 1

Abbreviations and Acronyms Used in Toxicokinetics

ADI	Acceptable daily intake
AF	Assessment factor
ALARA(P)	As low as reasonably achievable (practicable) In UK regulations relating to worker exposure In USA goal of risk management (USNRC regulations)
AUC	Area under the concentration-time curve
AUMC	Area under the moment curve
BCF	Bioconcentration factor
BEI	Biological Exposure Indices (ACGIH)
BEM	Biological effect monitoring
BOD	Biochemical oxygen demand
b.w.	Body weight
CMR	Carcinogenic, mutagenic and reproductive (toxicant)
CoMFA	Comparative molecular field analysis
Cyt	Cytochrome
CV	Ceiling value
DNA	Deoxyribonucleic acid
DNEL	Derived no-effect level
EC	Enzyme classification number or effective concentration
EC_n	Median effective concentration to n% of a population
EDI	Estimated daily intake
ED_n	Median effective dose to n% of a population
EEC	Estimated exposure concentration
EQS	Environmental quality standard
EED	Estimated exposure dose
EEL	Environmental exposure level
EMDI	Estimated maximum daily intake
GLP	Good laboratory practice
HSG	Health and Safety Guide (IPCS)
HQ	Hazard quotient
IC	Inhibitory concentration
i.c.	Intracutaneous
i.d.	Intradermal
i.m.	Intramuscular
inhl	By inhalation
i.p.	Intraperitoneal
I-TEF	International Toxicity Equivalency Factor
i.v.	Intravenous
K_M	Michaelis constant
K_{oc}	Organic carbon partition coefficient
K_{ow}	Octanol–water partition coefficient
LADD	Lifetime average daily dose
LC_n	Median concentration lethal to n% of a test population
LC_{50}	See LC_n
LD_n	Median dose lethal to n% of a test population
LD_{50}	See LD_n
LEL	Lowest effect level, same as LOEL
LOEL	Lowest-observed-effect level
LOAEL	Lowest-observed-adverse-effect level
LT_n	Median time for death of n% of a test population
LV	Limit value
MAC	Maximum allowable concentration
MEL	Maximum exposure limit
MF	Modifying factor
MOE	Margin of exposure
MPC	Maximum permissible concentration
MRL	Maximum residue limit
mRNA	Messenger ribonucleic acid
MSDS	Material safety data sheet
MTC	Maximum tolerable concentration
MTD	Maximum tolerable dose, Maximum tolerated dose
MTEL	Maximum tolerable exposure level
NADP(H)	Nicotinamide adenine dinucleotide phosphate (reduced)
ND_n	Median dose narcotic to n% of a population
NEL	No effect level, same as NOEL
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
NSC	Normalized sensitivity coefficients
PBT	Persistent, bioaccumulative and toxic
PEL	Permissible exposure limit
PBPK	Physiologically based pharmacokinetics modeling
$PM_{2.5}$	Particles in air of with a maximum aerodynamic diameter of 2.5 μm
PM_{10}	Particles in air of with a maximum aerodynamic diameter of 10 μm
PMR	Proportionate mortality rate, ratio
p.c.	Per cutim (Latin) = Through the skin
p.o.	Per os (Latin) = By mouth
POW	Octanol–water partition coefficient
PPAR	Peroxisome proliferator-activated receptor
PTWI	Provisional tolerable weekly intake
QSAR	Quantitative structure–activity relationship

3D-QSAR	Three-dimensional quantitative structure–activity relationship
QSMR	Quantitative structure–metabolism relationship
RD	Rate difference
RfC	Reference concentration
RfD	Reference dose
RNA	Ribonucleic acid
RR	Rate ratio
ROS	Reactive oxygen species
SAR	Structure–activity relationship
s.c.	Subcutaneous
SCE	Sister chromatid exchange
SMR	Standard mortality ratio
SMR	Structure–metabolism relationship
SNARL	Suggested no-adverse-response level
STEL	Short-term exposure limit
$t_{1/2}$	Half-life, half-time
TBPK	Toxicologically based pharmacokinetic modeling
TCDD	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin
TDI	Tolerable daily intake
TEF	Toxicity equivalency factor
TEQ	Toxicity equivalent
TL_n	See LT_n
TLV	Threshold limit value (ACGIH)
TMDI	Theoretical maximum daily intake
TWA	Time-weighted average
TWAC	Time-weighted average concentration
TWAE	Time-weighted average exposure
TWI	Tolerable weekly intake
UF	Uncertainty factor
V_{max}	Maximum velocity
vPvB	Very persistent and very bioaccumulative

Annexure 2

Abbreviations and Acronyms of Names of International Bodies and Legislation

ACGIH	American Conference of Governmental Industrial Hygienists
ATSDR	Agency for Toxic Substances and Diseases Registry
BCR	Bureau Communautaire de Référence (Bruxelles)
BIBRA	British Industrial Biological Research Association
CCFA	Codex Committee on Food Additives
CCPR	Codex Committee on Pesticide Residues
CDC	Centers for Disease Control and Prevention
CEC	Commission of the European Communities
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act (USA)
CHIP	Classification, Hazard Information and Packaging (UK)
COSHH	Control of Substances Hazardous to Health Regulations (UK)
CPL	Classification, Packaging and Labeling
EC	European Community, European Commission
ECB	European Chemicals Bureau
EEA	European Environmental Agency
EEC	European Economic Community
EINECS	European Inventory of Existing Chemical Substances
ELINCS	European List of New Chemical Substances
EPA	Environmental Protection Agency (USA), same as USEPA
EUROTOX	European Society of Toxicology
EUSES	European Uniform System for Evaluation of Substances
FAO	Food and Agricultural Organization
FDA	Food and Drug Administration (USA)
IAEA	International Atomic Energy Agency
IARC	International Agency for Research on Cancer
ICH	International Conference for Harmonization
ICRP	International Commission on Radiological Protection
ICSU	International Council of Scientific Unions (since 1998, International Council of Science)
IFCC	International Federation of Clinical Chemists
ILO	International Labour Organization
IPCS	International Programme on Chemical Safety, UNEP, ILO, WHO
IRIS	Integrated Risk Information System (USA)
IRPTC	International Register of Potentially Toxic Chemicals, now UNEP Chemicals
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
IUTOX	International Union of Toxicology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
NBS	National Bureau of Standards (USA), now NIST
NIH	National Institutes of Health (USA)
NIOSH	National Institute of Occupational Safety & Health (USA)
NIST	National Institute of Standards and Technology (USA), formerly NBS
NRC	National Research Council (USA)
OECD	Organization for Economic Cooperation and Development
OMS	Organisation Mondiale de la Santé, same as WHO
OSHA	Occupational Safety and Health Administration (USA)
RSC	Royal Society of Chemistry
REACH	Registration, Evaluation, and Authorization of Chemicals (EC)
SCOPE	Scientific Committee on Problems of the Environment (ICSU)
TOSCA	Toxic Substances Control Act (USA)
UNEP	United Nations Environment Programme
USEPA	United States Environmental Protection Agency, same as EPA
WHO	World Health Organization, same as OMS

Further Reading

Duffus JH (1993) *Pure and Applied Chemistry* 65: 2003–2122.

Nordberg M, Duffus JH, and Templeton DM (2004) *Pure and Applied Chemistry* 76: 1033–1082.