Veterinary Pharmacovigilance

Adverse Reactions to Veterinary Medicinal Products

Edited By
K.N. Woodward

WILEY-BLACKWELL

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To Diana, Alastair, Frances and Felicity
And to Jenny, Sally and Patsy, to Alfie and Molly,
and to Jack Russell Terriers everywhere.

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Preface

When a new veterinary medicinal product is launched into widespread use, adverse drug reactions may become apparent. These may be seen in the treated animal patients, in exposed users or as adverse effects on the environment. Additionally, they may manifest as excess residues of the drug in food of animal origin. As a consequence, legislation and regulatory approaches have developed across the globe to address these issues and to ensure that the continued safety of these products can be monitored and, where necessary, that regulatory actions can be pursued to assuage any concerns. All of these can be covered by the single term 'pharmacovigilance'.

This book is an attempt to survey and summarise current approaches to veterinary pharmacovigilance, to review the types of effects that may be seen and to examine some of the scientific principles involved. I hope it will prove useful in academia, in the regulatory environment and within the animal health industry.

Finally, I should like to pay tribute to one of my contributors, Ramzan Visanji, who died in October 2008. Ramzan, a person of tremendous courage, was a great colleague and a good friend. His advice and views will be sorely missed, as will his fine sense of humour.

Readers should note that the views expressed by the editor herein are solely the editor's views and they do not necessarily reflect the views of Intervet/Schering-Plough Animal Health.

K.N. Woodward
2008

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Introduction

D. Skilton

Veterinary Pharmacovigilance is the collection and assessment of information, including post-marketing surveillance of the adverse effects of veterinary medicines. An *adverse effect or reaction* to a veterinary product is one that is *harmful and unintended and which occurs at doses normally used in animals for the prophylaxis, diagnosis or treatment of disease or the modification of physiological function.*

Following the increase in knowledge and growth of technology in the veterinary sector, pharmacovigilance is now recognised as a vital component in the safe and efficacious use of veterinary medicines. The purpose of a good pharmacovigilance surveillance system is to ensure the safety of veterinary medicines once they are authorised and being used in the market place. The rapid identification of any adverse effects to medicines is essential and the data produced from the investigation should be assessed in order to reduce risks in the future use of the product where applicable.

When veterinary medicines are used according to the manufacturer's instructions, adverse effects to the products are extremely rare. Before a company can place a veterinary medicine in the market place there is a requirement under European Union (EU) and United Kingdom (UK) legislation for a Marketing Authorisation (MA) to be obtained. This MA is only granted after a detailed scientific assessment of the product data on quality, safety and efficacy. Part of the legislation covering the assessment requires the Marketing Authorisation Holder (MAH) to conduct clinical trials, which will provide product details on safety, efficacy and the potential for harmful side effects. Although clinical trials are controlled, they do not always provide full information on the effects of the product in all situations. The use of any medicine carries a risk of side effects which has to be considered against the benefit of using the product.

After the authorisation of a veterinary medicine, observation and feedback through appropriate pharmacovigilance should ensure the continued safety and efficacy of the product during its use in the field. In the EU an MAH must have permanently and continuously at his disposal an appropriately qualified person responsible for pharmacovigilance who resides in the member state. The information gained from post-authorisation surveillance is very important and the reports collected and collated can be used in further evaluation and assessment of the product.

In the UK the Veterinary Medicines Directorate (VMD), an executive Agency of the Department for Environment, Food and Rural Affairs

(DEFRA), is the regulatory authority responsible for pharmacovigilance for veterinary medicines through a national Suspected Adverse Reaction Surveillance Scheme (SARSS). The Scheme records and monitors all reported animal and human suspected adverse reactions (SARs), including environmental incidents, to veterinary medicines. The SARSS is a passive but valuable method of monitoring trends. Animal and human SARs should be reported to the VMD on yellow forms (MLA252A). In addition there is a green card (MLA1) to report environmental incidents, and a blue card (MLA2) to report suspected residues of antibiotics in milk.

The reports are analysed by the SARSS of the VMD and regular reports made to the Veterinary Products Committee (VPC). It is important that regular meetings are held between the MAHs and the VMD SARSS team to discuss issues of joint interest and in particular causality coding and trends in SARs. The assessment of the causal relationship between the suspected adverse reaction and the product is based on all the available information. The suspect reaction is then categorised using the ABON system of coding. This system codes the reaction as category A (probable) through to category N (unlikely). If a trend in adverse reactions emerges, then action could be taken by the regulatory authority. This action could involve the MAH being required to amend aspects of the authorisation such as product labelling, or even lead to product batch recall, suspension or withdrawal of the product.

The VPC is an independent scientific statutory committee established under the Veterinary Medicines Regulations, which are updated annually, to provide the Secretary of State with scientific advice on any aspect of veterinary medicinal products. As part of its remit the committee is required to promote the collection of information relating to suspected adverse reactions for the purpose of enabling scientific advice to be given on the use of products and their effects. The VPC appoints two of its members to liaise with the SARSS team at the VMD and these members provide a bimonthly report to the committee. Over the last few years the VPC has established a number of working groups, which have included groups reporting on *Feline and Canine Vaccination* published in 2002 and this was followed by a *Review of the Suspected Adverse Reaction Surveillance Scheme (SARSS)* in 2004.

The MAH that holds an MA is legally required to report a serious adverse reaction in animals, a suspected human adverse reaction after exposure to a veterinary medical product, and suspected unintended transmission of an infectious agent through a veterinary product following the administration of the product in the UK, to the regulatory authority within 15 days.

- A *serious adverse reaction* is one that results in death, is life-threatening, results in significant disability or incapacity, is a congenital anomaly/birth defect, or which results in permanent or prolonged signs in the animals treated.
- A *human adverse reaction* means a reaction that is noxious and unintended and that occurs in a human being following exposure to a veterinary medicine.

Each report is evaluated for any causal relationship between the product and the adverse reaction. The company must report all other adverse reactions in a Periodic Safety Update Report (PSUR). The format of these reports is dictated by EU guidelines and the report must include a scientific evaluation of the benefit : risk balance of the veterinary medicinal product. Recent revision of the pharmaceutical legislation in the EU has placed more emphasis on the risk management of authorised veterinary medical products through a greater reliance on the evaluation of PSURs whilst at the same time reducing the regulatory burden of MA renewals.

The EU also provides for other areas of pharmacovigilance in addition to adverse reactions occurring in animals and humans. These include: lack of expected efficacy, off-label use/misuse, reported violations of approved residue limits and environmental problems.

Incidents involving suspected lack of expected efficacy should normally be reported in the PSUR. EU guidance, however, makes provision for

reporting incidents within 15 days in certain specific circumstances. The VMD considers these circumstances to include a lack of efficacy associated with possible development of antimicrobial or anthelmintic resistance, a very important consideration in the use of veterinary medicines. In 2006 the VMD received two reports of suspected lack of efficacy to ectoparasiticides in salmon, a possible indication of resistance to emamectin in sea lice. The VPC has discussed the possible development of sea lice resistance to emamectin and cypermethrin and expressed its concern over resistance issues not being detected promptly due to historic reporting. Intensive rearing of salmon in Norway in the 1970s led to the first reports of sea lice infestations. More recently outbreaks in farmed fish in Scotland and Chile have produced serious welfare problems and great economic loss to the aquaculture industry. Avermectins have been used to control sea lice infestations in salmon and concern over the toxicity of ivermectin led to the development of the less toxic emamectin benzoate.

All reporting of suspected adverse reactions, other than those reported by the MAH, is voluntary, although there is a professional duty on the veterinary surgeon to report any suspected adverse reaction to the MA holder or to the VMD. Veterinary surgeons are usually the first point of contact when an adverse reaction to a veterinary medicine is suspected. In 2006 the VMD SARSS received a total of 2,384 reports involving SARs in animals. Veterinary surgeons were the main source of reports received by the VMD with 50.0%, followed by the MAH with 47.6%. Only 2.2% of reports were made direct by the general public. Promoting pharmacovigilance involves motivating people to report suspected incidents. Prompt and accurate reporting of adverse reactions is essential so that a continuous assessment can be made of the balance between risk and benefit of the product in use. Accurate details, including laboratory analysis and post mortem reports where applicable, are essential to enable a full assessment of the reaction. Many investigation reports conclude with an ABON coding of 'O' (unknown) where there is insufficient evidence to make an accurate assessment on causality.

Veterinary pharmacovigilance is also important in the area of unauthorised use of veterinary medicines. The SARSS team report that for 2006 there were 185 reports involving unauthorised use of an authorised product. The majority of cases involve use in an unauthorised species or overdosing. Other reactions occur from unauthorised route of administration, failure to observe written warnings or contraindications.

The potential adverse effect of retinal degeneration after the use of enrofloxacin in cats is well documented. The Summary of Product Characteristics (SPC) states that 'retinotoxic effects including blindness can occur when the recommended dose is exceeded'. In 2006, however, the SARSS received ten reports of blindness in cats involving the use of enrofloxacin and eight of these cases involved the administration of an overdose.

Unauthorised use in the feline of canine 'spot-on products' containing permethrin is one example that has received publicity in the past and continues to cause concern. Permethrin is a safe and effective product when used according to the SPC produced by the MAH in the canine species. The feline species, however, is particularly susceptible to the effects of permethrin. Despite warnings on product literature, a significant number of cats have been exposed to the toxic effects (including convulsions, twitching and tremors) of the product in that species. These effects have been shown both from direct application (as a spot-on treatment) and from secondary exposure through contact with treated dogs. A recent Veterinary Poisons Information Service (VPIS) study on 286 cases found that 96.9% of cats exposed to permethrin developed clinical effects and 10.5% died or were euthanised (Sutton *et al.*, 2007). These cases underline the importance of using authorised products in the stated species and of following the manufacturer's instructions, advice and warnings on the correct use of the product.

Regulatory and current economic factors have led over recent years to the withdrawal from the

market place of many products where there are limited sales. Indications of use for other products have been restricted to use in the main species. These developments have had an effect on the medicine availability for what are termed the minor use, minor species (MUMS) where no authorised medicines exist for use in those species. These species include, for example, rabbits, goats, ostriches and bees. If these species are left untreated or are treated with unauthorised products, then animal welfare problems could arise. The lack of authorised medicines in certain species has led to the regulatory authority allowing the administration of a veterinary medicinal product outside the terms of an MA in order to meet animal welfare requirements and to avoid unacceptable suffering. These provisions (the 'cascade') allow the use of products authorised in a different animal species or for another condition in the same species. If no such product is suitable then a product either authorised for human use or authorised in another member state may be used. A recent letter in the *Veterinary Record* from the SARSS team at the VMD records some of the suspected adverse reactions reported to human medicines when used in animals (Spagnuolo-Weaver, 2007). Benefit : risk assessments and pharmacovigilance become even more important when products are used under the 'cascade' provisions.

Vaccination in animals and suspected adverse reactions, including lack of efficacy, continues to receive publicity. The VPC working group in 2002 concluded that although adverse reactions to vaccination, including lack of efficacy, occasionally occur, the overall benefit : risk analysis strongly supports their continued use (Gaskell *et al.*, 2002). The working group considered in depth the monitoring of adverse reactions, including the advantages and disadvantages of surveillance schemes. These schemes are useful in monitoring trends in a population over a period of time, although under-reporting is likely to be a feature of such schemes. Vaccination is a very effective way of controlling and preventing significant diseases, and feedback to a central base on the effectiveness of such a programme is important in assessing appropriate control for the future.

Over recent years there has been an increase in the number of reports involving suspected lack of efficacy to parvovirus vaccines. There were eight reports submitted to the VMD in 2003, 15 reports in 2004 and 32 reports in 2005. In 2006, following a reported increase in cases of parvovirus in vaccinated dogs, the VMD requested the submission of safety reports from all MAHs with authorised vaccines containing parvovirus. Further investigation is required to ascertain whether this trend is associated with a lack of response to vaccination. The true position with regard to the disease status in the field is unknown.

In the USA in 1991 a higher than expected number of sarcomas in cats were reported at the injection sites of commonly used vaccines. This led in 1996 to the formation of the Vaccine-Associated Feline Sarcoma Task Force (VAFSTF), which included various representatives of the veterinary organisations plus veterinary researchers and clinicians in the USA. The aetiology of vaccine-associated sarcomas in cats is very complex, although there is evidence supporting the role of inflammation in the development of these lesions.

Vaccine-associated sarcomas in cats are very complex, although there is evidence supporting the role of inflammation in these lesions. There is also historical evidence that a change from live to killed adjuvanted rabies virus vaccine and increased number of antigens available (FeLV vaccine) coincide with an increase in the development of sarcomas at the injection site. Manufacturers of vaccines continue to work towards the development of new and different approaches in vaccine production and route of administration. The aim is to provide maximum protection of a species with minimum risk to the individual, and the veterinary surgeon should continue to advise cat owners of the appropriate vaccination protocol for the individual cat. The VAFSTF has concluded its official investigation on this issue, although individual researchers will no doubt continue to study this very complex subject.

Recently in the UK there have been claims that canine vaccination is responsible for illness in a number of dogs within 3 months of vaccination. An independent and scientifically peer-reviewed epidemiological investigation, however, has produced evidence that demonstrates the absence of any deleterious association between routine vaccination and signs of ill health (Edwards *et al.*, 2004). Vaccination triggers the body's immune system to produce a protective immune response. The stimulus required is not related to breed or body mass. There is always a potential for adverse reactions in any species and the VPC has stressed the importance of continued pharmacovigilance. The VPC working group on this matter emphasised that surveillance schemes, and the UK VMD SARSS in particular, provided a very valuable resource for monitoring adverse reactions.

In the UK all human SARs are considered by the Appraisal Panel for Human Suspected Adverse Reactions to veterinary medicines, which is a sub-committee of the VPC. The Appraisal Panel's terms of reference are to evaluate all suspected adverse reactions to veterinary medicinal products in humans. The Panel plays a key role in identifying trends and signs of emergent problems, generating hypotheses as to possible causes of these trends, and monitoring the consequences of recommendations for changes in working practices or use. The Panel considers reports of human suspected adverse reactions to veterinary medicines received by the VMD under the SARSS and reports its findings to the VPC. Whenever possible, a report to the Appraisal Panel will include further information obtained from the reporter of the SAR. The VMD obtains follow-up information on individual cases by questionnaire, letter and telephone.

The Appraisal Panel considers all serious human SARs. A human SAR is considered serious if it involves one or more of the following:

- the death of a person exposed to a veterinary medicine;
- a person having in-patient hospital care as a result of exposure to an animal medicine;
- hospital out-patient care if it involves significant medical intervention (such as in the treatment of injection site injuries from vaccines containing mineral oil adjuvants);
- persistent or irreversible symptoms.

The Appraisal Panel does not attribute causality in individual cases but collectively assesses reports in relation to the type of veterinary medicine and circumstances of use. However, in identifying trends it is sometimes necessary to establish the significance of a SAR and/or validate the data. In such cases the Panel may undertake individual case assessment to assist in identifying trends and to generate hypotheses as to the possible causes of these trends. In order to increase the objectivity and the reliability of these reports, medical practitioners' participation in the scheme is encouraged.

In 2006 the VMD received 126 reports (104 in 2005) of human SARs due to accidental or occupational exposure to veterinary medicines; 87.3% of these reports came through the MAHs (Veterinary Products Committee and its Sub-Committees, 2006). Half of the reports received related to the use of ectoparasiticides and endectocides. Although the number of reports of human SARs, particularly non-serious SARs, received had increased in 2006, under-reporting continued to give the Appraisal Panel concern. In considering ways that this could be improved, members were advised that the Health and Safety Executive, which liaised regularly with the National Proficiency Test Council on the content of qualifications, would recommend that the reporting of SARs to veterinary medicines should be included in the appropriate qualifications and assessment schedules.

Safety to humans using veterinary medicines is an important priority. Micotil (Tilmicosin injection) is a recognised treatment for bovine respiratory disease (BRD) and the deaths of two farmers in North America have been associated with the accidental injection of Micotil. There have also been serious adverse reaction reports in the EU and as a consequence the EU has recommended additional safety warnings on the product.

Although the hazards associated with Micotil are well understood by those who administer it in the UK, the EU has made a decision to restrict the administration of the product so that only veterinary surgeons can administer it to animals. The use of this product emphasises the need for extreme caution in the administration of all veterinary medicines in order to avoid accidental self-injection by the user.

In the UK there have been reports of accidental self-injection of vaccines containing mineral oil adjuvants. It is recommended that needles should only be connected to the syringe when filling or giving the injection, and animals should always be properly restrained when administering the medicine. The VPC published a letter in the *Veterinary Record* highlighting the dangers of self-injection after receiving information that fewer veterinary needle stick injuries are reported to the VMD than there are enquiries made to the National Poisons Information Service (NPIS) for advice about how to treat them (Skilton and Thompson, 2005).

In 1994 the VPC recommended that a sub-committee, the Medical and Scientific Panel (MSP), comprising medical and scientific experts, should be established to evaluate and co-ordinate research on organophosphate (OP) sheep dips in relation to possible human exposure. The Panel also advises on any additional work that may be needed to elucidate the potential long-term effects on humans of OP sheep dip. In 2006 the Panel considered 39 published papers and concluded that none of them provided new evidence of a link between low-level exposure to OPs and health effects. The panel also reviewed the VMD response to a consultation on a review of diazinon by the Australian Pesticides and Veterinary Medicines Authority (APVMA).

The reporting of environmental incidents became part of the UK SARSS in 1998. The majority of reports come from the Environment Agency (EA), the Scottish Environment Protection Agency (SEPA), the Environment and Heritage Service, Northern Ireland, and the Wildlife Incident Investigation Scheme. The SARSS team also receive reports from the MAHs and the general public. In 2006 the VMD received reports on 62 environmental incidents, the majority involving the aquatic environment. Many of the reports received were historical relating to incidents in previous years. As a result of the number of reports received involving the use of cypermethrin sheep dips in areas of Wales, the MAs of these products were suspended in February 2006. The main cause of these incidents when identified was due to spent dip entering a watercourse.

Environmental risk assessment is unlike human or target species risk assessment because of the much wider range of species and exposure pathways that need to be considered. Therefore a regulatory scheme that does not involve credible post-authorisation monitoring is likely to suffer from an unknown number of false negatives, in which the environmental risks of chemicals are underestimated. Evidence of this is available from experiences with pesticides, biocides and industrial chemicals risk assessment. There is a need for a more active strategic monitoring of the environmental fate and effects of those veterinary medicines that have the potential to cause harm to the environment. Most veterinary medicines, however, are likely to pose little risk to the environment because of the way they are used (e.g. in individual companion animals) or because of their intrinsic properties (e.g. low toxicity or environmental persistence).

International collaboration is fundamental to good pharmacovigilance. The European Medicines Agency (EMEA), through its veterinary scientific committee, the Committee for Medicinal Products for Veterinary Use (CVMP), is responsible for post-marketing surveillance of veterinary medicinal products in the EU that reach the market by authorisation through the centralised procedure. EU pharmacovigilance was strengthened in 2002 in Madrid when a workshop, organised by EMEA, the International Federation for Animal Health – Europe (IFAH-Europe) and the Federation of Veterinarians of Europe (FVE), held presentations and discussion meetings on all aspects of mutual interest relating to veterinary pharmacovigilance. The workshop

identified the need to improve awareness of the EU pharmacovigilance system and to improve communication between all stakeholders. There was a need to facilitate and increase reporting, improve data quality and ensure consistency and standardisation in the information and reports produced. One of the points to emerge from the Madrid Workshop was the importance of feedback, subject to issues of confidentiality, to reporters of SARs. A pharmacovigilance scheme is likely to be most successful if reporters receive information about the outcome of their reports. Following this workshop the various issues and conclusions were considered by the CVMP with the advice of its Pharmacovigilance Working Party (PhVWP-V) and proposals were agreed to promote veterinary pharmacovigilance across the EU. Further progress was achieved in 2006 when the European Surveillance Strategy (ESS) group for veterinary medical products of the Heads of Veterinary Medicines Agencies agreed a plan for better harmonisation in the regulation through pharmacovigilance between the regulatory authorities.

As the importance of pharmacovigilance became recognised prominence was given in the changing legislation. Directive 2004/28 EC of the European Parliament and of the European Council amends Directive 2001/82/EC on the Community code relating to veterinary medicinal products. The legislation now puts more emphasis on the safety of products, through pharmacovigilance, and the provisions now encourage prompt reporting of SARs. EMEA through the CVMP evaluates all products authorised through the centralised procedure. An MA granted under this procedure applies simultaneously to all EU member states. The number of SARs reported to EMEA in 2006 was approximately twice that received in 2005, possibly associated with a greater awareness of the need to report adverse events.

Sharing of information on adverse reactions is strongly encouraged and a central EU database has been established to allow for electronic reporting. This is now obligatory for all MA holders and regulatory authorities within the EU. EudraVigilance Veterinary is a central computer database created by the EMEA and contains adverse reaction reports to veterinary medicines authorised throughout the EU. These reports are received from the pharmaceutical companies and the EU Regulatory Authorities. The development of an electronic database in the EU for monitoring pharmacovigilance and adverse reactions is a new development and requires the input of accurate and quality data to enable the production of reliable and valuable information on the adverse reactions to all authorised veterinary medicines.

A list of clinical terms for reporting suspected adverse reactions in animals to veterinary medicinal products (VEDDRA), using codes, has been specifically developed by the CVMP and its Pharmacovigilance Working Party for the electronic reporting of adverse reactions in animals to veterinary medicines. Hopefully the harmonisation of data through EudraVigilance and increased transparency of information will benefit the MAH, the regulatory authorities and the public at large.

On the global front, the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) is a trilateral (EU, Japan, USA) programme aimed at harmonising technical requirements for veterinary product registration which was launched in 1996. One of the objectives of the VICH is:

> '. . . by means of a constructive dialogue between regulatory authorities and industry (to) provide technical guidance enabling response to significant emerging global issues and science that impact on regulatory requirements within the VICH regions'.

The VICH has a Steering Committee which drives the harmonisation process. This Committee has recently reviewed the guidelines VICH GL 24, 29 and 30 relating to pharmacovigilance of veterinary medicinal products, produced by its Expert Working Group. The VEDDRA terminology for animal and human adverse reaction reports has been agreed by the VICH

Pharmacovigilance Working Group as a suitable format for a clinical dictionary, allowing standardisation in the analysis of reports.

All stakeholders in veterinary medicines benefit from a harmonised approach to pharmacovigilance with a common system and standardised definitions and terminology. Many MAHs operate worldwide and a pooling of knowledge on a particular veterinary medicine and its use in the field will only enhance the safety profile.

Veterinary medicines have a valuable role to play in the health and welfare of animals and humans. This role is enhanced by the presence of a good pharmacovigilance surveillance programme, which allows accurate monitoring of all authorised veterinary medicines. When unexpectedly a serious risk to health and welfare arises, rapid recall or removal of a product from the market place is essential. In less serious cases, amendments to the SPC or modification to product labelling is sufficient to allow continued safe use. The success and benefit of any pharmacovigilance system, however, requires the constant vigilance and co-operation of all the stakeholders.

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Further reading

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Skilton, D., Collingborn, R., Crane, M., Levy, L.S. and Ray, D. (2004) *The Suspected Adverse Reaction Surveillance Scheme – A Report of a Working Group of the Veterinary Products Committee*. Available at http://www.vpc.gov.uk.

1 Elements of veterinary pharmacovigilance

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Pharmacovigilance in the human medicines sector is a well-established discipline. So well established in fact that reports of adverse reactions to medicinal products are relatively common in general and specialist medical journals either as case reports or as detailed epidemiological studies. There are numerous text books on the topic or on related areas such as pharmacoepidemiology and a number of dedicated journals such as *Drug Safety* and *Pharmacoepidemiology and Drug Safety*, while publications such as the *Journal of Clinical Epidemiology* also regularly cover the subject. So what exactly is pharmacovigilance?

Pharmacovigilance has been described as:

> '. . . a neologism created by the European Union to cover procedures involved in the detection of unwanted adverse effects causally related to the administration of therapeutic drugs' (Fletcher, 2000).

Regardless of whether or not the author intended a degree of cynicism or even sarcasm in this comment, it is quite a useful description, if not a definition. However, the term 'therapeutic drugs' is probably better replaced by medicinal products or, for the purposes of this book, veterinary medicinal products, as the discipline of pharmacovigilance covers the whole panoply of agents, including therapeutic and prophylactic drugs, vaccines and other immunological products and drugs used to alter physiological status such as those used to synchronise oestrus or promote growth in animals and drugs used as contraceptive agents in humans.

In fact *pharmacovigilance* is a relatively new term in the veterinary context for a well-established concept, namely the gathering of information on adverse reactions which may occur after the administration of medicinal products. Perhaps surprisingly, although the term is now widely used, there is very little by way of a formal definition. Even the Council for International Organisations of Medical Sciences' and the World Health Organisation's otherwise excellent document entitled *Reporting Adverse Drug Reactions*, which is subtitled *Definitions of Terms and Criteria for Their Use*, finds few places in its 146 pages to even mention the term *pharmacovigilance*, and none to define it (Bankowski *et al*., 1999).

The European Union's Directive 2001/82/EC (as amended) requires that:

> '. . . member states shall establish a veterinary pharmacovigilance system that shall be used to collect information useful in the surveillance of veterinary medicinal products, with

particular reference to adverse reactions in animals and human beings related to the use of veterinary medicinal products, and to evaluate such information scientifically'.

However, it fails to give a concise definition.

Yet all is not lost. The major aims of pharmacovigilance have been identified for human medicines (Stephens, 2000), and these can be readily adapted for veterinary medicines:

1. Identification and quantification of previously unrecognised adverse drug reactions.
2. Identification of subgroups of patients at particular risk of adverse drug reactions, e.g. relating to species, breed, age, gender, physiological status and underlying disease.
3. Continued monitoring of the safety of a product in each species for which it is authorised, to ensure that the risks and benefits remain acceptable. This should include extension of monitoring to new indications and new species.
4. Comparing the adverse reaction profile with those of products in the same therapeutic class, both within and across species.
5. Detection of inappropriate prescription and administration. With respect to the latter, administration by specific groups, e.g. farmers or the public, may need to be monitored.
6. Further investigation of a drug or product's toxicological, pharmacological or microbiological properties in order to understand, where possible, the mechanisms underlying adverse drug reactions.
7. Detection of drug–drug interactions. This is particularly important for new drugs that are then co-administered with established products or even other new drugs.
8. Provision of appropriate information on adverse drug reaction data and drug–drug interaction information to veterinarians and others involved in the treatment of animals, e.g. veterinary nurses, farmers and other animal owners.
9. Provision of information to discount so-called 'false positive' reports.
10. Provision of adverse drug reaction data from permitted off-label use, e.g. under the cascade permitted in EU veterinary legislation (this permits a veterinarian or someone under his or her supervision, with a number of restrictions, to prescribe a veterinary medicine authorised in another EU member state or, if unavailable, a medicine authorised for human use or, if unavailable, a medicine prepared extemporaneously, in those circumstances where there is no authorised veterinary product available for the condition in an animal or small number of animals).
11. Identification of adverse drug reactions in humans following inadvertent exposure, e.g. occupationally or otherwise (accidental exposure or suicide or homicide attempts).

To these, others can be usefully added, although to some extent these may depend on specific national or multinational legislative requirements:

12. Adverse effects of veterinary medicinal products on the environment and on organisms in the environment.
13. The violation of permitted residue limits of veterinary medicines in food of animal origin such as meat, milk and honey.
14. Legislation and guidelines governing the requirements of pharmacovigilance.
15. Methodologies for dealing with pharmacovigilance data (e.g. databases, electronic reporting and other reporting systems).

Taking all of these into account, and perhaps put more simply, pharmacovigilance may also be defined as the process of evaluating and improving the safety of marketed medicines (Waller *et al.*, 1996), while pharmacoepidemiology, one of the disciplines within pharmacovigilance and the application of the principles of epidemiology to drug safety, can be seen as the completion of the safety evaluation of a drug that was started before the product was authorised

(Bégaud and Dangoumau, 2000). It includes data collection, information flow, knowledge of relevant regulations, product data and the overall management of relevant information (Allan, 1992a–c). The process of safety evaluation and continued evaluation through pharmacovigilance is illustrated in *Figure 1.1*.

The events following the use of the drug thalidomide in humans where birth defects (phocomelia) occurred when pregnant women were treated with the drug exemplify not only the serious nature that adverse drug reactions can take, but also the essence of pharmacovigilance in the detection of such adverse events. Indeed, the thalidomide tragedy led to the establishment of the regulation of human and veterinary medicines in the UK with the introduction of the Medicines Act 1968. Similarly, a disaster in the USA where the solvent diethylene glycol, used in a medication known as Elixir of Sulphanilamide, caused the deaths of 73 people (and associations with a further 20) in 1937 was the engine behind the passing by Congress of the Food, Drug and Cosmetic Act in 1938 (Mann, 1993; Gad and Chengelis, 2001; Collins, 2004; Barr *et al*., 2007). Human medicine has since been marked by drug withdrawals and fatalities caused by medicines (Routledge, 1998; Buajordet *et al*., 2001; Preskhorn, 2002) and these contribute in a negative manner to both the economics and the standing of the industry (Khong and Singer, 2002; Lundquist and Jönsson, 2004).

It is evident that these early adverse drug reactions were underpinned by the toxicity of the chemicals involved. However, while this may be specific for adverse drug reactions where toxicity is the underlying cause, many adverse drug reactions are not related to toxicity. In fact this is particularly true with vaccines where the adverse reaction may be associated with a biological origin rather than a chemical origin, such as reversion to virulence leading to disease, or anaphylaxis arising from foreign proteins present in the products concerned. Overall, the term *pharmacovigilance* is perfectly adequate to describe the scientific study and follow-up of adverse drug reactions, whatever their underlying aetiologies, in humans and animals, including structured post-marketing surveillance activities. Indeed, there are now other, perhaps less-well recognised 'vigilance' disciplines associated with other areas of product safety including toxicovigilance (the study of adverse effects of chemicals in individuals and populations) (Belhadj-Tahar *et al*., 2003; Descotes, 2003; Keck *et al*., 2004; Descotes and Testud, 2005; Watson *et al*., 2005), cosmetovigilance (the corresponding study of cosmetics) (Tissier and Lepagnol, 2002; Di Giovanni *et al*., 2006), pharmacoenvironmentology (adverse effects of drugs on the environment) (Rahman *et al*., 2007) and, perhaps bizarrely, vaccinovigilance, the study of adverse effects following vaccination (Lankinen *et al*., 2004). Most observers would regard the latter and indeed pharmacoenvironmentology as simply parts of pharmacovigilance. The concept has even been suggested for the monitoring of food products (van Puijenbroek *et al*., 2007; Hepburn *et al*., 2008).

It should be recognised that there were drug disasters in human medicine prior to both the thalidomide and sulphanilamide episodes. Perhaps more importantly, these have continued to occur since the introduction of modern regulatory frameworks, thus emphasising the need for the continued refinement of pharmacovigilance systems. It is perhaps worth emphasising that the major difference between the pre-thalidomide era and now is that not only has pre-clinical (including toxicological) testing improved and the models used have become better defined, but also formal pharmacovigilance systems have been introduced. These have subsequently been honed and refined and so problems with the use of human medicines come to light more readily and are dealt with accordingly (D'Arcy, 1993, 2000). Now, at the beginning of the twenty-first century, several of these spontaneous adverse reaction reporting systems have been in place for many years. A good example of this is the UK's 'yellow-card' system. This card is completed by physicians when they note adverse events in patients under their care, and is returned to the UK's regulatory authority for human medicines, the Medicines and Healthcare Products

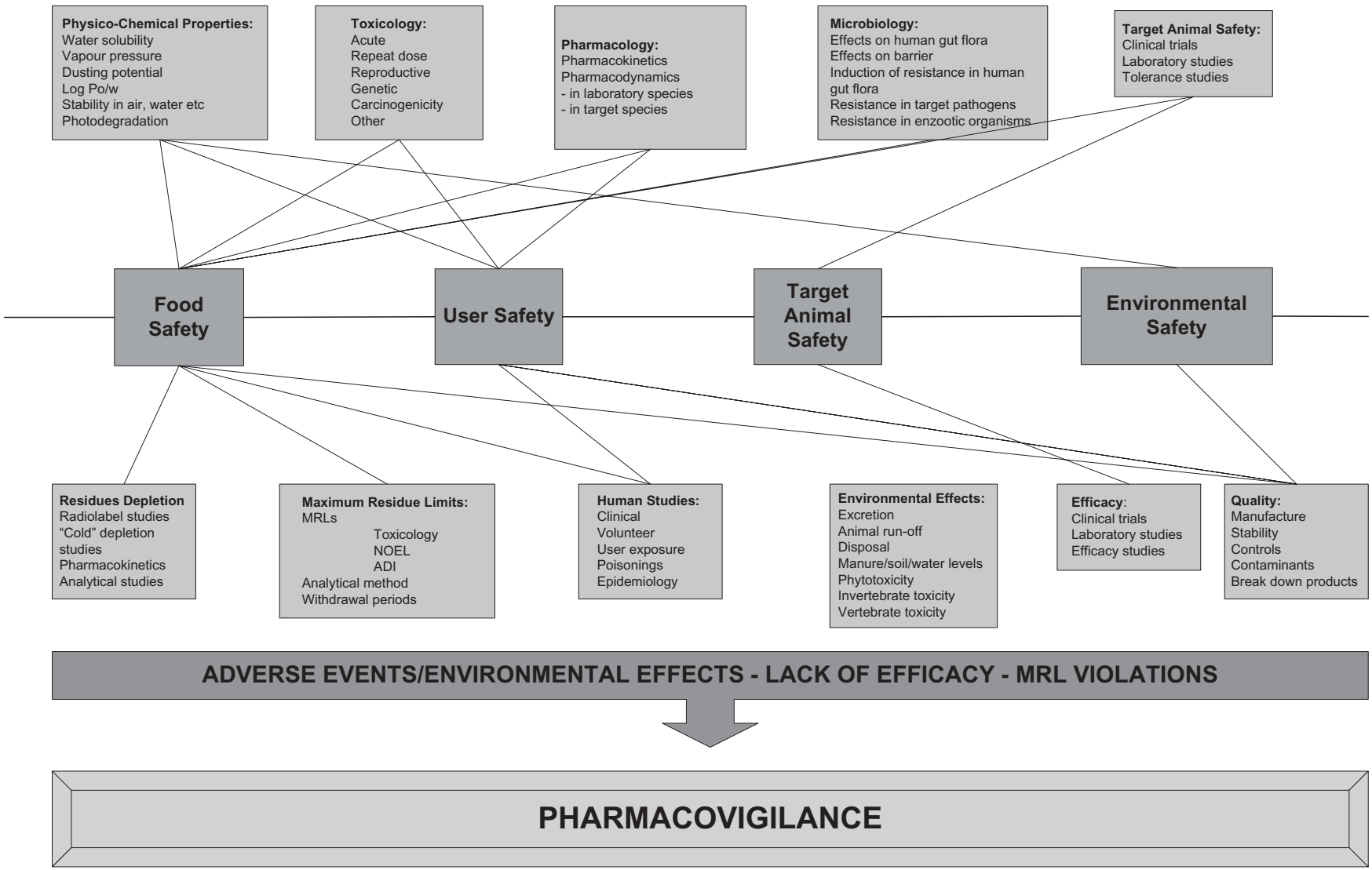

Fig. 1.1 Pharmacovigilance and the process of continuous assessment.

Regulatory Agency (MHRA, formerly the Medicines Control Agency – MCA).

Quite obviously, animals too are susceptible to the side effects of drugs. Indeed, some species may be particularly sensitive to the toxic effects of some specific drugs (and other chemicals). For example, the cat has a very low capacity to conjugate paracetamol (acetaminophen) because of its low glucuronyl transferase activity. Hence, cats are extremely sensitive to the toxic effects of paracetamol, and what is a therapeutic dose in other species may prove to be a lethal dose in the cat (Campbell and Chapman, 2000: 89–96). Similarly, dogs appear to be more sensitive to the effects of non-steroidal anti-inflammatory drugs (NSAIDs) on the gastro-intestinal tract than do other species (Campbell and Chapman, 2000: 31–38, 152–162).

However, many adverse events in animals are subtler than might be inferred from these examples. Rather than highlighting species that might be less tolerant to a particular substance or formulation, they are more likely to be seen as events in intolerant or less tolerant individuals or subpopulations of individuals, in a species that otherwise tolerates the product well. Due to these concerns, veterinary regulatory authorities around the world have introduced their own spontaneous reporting schemes. For some years, the UK scheme has served as an example and model system for regulatory authorities in other countries to adapt and adopt to fit their own requirements. Indeed, it was in existence and operating effectively long before many other countries had anything in place at all, and it has been reporting its findings since 1987. It will be used here, along with other examples, to exemplify many of the positive requirements of a pharmacovigilance scheme, as well as some of the more negative points, common to all.

The purpose of this book is to help place veterinary pharmacovigilance firmly on the scientific map. In doing so it will examine pharmacovigilance regulatory requirements and systems from across the world, as well as consider examples of adverse effects of veterinary medicinal products on animals, on exposed humans and on the environment. This latter aspect is of growing importance. There is now substantial evidence that human pharmaceuticals are entering the environment to an increasing degree and these are being found in sewage, river water and sediments (Hignite and Azarnoff, 1977; Christensen, 1998; Halling-Sørensen *et al*., 1998, 2000; Zuccato *et al*., 2000; Daughton, 2001; Castiglioni *et al*., 2004; Carlsson *et al*., 2006a, b; Hao *et al*., 2006; Liebig *et al*., 2006; Rivett *et al*., 2006; Williams and Cook, 2007). At high enough concentrations, some of these substances have the potential to exert harmful effects on the environment and the organisms in it (Beasley and Schaeffer, 1989; Halling-Sørensen *et al*., 2000; Glassmeyer and Shoemaker, 2005; Wolf and Wolfe, 2005; Yoshimura and Endoh, 2005; Robinson *et al*., 2005; Fent *et al*., 2006; Sumpter, 2007) and this may be exacerbated by mixtures of chemicals (Cleuvers, 2004; Eggen *et al*., 2004). Some may have the potential to harm human health, even at the low levels found in the environment (Henschel *et al*., 1997; Christensen, 1998; Sharpe, 2000; Pawlowski *et al*., 2003; Anonymous, 2004). This has led to the tighter regulation of human pharmaceutical products in a number of countries from the point of view of environmental effects (Calow, 1998; Stuer-Lauridsen *et al*., 2000; Länge and Dietrich, 2002; Straub, 2002; Mattson, 2007; Mattson *et al*., 2007; Montforts *et al*., 2007; Spindler *et al*., 2007; Webber and Spindler, 2007; Yoshioka, 2007; Adler *et al*., 2008) and the development of regulatory guidelines (O'Brien and Dietrich, 2004; Shaw and Barrett, 2004).

Veterinary medicines, including vaccines derived from biotechnology, also have the capacity to enter the environment and these too are subject to regulation, risk assessment and guidelines as they have the capacity to affect environmental and human health (Pastoret *et al*., 1995; Chung *et al*., 1999; Koschorreck *et al*., 2002; Longand Crane, 2003; Montforts *et al*., 2004; Woodward, 2005; Boxall *et al*., 2006; Sarmah *et al*., 2006; Robinson, 2007). The recent withdrawal of cypermethrin-based sheep dips in the UK because of environmental contamination and associated

adverse environmental effects serves as an example of what might happen – both from a scientific and regulatory viewpoint, if this area of veterinary pharmacovigilance is transgressed (Anonymous, 2006). This is an increasingly important area of veterinary pharmacovigilance and, consequently, one that is dealt with in this book. Indeed, the issue of pharmaceuticals in the environment and their potential effects on humans and other organisms has led to the coining of the terms environmental pharmacology or ecopharmacology (Kümmerer and Velo, 2006; Rahman and Khan, 2006).

Hopefully therefore it will serve as an invaluable tool to those working in clinical veterinary medicine, toxicology, occupational health, the environmental sciences and regulatory areas. The teaching of pharmacovigilance to cover human medicines is in its infancy (Evans, 2007; May, 2007). It is hoped that this book may help to drive educational initiatives for veterinary pharmacovigilance.

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2 Veterinary pharmacovigilance in the European Union

K.N. Woodward

Regulation of veterinary medicines in Europe

Regulation is very often preceded by disaster (Illing, 1999, 2001) and the thalidomide episode, the events surrounding it and to some extent previous reactions (e.g. the Elixir of Sulphanilamide disaster and the adverse events associated with phenacetin use in humans) led directly to the regulation of human and veterinary medicines in many countries (D'Arcy, 2000). The historical background in the UK typifies the introduction of many regulatory schemes, although there had previously been attempts to control 'drugs' as early as the reign of Henry VIII, while the Food and Drugs Act of 1925 placed a degree of control over the quality of medicinal products (Cuthbert *et al*., 1978; Harrison, 1986a).

Following on from the events surrounding thalidomide, the Committee on Safety of Drugs, usually referred to as the Dunlop Committee after its Chairman, Sir Derek Dunlop, was established in the UK. This Committee had no regulatory powers, but it worked with the pharmaceutical industry in a voluntary manner. Veterinary drugs, like their counterparts in human medicine, had also been controlled in the UK by a voluntary scheme. Later, more legislative measures were examined and considered, and this ultimately resulted in the passing by Parliament of the Medicines Act 1968. Until relatively recently, this formed the basis for the regulation of both veterinary and human medicinal products in the UK (Cuthbert *et al*., 1978). Expert advice on veterinary medicines is provided largely by the Veterinary Products Committee (VPC), the counterpart of the perhaps better known Committee on Safety of Medicines (CSM) for human medicinal products. Both committees were established under Section 4 of the Medicines Act and both committees are made up of independent members, largely drawn from academia and research centres and they provide a source of unbiased advice for government ministers who together form the Licensing Authority (Brinley Morgan, 1983; Harrison, 1986b; Woodward, 1993).

Applications for marketing authorisations for veterinary medicinal products are dealt with by the Veterinary Medicines Directorate (VMD) in the UK. This agency deals with all types of veterinary medicines including pharmaceuticals, ectoparasiticides and biological products. The VMD now operates under the auspices of the Department for Environment, Food and Rural Affairs (DEFRA) (Woodward, 1991, 1993, 2000). While regulatory systems in other EU countries

Table 2.1 European Union countries in 2008.

Country	*Symbol*	*Accession date*	*Population (million)*	*Number of MEPs*
Belgium	BE	1958	10.6	24
France	FR	1958	63.4	78
Germany*	DE	1958	82.3	99
Italy	IT	1958	59.1	78
Luxembourg	LU	1958	0.5	6
Netherlands	NL	1958	16.3	27
Denmark	DK	1973	5.5	14
Ireland	EI	1973	4.2	13
United Kingdom	UK	1973	60.6	78
Greece	EL	1981	11.1	24
Spain	ES	1986	45.1	54
Portugal	PT	1986	10.6	24
Austria	AT	1995	8.3	18
Finland	FI	1995	5.3	14
Sweden	SE	1995	9.1	19
Cyprus	CY	2004	0.8	6
Czech Republic	CZ	2004	10.3	24
Estonia	EE	2004	1.3	6
Hungary	HU	2004	10.1	24
Latvia	LV	2004	2.3	9
Lithuania	LI	2004	3.4	13
Malta	MT	2004	0.4	5
Poland	PO	2004	38.1	54
Slovakia	SK	2004	5.4	14
Slovenia	SI	2004	2.0	7
Bulgaria	BG	2007	7.7	18
Romania	RO	2007	21.6	35

*Originally acceded as West Germany but 'Germany' now covers the former East and West Germany countries following reunification.

differ in detail from those that operate in the UK, they have many similarities, and many employ the expert committee approach.

The European Union is an association of European countries which constitute the member states. It began as the European Economic Community in 1958 with six member states (Belgium, France, (West) Germany, Italy, Luxembourg and The Netherlands) and has expanded since, culminating in the accession of Romania and Bulgaria in January 2007 (*Table 2.1*). The 27 EU countries are joined by three of the four European Free Trade Area countries (Iceland, Norway and Liechtenstein but excluding Switzerland) to make up the European Economic Area (EEA) block. The EEA countries share the 'four freedoms' enjoyed by the EU members – free movement of goods, free movement of persons, free movement of services and free movement of capital. Much of EU legislation, including many aspects of EU pharmaceutical law, is aimed at promoting these four freedoms, especially by removing barriers to trade, frequently through harmonisation of requirements and standards. A good example of this is the establishment of common Maximum Residue Limits (MRLs; see Chapter 23) which, in addition to conferring elements of consumer safety, also serve to remove barriers to trade in food animal produce within the EU and EEA.

Iceland, Norway and Liechtenstein participate in many of the EU's procedures, including the mutual recognition, decentralised and centralised

procedures, but are not members of the EU and have no Members of the European Parliament (MEPs) at Brussels or Strasbourg (*Table 2.1*).

In 2008, there are currently 27 EU countries, but candidate countries for future accession include Turkey, Croatia and the former Yugoslav Republic of Macedonia. These countries are subject to EU-wide treaties and share many laws and procedures, including those that apply to veterinary and human medicinal products.

The European framework for the regulation of veterinary medicinal products, including the application of pharmacovigilance requirements, can be viewed in three distinct phases: prior to 1995 when national procedures predominated, 1995–2004 when the EU's new procedures became effective, and post-2004 following the revision of EU pharmaceuticals legislation (Woodward, 2005a). This is illustrated in *Figure 2.1*.

The European situation prior to 1995

Directive 65/65/EEC of 1965 was the first of the European pharmaceutical directives and it formed the basis of subsequent directives and regulations which governed the authorisation of both veterinary and human medicinal products in the EU (Sauer and Hankin, 1987; Cartwright, 1991a). The two major directives that formed the backbone of the European legislation on veterinary medicines were Directives 81/851/EEC and 81/852/EEC. The former established the basic regulatory framework for veterinary medicines in the EU while the latter set out the testing requirements to ensure safety, quality and efficacy – the three criteria on which human and veterinary medicines are universally assessed. Examples of aspects of each of these are given in *Table 2.2*.

Table 2.2 Examples of the major elements of quality, efficacy and safety (including residues).

Quality	Manufacturing methods and dosage form
	Analysis
	Composition
	Control of starting materials
	Control of finished product
	Stability/shelf life
	Containers, cartons and packaging
	Labelling and product literature
	Quality relating to safety (toxic contaminants, toxic degradation products, microbiological contaminants)
	Sterility (where appropriate)
Efficacy	Pharmacodynamics
	Pharmacokinetics
	Laboratory studies, e.g. in vitro effects on pathogens
	Laboratory trials of efficacy
	Clinical field trials
Safety	Consumer safety*
	Operator safety** (to veterinarians, farmers, pet owners, others)
	Environmental safety†
	Target animal (patient) safety
	Residues
	Pharmacokinetics
	Residues depletion (radiolabelled and conventional studies)
	Analytical methods for residues determination and surveillance

*Largely toxicology data.

**Largely toxicology and operator exposure data.

†Environmental toxicology, exposure and persistence/degradation data.

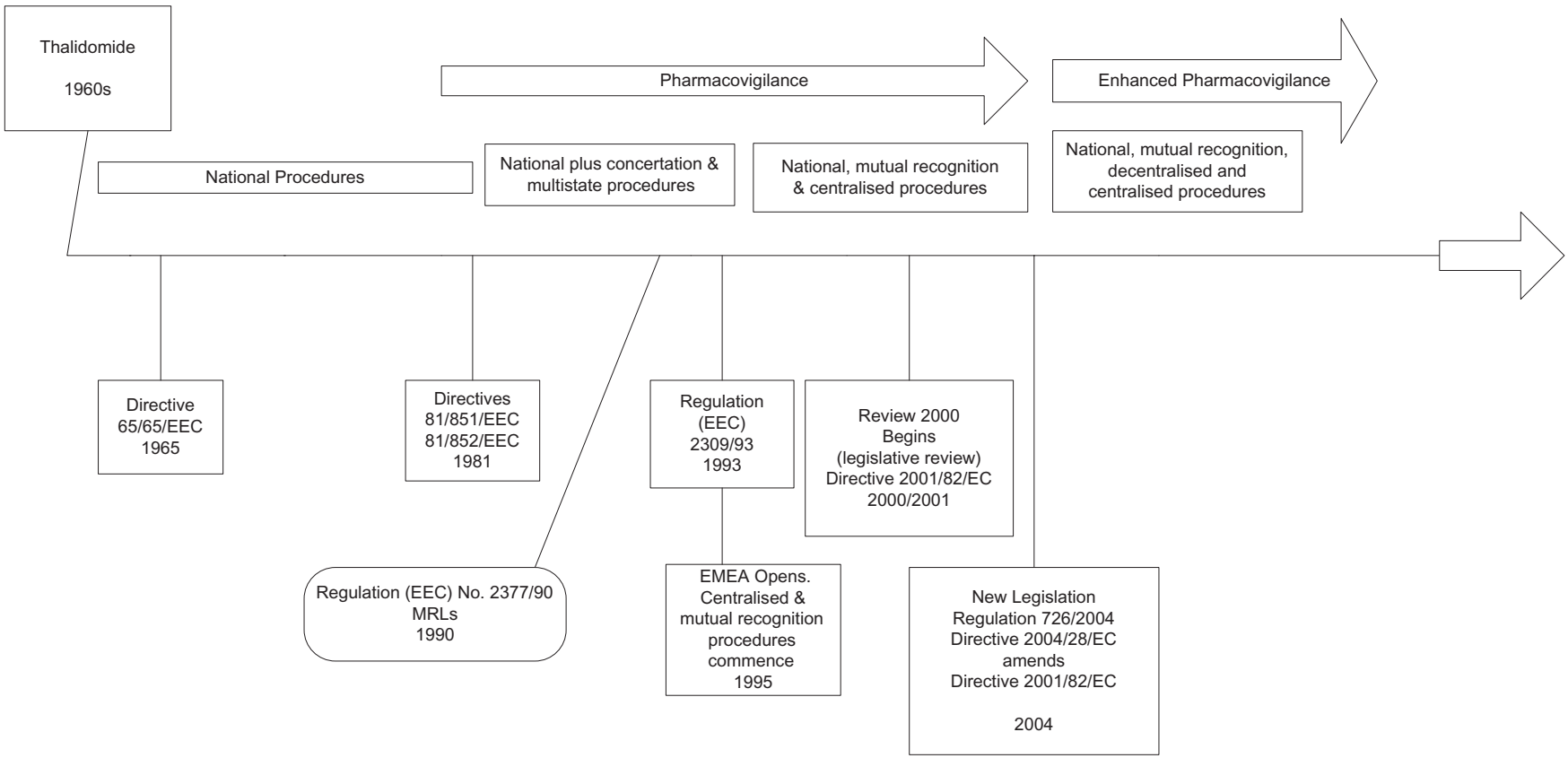

Fig. 2.1 Development of European medicines legislation, 1960–2007.

Importantly, Directive 81/851/EEC also created provision for the main European advisory committee on veterinary medicines, the Committee for Veterinary Medicinal Products (CVMP), which was formed in 1983. The legislative provisions of Directives 81/851/EEC and 81/852/EEC were subsequently transposed into the legal frameworks of the member states, and in the UK this meant legislation in the form of Statutory Instruments under the Medicines Act.

For the most part, applications continued to be considered and authorisations were granted in the member states as purely national authorisations, but in accordance with the requirements of the directives. However, two European Community procedures were also available. One of these, the so-called concertation procedure, was introduced by Directive 87/22/EEC. This procedure was compulsory for products regarded as high-technology products, such as those derived from recombinant DNA technology or from methods involving hybridoma or monoclonal antibody techniques. It was optional for other products including products containing substances new to veterinary medicine in Europe. Concertation procedure applications were considered by the CVMP meeting in Brussels, under the auspices of Directorate General (DG) III, now DG Enterprise, of the European Commission. What emerged was an opinion of the CVMP which could include a recommendation that the product should be authorised. However, this opinion was not binding on member states and they could, if they so wished, ignore it in part or even ignore it entirely (Cartwright, 1991b).

The other procedure was the so-called multi-state procedure which was based on a provision in Directive 81/851/EEC as amended by Directive 90/676/EEC. Here a marketing authorisation was first obtained from one of the member states in accordance with national procedures. The holder of the authorisation could then apply to at least two other member states using the dossier approved by the first as the basis for the subsequent applications. It was then up to those subsequent member states to grant the authorisations or to give reasoned objections as to why they would not. Under the latter circumstances, the matter was referred to the CVMP for an opinion. Again this opinion was not binding (Cartwright, 1991c).

The lack of binding decisions meant that European member states were able to interpret the outcome of the multi-state and concertation procedures as they saw fit. It was probably this absence of binding opinions, coupled with difficult regulatory experiences endured by those companies who made applications, which resulted in a relatively poor uptake of both procedures by the veterinary pharmaceutical industry. Certainly, the human pharmaceutical industry made greater use of these under the corresponding provisions governing human pharmaceutical products (Jefferys, 1995).

A third procedure, which for reasons that will become obvious has no counterpart for human medicines, was the introduction of a Council Regulation governing MRLs. This subject is discussed in more detail in Chapter 23. Council Regulation (EEC) No. 2377/90 was introduced on 26 June 1990 and it brought with it European Community requirements for the establishment of MRLs for veterinary drugs used in food-producing animals.

The new procedures – 1995 to 2004

Council Regulation (EEC) No. 2309/93 of 1993 introduced a number of the fundamental changes affecting both veterinary and human medicines regulation in the EU. This Regulation introduced radically new procedures for the authorisation of medicinal products and established the European Medicines Evaluation Agency (EMEA) (Jefferys, 1995; MacFarlane *et al*., 2007).

The EMEA began operations in January 1995 as an agency of the European Commission. In doing so, it took over the responsibility for the assessment marketing authorisation applications for both veterinary and human medicinal products under the Centralised Procedure (see later)

and for the evaluation of MRL applications. As a consequence, the CVMP and its counterpart for human drugs, the Committee for Proprietary Medicinal Products (CPMP), and all of their working parties, including the one that deals with safety, residues and MRLs, now began to meet at the EMEA which is based in Canary Wharf in London's Docklands area.

The role and composition of the CVMP (and CPMP) also changed. Previously, the committee consisted of two representatives from each member state who represented their national authorities. As a direct result of the Regulation mentioned above, and the establishment of the EMEA in London, members were subsequently appointed as experts from each country who, although they may still be chosen from national authorities, nevertheless served as individual experts in their own right. The Committee continued to provide advice on scientific, policy and legislative issues; one of its main functions being to adopt opinions, for example on marketing authorisation applications and MRLs, which subsequently become Decisions of the European Commission (Woodward, 1997).

The centralised procedure

This evolved from the old concertation procedure and is allowed for under terms and conditions originally set out in Regulation No. 2309/93 (but now in Regulation (EC) No. 726/2004). Unlike the concertation procedure, the outcome is binding on member states (Jefferys, 1995). In fact, it goes beyond the scope of the old concertation procedure as the resulting outcome of the centralised procedure is an EU-EAA-wide marketing authorisation issued by the European Commission on the basis of a positive CVMP opinion.

The assessment of centralised applications for veterinary medicinal products is dealt with using the rapporteur and co-rapporteur system within the CVMP. The rapporteur and the co-rapporteur can appoint assessment teams from a list of 'European experts' accredited to the EMEA. These experts may consider the general areas of safety, quality and efficacy or they may examine more detailed aspects of these issues such as residues depletion, analytical methods or ecotoxicity. The applications had to be in accordance with the general requirements of Articles 5, 5a and 7 of Directive 81/851/EEC and be accompanied by supporting data on safety, quality and efficacy set out in Directive 81/852/EEC as amended by Directive 92/18/EEC. As with the MRL procedure, an opinion is given by the CVMP and the decision is adopted into EU law through the Regulatory Committee procedure. The marketing authorisation itself is issued by the European Commission.

Products that are intended for authorisation through the centralised procedure must have MRLs (as do those that are considered under the national, mutual recognition and, more recently, decentralised procedures; see later) if they are intended for use in food-producing animals. Each procedure has its own separate legal basis, but, nevertheless, the EMEA has recommended that the MRL be applied for prior to the submission of the marketing authorisation.

The scope of the centralised procedure was originally detailed in the Annex to Regulation (EEC) No. 2309/93. Products that fell into Part A of the Annex had to follow this route; there was no choice. These included products derived from recombinant DNA technology, for the controlled expression of genes in prokaryotes and eukaryotes, and from hybridoma and monoclonal antibody methods. In addition, for veterinary medicines, products intended to promote growth, or to enhance yield (for example, of milk), had to follow the centralised route.

The centralised route was optional for products covered by Part B of the Annex. For veterinary medicinal products these options included:

- products developed from biotechnology that, in the opinion of the EMEA, constitute a significant innovation;

- products administered by means of a new delivery system that, in the opinion of the EMEA, constitute a significant innovation;
- products presented for an entirely new indication that, in the opinion of the EMEA, is of significant therapeutic interest;
- products, the manufacture of which employs processes that, in the opinion of the EMEA, demonstrate a significant advance such as two-dimensional electrophoresis under microgravity;
- products intended for use in food-producing animals containing a new active substance that, on the date of entry into force of the regulation, was not authorised by any member state for use in food-producing animals.

However, these categories have changed subtly following the recent review of the EU legislation and the publication of Regulation (EC) No. 726/2004 (see later).

The benefits of the centralised procedure are clear. Applicants can pay a single fee to a single agency and obtain an authorisation for a product in all EU member states. The disadvantage is the not inconsiderable fee that currently applies, but this can be offset against the total sum of each of the national fees in individual EU countries. In addition, there is the advantage of discussions with only one set of officials rather than with numerous national authorities, and a single set of queries and questions. Experience of animal health and human pharmaceutical companies over more than 10 years suggests that this is a useful and successful method for obtaining EU-wide marketing authorisations.

The mutual recognition procedure

The mutual recognition procedure (or decentralised procedure as it was often confusingly known) was originally allowed for under Article 17 of Directive 81/851/EEC (as amended). Under this procedure, the applicant obtains initial authorisation in one EU member state, the so-called Reference Member State (RMS), through the national procedure in that State, and then requests mutual recognition of this authorisation in the other member states of interest to the applicant – the Concerned Member States (CMS).

The mutual recognition procedure replaced the old multi-state procedure and, unlike the latter, the decision is binding on member states. It enables an applicant to obtain a marketing authorisation in more than one member state without, in theory at least, the complexities of multiple applications at the national level. The procedure can also be initiated by a member state where parallel multiple applications are made by the applicant to several member states. If a member state is informed by the applicant of such multiple applications, that member state may choose to suspend its own procedures and recognise the authorisation granted by another member state.

Alternatively, and the usual approach, the applicant may initiate the procedure and ask one or more CMS to recognise an authorisation granted in the RMS. Under these circumstances, the assessment report produced by the RMS must be updated (on the basis of data supplied by the applicant) and this must be supplied to each CMS. On receipt of the assessment report, the CMS should then mutually recognise the application granted in the RMS. In practice, the applications tend to be re-evaluated in each individual CMS, often resulting in substantial numbers of additional questions and comments for the applicant to consider prior to individual national marketing authorisations being granted.

It is important to recognise that the EMEA and the CVMP are not usually concerned with this procedure. It is largely the province of the EU member states and the authorisations issued are individual national authorisations. An exception arises when member states cannot agree on aspects of safety, quality and efficacy, and mutual recognition cannot therefore be achieved. In these circumstances, the applicant has the option to withdraw the application in the country or countries that have raised seemingly insurmountable

Table 2.3 Basis of veterinary legislation in the European Union.

National authorisations	Directive 2001/82/EC, as amended by Directive 2004/28/EC. National authorisations are limited to one EU member state; if more than one member state is required, the mutual recognition procedure or the centralised procedure must be used. Authorisations are issued by the national authority involved
Mutual recognition and decentralised procedures	Directive 2001/82/EC, as amended by Directive 2004/28/EC. Authorisations are issued by each national authority
Centralised procedure	Authorisations are issued under Regulation (EC) No. 726/2004, subject to the requirements set out in Directive 2001/82/EC as amended by Directive 2004/28/EC. Applications are considered by the EMEA/CVMP. Authorisations are issued by the European Commission based on positive opinions from the CVMP. Compulsory for certain types of product such as those intended for yield enhancement in food-producing animals
Maximum residue limits (MRLs)	Regulation (EEC) No. 2377/90. Establishes MRLs based on toxicology, pharmacology, microbiology and residues depletion data for 'pharmacologically active ingredients' used in veterinary medicinal products intended for use in food-producing animals. This regulation is currently subject to review

objections or the application will be referred through the EMEA and the opinion of the CVMP will be sought. This is known as referral (or arbitration) for obvious reasons. The outcome of this referral is binding on member states and it can affect the application even in the RMS or other member states where the authorisation has already been granted. At its worst outcome (for the applicant), if the arbitration decision was that the product should not be authorised, then not only would the CMS not grant the application, but also the RMS would need to revoke the existing authorisation. However, to date, referrals have resulted in CVMP opinions that were largely in line with the decisions of the RMS and the majority of CMS. It is also important to recognise that for products intended for use in food-producing animals, EU MRLs must be in force before mutual recognition can begin.

Until the end of 1997, the mutual recognition procedure was optional for all applications for which the centralised procedure is not compulsory. However, from 1 January 1998 the procedure became mandatory for applications for marketing authorisations made in more than one EU member state.

Over the period 2000 to 2004 the veterinary and human pharmaceutical legal texts were subject to substantial review and revision as described later. As Directives 81/851 and 81/852 had been amended several times over the period 1985 to 2000, the European Commission published what were known as the codified text for veterinary legislation for the purposes of facilitating this review. A similar exercise was undertaken for the human medicines legislation. For veterinary legislation, this meant that all the existing provisions of the two major Directives, and all of the amendments in force at the time, were drawn into one Directive, Directive 2001/82 EC, and all the previous Directives were repealed. In March 2004 this review process was completed when Directive 2004/28/EC amended Directive 2001/82/EC and Regulation (EC) No. 2309/93 was repealed and replaced by Regulation (EC) No. 726/2004. Hence, the current regulatory framework can be summarised as shown in *Table 2.3*. Similar changes were made to the human pharmaceutical legislation (Lisman and Schoonderbeek, 2005; MacFarlane *et al*., 2007).

Directive 2004/28/EC introduced a degree of complication for, in addition to the existing

mutual recognition procedure, it introduced a new system – the decentralised procedure. This is an application route with many similarities to the mutual recognition procedure, but it involves simultaneous submission of an application to several or all member states, with one being identified as the reference member state. It is a method of application that has several advantages over the mutual recognition system, and it is gaining popularity, at least with human products (Fisher and Woods, 2008). However, it can only be used for new products. If a marketing authorisation already exists for a product, then the mutual recognition process must be used to obtain further authorisations in other EU countries.

Maximum Residue Limits (MRLs)

No new marketing authorisation may be granted in the EU for a product intended for use in food animals until MRLs have been established for its 'pharmacologically active' ingredients. Existing products were also subject to review under this legislation. These actions have come about because of the effects of Council Regulation No. (EEC) 2377/90 mentioned earlier. This legislation set out to ensure that the safety of pharmacologically active ingredients used in veterinary medicines intended for use in food-producing animals was adequately reviewed and that consumers of food of animal origin were thus offered adequate protection from any potential harmful effects.

The scope of Council Regulation No. (EEC) 2377/90 is indeed more far reaching than at first it might appear. There are four annexes to the Regulation, as set out below:

Annex I: full MRLs
Annex II: no MRLs required
Annex III: provisional MRLs
Annex IV: no MRLs possible on consumer safety grounds.

MRLs in Annex I are full MRLs for which no further action is required. Annex III on the other hand is for provisional MRLs where further information is considered necessary by the CVMP. They can be established for periods of up to 5 years and extended for a further 2-year period. In general the expiry date is established on the basis of the time period that the CVMP considers is necessary for the applicant to complete the work. Substances in Annex II are those for which it is considered that there is no undue risk to human health – because the substance is of very low toxicity, or it is used in small numbers of animals, or it is rapidly metabolised, detoxified and excreted, or a combination of these factors. Annex IV is the destiny of those substances that are considered to pose a risk to consumer safety or where there is insufficient data to assuage specific safety concerns. Substances in Annex IV may not be used in veterinary medicinal products intended for use in food-producing animals.

The annex entries are published in the *Official Journal of the European Union* (*OJ*) as amending Commission Regulations to the original Council Regulation, and several such amendments have been published. This topic is addressed in more detail in Chapter 23.

Pharmacovigilance

The legal basis for pharmacovigilance for veterinary medicinal products in the European Union was originally established by Directive 81/851/EEC, Article 42, as amended by Directive 93/40/EEC and Commission Regulation (EC) No. 540/95. Additional requirements were imposed by Council Regulation (EEC) 2309/93 (Boisseau, 1994). However, Directive 2001/82/EC as amended by Directive 2004/28/EC and Regulation (EC) No. 726/2004 now form the legal framework. Nevertheless, to understand the development of recent requirements and accompanying guidance, it is important to recognise the impact of Directive 2001/82/EC prior to its recent amendment and that of Regulation (EEC) No. 2309/93 prior to being repealed by Regulation (EC) No. 726/2004.

Each of the three authorisation procedures then available – national, mutual recognition and

centralised – places responsibilities for the gathering of pharmacovigilance data on different entities. The national procedure relies entirely on the national authority in each member state. This is similar to that for the mutual recognition system whereby the national authority in each EU member state where there is a marketing authorisation is responsible for the collection of data. This is not surprising since each marketing authorisation, despite the use of the mutual recognition system to obtain it, is a national authorisation issued by each EU national authority. However, it is the responsibility of the reference member state, i.e. the one where the product was initially authorised and which then took the lead role in the mutual recognition process, to take overall responsibility for pharmacovigilance. With the centralised procedure, the rapporteur and co-rapporteur, who initially had the responsibility for guiding the product through the various stages of CVMP debate, take some responsibility, although the national authorities collect and collate the data. However, if the pharmacovigilance data mean that changes are needed to the terms of the CVMP's original opinion and the Commission's subsequent decision, then, and following a further CVMP opinion, the European Commission and its procedures must be involved (Wood, 1998a, b; Woodward, 2005a).

The legal texts are supported by a number of guidelines, the most important of which is Volume 9 in the *Rules Governing Medicinal Products in the European Community* (European Commission, 2001a). This describes in detail the requirements for adverse drug reaction reporting for both veterinary and human medicines. However, there are a number of other guidelines produced by the CVMP, including some developed through the VICH initiative (see later) and released through the EMEA, and these are listed in *Table 2.4*. These guidelines provide invaluable advice to industry (Clayton, 2006) and to others involved in the regulatory process. Volume 9 is the subject of extensive revision and only a draft version is currently available (EMEA/CVMP/PhVWP/430286/2007 – draft 13 as Volume 9B) (Volume 9A covers human pharmacovigilance). This draft was subject to external discussion with interested parties in June 2008. It was extensively revised and, as draft 14, sent for further consultation in July 2008. Publication (on the Commission's website) is expected in early 2009.

This draft version does, however, provide some useful insight into its final form. It is divided into four parts, covering general guidance for marketing authorisation holders, guidelines for competent authorities and the EMEA, guidance on electronic reporting and guidance on communication of pharmacovigilance with health care professionals. It is extremely detailed and in some parts quite prescriptive, although many of the comments made during the consultation on draft 13 have been taken into account in draft 14.

Requirements of Directive 2001/82/EC

The Directive sets out the essential requirements for pharmacovigilance for all veterinary medicinal products authorised in the EU. In addition, it specifies the responsibilities of various parties involved in pharmacovigilance activities. However, it begins (Article 1) by providing some definitions. More details on these definitions and on the content of the Directive are provided in Volume 9 and some of the guidelines mentioned in *Table 2.4*. The definitions are:

- *Adverse reaction*: a reaction that is harmful and unintended and which occurs at doses normally used in animals for the prophylaxis, diagnosis or treatment of disease or the modification of physiological function.
- *Human adverse reaction*: a reaction that is noxious and unintended which occurs in a human being following exposure to a veterinary medicinal product.
- *Serious adverse reaction*: an adverse reaction that results in death, is life-threatening, results in significant disability or incapacity, is a congenital anomaly/birth defect, or that results in permanent or prolonged signs in the animals treated.

Table 2.4 Major EU Guidelines and EMEA SOPs and other publications relating to pharmacovigilance.

Guideline	*Purpose*
Current Guidelines	
Volume 9B of the Rules Governing Medicinal Products in the European Union – Guidelines on Pharmacovigilance for Medicinal Products for Veterinary Use	Provides general guidance for competent authorities and the EMEA, marketing authorisation holders, information on electronic exchange of data and communication with health care professionals
EMEA/CVMP/183/96-FINAL-Rev. 1 Pharmacovigilance of veterinary medicinal products	Provides general guidance on pharmacovigilance for veterinary medicinal products
EMEA/CVMP/345/98-Rev. 1-FINAL Procedure for competent authorities for pharmacovigilance information of veterinary medicinal products	Describes procedures for regulatory authorities to follow in complying with and implementing EU pharmacovigilance requirements. Also largely superseded by Volume 9
EMEA/CVMP/413/99-FINAL-Rev. 5 CVMP VEDDRA list of clinical terms for reporting suspected adverse reactions in animals to veterinary medicinal products	Sets out in detail preferred terms for use in pharmacovigilance reporting, including system organ class and preferred medical terminology, for adverse reactions in animals
EMEA/CVMP/605/00-FINAL Position paper on PSURs for centrally authorised veterinary medicinal products	Sets out the EMEA/CVMP view on issues relating to adverse reaction reporting for veterinary medicinal products authorised through the centralised procedure
EMEA/CVMP/227/01-FINAL PSURs for centrally authorised veterinary medicinal products: procedure on PSUR submission and evaluation for non-marketed products	Provides specific guidance on procedures for PSUR drafting and submission for products authorised through the centralised procedure but which are not marketed
EMEA/CVMP/601/02-FINAL Points to consider regarding reporting of suspected serious adverse reactions to veterinary medicinal products. Common EU reporting form for marketing authorisation holders	Provides specific guidance on reporting serious adverse reactions and, as the title suggests, provides a common reporting form for use throughout the EU
EMEA/CVMP/552/03-FINAL Causality assessment for adverse reactions to veterinary medicinal products	Provides advice and guidance on ascribing causality in accordance with the ABON classification system. Non-algorithmic
EMEA/CVMP/900/03-FINAL Guideline on a strategy for triggering investigations preceding regulatory actions by EU competent authorities	Provides guidance to European regulatory authorities on a harmonised approach to regulatory action resulting from pharmacovigilance findings
EMEA/CVMP/891/04-Rev. 3 List of clinical terms for reporting suspected adverse reactions in human beings to veterinary medicinal products	As the name suggests, sets out lists of preferred terms, etc. for suspected adverse reactions in humans following exposure to veterinary medicinal products, in parallel with EMEA/CVMP/413/99-FINAL-Rev. 5
EMEA/CVMP/280/04 Guideline on EudraVigilance Veterinary – XML Schema Definition (XSD) Version 2.0.0 and Veterinary Acknowledgment XSD Version 1.0.0	Guidance on standards to exchange safety reports on adverse reactions

Table 2.4 *Continued*

Guideline	*Purpose*
EMEA/CVMP/159/04 Crisis management plan regarding safety issues for centrally authorised products for veterinary use	Sets out provisions for EMEA actions arising from safety issues for products authorised under the centralised procedure
EMEA/CVMP/PhVWP/110607/2005 Veterinary pharmacovigilance in the EU – a simple guide to reporting adverse reactions	Developed from consultation – see below for details – what should be reported, how and significance of reporting
EMEA/CVMP/557/04-Consultation A simple guide to veterinary pharmacovigilance	Promotes pharmacovigilance to animal health professionals (veterinarians, veterinary nurses, pharmacists) to improve pharmacovigilance reporting in the EU
EMEA/CVMP/893/04-UK Guideline on EU veterinary suspected adverse reaction report form for veterinary and health professionals	Format for form to be used by human and veterinary health professionals to report adverse drug reactions in animals and exposed humans, lack of expected efficacy and environmental incidents
EMEA/123352/2004-Rev. 3 Call for comments on standard lists for EudraVigilance Veterinary (July 2008)	'Permanent' call for comments on annual updates to EudraVigilance Veterinary, and proposed amendments
EMEA/CVMP/PhVWP/4550/2006-CONSULTATION Guideline on management and assessment of PSURs of veterinary medicinal products	Provides guidance on assessment of adverse reactions, including suspected lack of expected efficacy, and other data contained in PSURs
EMEA/CVMP/PhVWP/145320/2005-FINAL Concept paper on a PSUR guideline for veterinary medicinal products	Provides considerations on content of a proposed guideline on approach to be taken in assessing PSUR data for all veterinary medicinal products, e.g. those authorised through mutual recognition across the EU
EMEA/CVMP/PhVWP/110607/2005 Veterinary pharmacovigilance in the EU – a simple guide to reporting adverse reactions	Provides a guide to reporting suspected adverse reactions for human and animal health professionals
EMEA/CVMP/68614/2006-CONSULTATION Concept paper for use of data in EudraVigilance Veterinary	Recommends a guideline on surveillance of pharmacovigilance data stored within EudraVigilance Veterinary, its use for regulatory purposes and provision of data to the public
EMEA/CVMP/PhVWP/288284/2007 Guidance notes on the use of VEDDRA terminology for reporting suspected adverse reactions in animals (version 2)	Provides guidance on use of VEDDRA, including system organ class, higher, preferred and lower level terms, death, etc.
EMEA/CVMP/PhVWP/271983/08 List of changes to VEDDRA for 2008	As per title
VICH-EMEA Guidelines	
EMEA/CVMP/VICH/547/00 VICH Topic GL24. Guideline on pharmacovigilance of veterinary medicinal products: management of adverse event reports (AERs)	Provides guidance on aspects of reporting, definitions, serious adverse reactions, information flow and report submission to authorities
EMEA/CVMP/VICH/646/01 VICH Topic GL 29. Pharmacovigilance of veterinary products: management of periodic summary update reports (PSURs).	Covers all areas relevant to design, drafting and submission of PSURs

Table 2.4 *Continued*

Guideline	*Purpose*
EMEA/CVMP/VICH/647/01-Rev. 1-CONSULTATION VICH Topic GL30. Guideline on pharmacovigilance of veterinary medicinal products: controlled list of terms	Covers terms used in pharmacovigilance and adverse drug reaction reporting
EMEA/CVMP/VICH/355996/2005-CONSULTATION VICH Topic GL42. Guideline on pharmacovigilance of veterinary medicinal products: data elements for submission of adverse event reports	Sets out major categories of data to be reported
EMEA Standard Operating Procedures (SOP)	
CVMP/SOP/693/99-Rev. 1 Procedure for management of 15-day suspected adverse reaction (SAR) reports to a centrally authorised veterinary medicinal product	Describes procedure to be followed for expedited reports (e.g. serious) for products authorised through the centralised procedure, and appropriate responsibilities
SOP/V/4019 Standard operating procedure on annual review of standard lists to be used in EudraVigilance	Sets out procedures to be followed in annual updating of lists of terms such as VEDDRA
SOP/V/4033 SIAMED-related data validation of new veterinary centralised procedures	Describes the process for validating data on products authorised through the centralised procedure for inclusion in SIAMED, an EMEA database
SOP/V/4023 Management of periodic safety update reports	Sets out procedures to be followed in dealing with and assessing PSURs submitted to the EMEA for products authorised through the centralised procedure
SOP/INSP/2019 Co-ordination of pre-approval G×P inspections	Sets out procedures to be followed in the event of inspections being required for GLP, GCP or GMP prior to approval of a product submitted for authorisation through the centralised procedure. Could be used or adapted for pharmacovigilance inspections, currently the responsibility of EU member states
Guidelines of historical interest	
EMEA/CVMP/141/98-Rev. 2 FINAL Revised rapid alert system (RAS) and non-urgent information system (UNIS) in veterinary pharmacovigilance	Alerts European regulatory authorities on pharmacovigilance information regarding veterinary medicinal products of action that may be required to protect animal or public health
EMEA/CVMP/143/99-Rev. 1 Conduct of pharmacovigilance for veterinary medicinal products authorised through the mutual recognition procedure	Provides specific guidance for pharmacovigilance activities through the mutual recognition procedure. Much of this is now covered by Volume 9
EMEA/CVMP/044/99-FINAL Guideline for the conduct of post-marketing studies of veterinary medicinal products	Provides guidance on the possible requirements for post-marketing surveillance studies, and the types of study that could be required

Table 2.4 *Continued*

Guideline	*Purpose*
EMEA/CXMP/PhVMP/2056/99 Note for guidance on electronic exchange of information for human and veterinary medicinal products in the EU (note: this is a joint CVMP and CPMP guideline prepared with input from the Pharmacovigilance Working Party)	Provides information relating to the electronic exchange of data between industry, regulatory authorities and the EMEA
EMEA Public Bulletins	
EMEA/CVMP/PhVWP/72829/2007 EMEA Public Bulletin 2007 on Veterinary Pharmacovigilance	
EMEA/CVMP/PhVWP/73213/2006 EMEA Public Bulletin 2006 on Veterinary Pharmacovigilance	
EMEA/CVMP/226674/2005 EMEA Public Bulletin 2005 on Veterinary Pharmacovigilance	
EMEA/CVMP//066/05-FINAL; EMEA/CVMP/138552/2004 EMEA Public Bulletin 2004 on Veterinary Pharmacovigilance	
EMEA/CVMP/359/04 EMEA Public Bulletin 2003 on Veterinary Pharmacovigilance	

- *Unexpected adverse reaction*: an adverse reaction, the nature of which, severity or outcome of which is not consistent with the summary of product characteristics.
- *Post-marketing surveillance study*: pharmacoepidemiological study or a clinical trial carried out in accordance with the terms of a marketing authorisation conducted with the aim of identifying and investigating a safety hazard relating to an authorised veterinary medicinal product.
- *Off-label use*: off-label use of a veterinary medicinal product that is not in accordance with the summary of product characteristics, including the misuse and serious abuse of the product.

The Directive (Article 72) places a requirement on member states to 'take all appropriate measures to encourage the reporting' of adverse reactions to the regulatory authorities. It also requires that member states 'shall impose a requirement' for veterinarians and other health care professionals to report serious and unexpected suspected adverse reactions and adverse reactions in humans exposed to veterinary medicinal products to the regulatory authorities. In doing so, it does not exclude others from submitting reports. Article 73 requires member states to establish pharmacovigilance reporting systems in their territories and to collect data on adverse reactions in animals and humans, and to evaluate these 'scientifically'. In addition to the adverse reactions defined in Article 1, it explains that the following are also included:

- lack of efficacy;
- off-label use;
- validity of withdrawal periods, i.e. violations of MRLs;
- environmental problems arising from the use of veterinary medicinal products.

Article 74 requires that marketing authorisation holders shall have at their permanent disposal a Qualified Person for Pharmacovigilance. No guidance is provided in the Directive or associated guidance as to what might constitute 'Qualified', but the duties of this individual are clear. They include a requirement to establish and maintain an information system that ensures that all suspected adverse reactions are reported to personnel of the company concerned, to prepare

Periodic Safety Update Reports (PSURs), to respond to regulatory authorities for requests for information and to provide authorities with data derived from post-marketing surveillance studies (Borner *et al*., 2006).

The Directive is clear that the requirement on the marketing authorisation holder to maintain records on all adverse reactions also applies to those that have occurred in countries outside of the EU ('third countries'; Article 75). However, one of the most important requirements is that the marketing authorisation holder must report all serious adverse reactions in animals and all human adverse reactions to the authority in the member state where they occurred, immediately or at the latest within 15 calendar days (not working days), after receipt of the information by the qualified person for pharmacovigilance. These are referred to as expedited reports. Similarly, this requirement also extends to all serious adverse reactions and all human adverse reactions that occur in third countries. For products that were authorised under the mutual recognition system or under the defunct concertation procedure (products covered by the latter were converted to mutual recognition product when the new procedures came into force), then the adverse reactions reports must be submitted to the reference member state. Article 75 imposes the requirement for PSURs. These must be supplied on request to a member state regulatory authority, or at the following intervals:

- six monthly – first 2 years;
- then annually – for 2 years;
- then at the time of first renewal of the marketing authorisation, that is at 5 years;
- thereafter, at each 5-yearly renewal.

However, if different time intervals can be justified, the marketing authorisation holder may ask the authorities to permit these. In fact the periodicity has now changed due to the changes in the legislation (see later).

PSURs must detail all the adverse events that have occurred both within the EU and in the rest of the world where the product is authorised. PSURs are timed from the so-called EU birth date, i.e. from the date when the product was first authorised in the EU. The PSURs must contain certain specific information. In addition to the normal administrative data, such as the name and nature of the product, this information must include:

- the current summary of product characteristics;
- worldwide authorisation status;
- details of any regulatory decisions taken for safety reasons;
- sales volume;
- individual case histories (line listings) and incidence calculations;
- suspected adverse reactions;
- published adverse reactions (including databases searched);
- overall safety evaluation:
  - evidence of previously unidentified toxicity
  - increased frequency of known toxicity
  - drug interactions
  - extra-label use
- details of any reactions in humans;
- a conclusion(s) and re-evaluation of benefit: risk assessment.

It is not difficult to see that the pursuit of PSURs could indeed be a time-consuming occupation for both the regulated and the regulators! Nevertheless, these PSURs are recognised as valuable devices for assessing the safety profiles of marketed drugs (Klepper, 2004).

Post-marketing surveillance studies are not common in veterinary medicine. They have major resource implications, particularly for the animal health industry rather than for the regulatory authorities. The guidelines, depending on the circumstances, recommend observational cohort studies, case control studies, group surveillance and even clinical trials, if these seem appropriate to further investigate adverse events seen during use of the veterinary medicinal product in question. Such studies can be difficult to design and conduct, are expensive and may be very difficult to interpret. In addition, there are innate difficulties involved in recruiting animal patients

as there are no records available from anything like a national health service provider, and prescription monitoring is not currently possible.

With the potential for data exchange required by the Directive, it is perhaps not surprising that Article 76 requires the EMEA and the member states to establish a network for 'data processing' to allow them to collect pharmacovigilance data. In fact, the Directive foresees the use of this network in aiding compliance with the 15-day requirement for serious and human adverse reactions. This Article also requires member states to notify the marketing authorisation holder of serious or human adverse reactions which have occurred within their territories. The concept of data exchange is taken further in Article 77. It requires the establishment of an interchange of data within the EU and stipulates that the European Commission, in consultation with the EMEA, member states and 'interested parties' (the latter is not defined but is usually understood to include industry), shall draw up guidance on the 'collection, verification, and presentation of adverse reactions, including guidance for electronic exchange' using 'internationally recognised terminology'. It also requires that this guidance be published in Volume 9B of the *Rules Governing Medicinal Products in the European Community*.

As mentioned in *Table 2.4*, there are several guidelines associated with electronic exchange of information and two of these, on electronic exchange per se (EMEA/CXMP/PhVWP/2056/99) and data elements to include in electronically submitted adverse reaction reports (EMEA/CVMP/065/03-Rev.1), are intended to work in concert with the guideline on terminology (VEDDRA, EMEA/CVMP/413/99-FINAL). In practice, the implementation of these guidelines fulfils some of the aims and aspirations of the Directive, as discussed above.

The veterinary version of a data processing network known as EudraVigilance was introduced for the main part in 2006. EudraVigilance for human medicinal products uses the MedDRA (Medical Dictionary for Regulatory Activities) medical terminology and coding system (Wood and Coulson, 1993; Brown *et al*., 1997; Brown and Douglas, 2000). Systems such as MedDRA should allow standardisation of medical terminology including system organ classes, disease states and drug and adverse reaction terms (Brown *et al*., 1999; Goldman, 2002; Aronson and Ferner, 2005) and should minimise problems encountered with other systems (Saltzman, 1985; Strathman, 1986; Schneiweiss, 1987; Sills, 1989; Joseph *et al*., 1991).

The EudraVigilance system for veterinary medicinal products uses the VEDDRA (Veterinary Drug Dictionary for Regulatory Activities) system. This serves a similar purpose to MedDRA but is far less complex and, for obvious reasons, is orientated towards veterinary terminology and use. The proposed guideline on species and breeds mentioned in *Table 2.4* is intended to provide some degree of standardisation in this area. Even now, familiar species may be entered on adverse reaction reports under a variety of names. Examples include dog, canine, puppy, bitch or a specific breed name, and cattle, cow, bull, bullock, bovine or calf.

However, products such as MedDRA are not without their limitations. First, experience with MedDRA has shown that there is potential for constraint of information by the use of standardised terms (Brown and Clark, 1996), and further confusion may arise when preferred terms represent different medical concepts (Brown, 2002). Moreover, there may be multiple locations for specific terms within a system organ class, with a concomitant lack of recognition of group terms (Brown, 2003). Multiple preferred terms can be confounding when searching databases. For example, MedDRA has 13 terms for urticaria and 18 for convulsions (White, 1998). Coding of relatively simple narrative from a medical history can be misconstrued and, although technically correct, may be medically misleading (Doan, 2000). Hence, appropriate caution must be exercised in both the choice of system and use.

All of this emphasises the need for careful input, analysis, retrieval and control of data when using any coding technique. The VEDDRA

system, as already mentioned, is less complex than MedDRA and so perhaps some of the more obvious pitfalls can be avoided. More details on VEDDRA can be found at the EMEA and the veterinary EudraVigilance websites (http://www.emea.europa.eu/ and http://eudravigilance.emea.europa.eu/veterinary/index.asp).

The EudraVigilance system is a complex computer network or telematics database system that allows EU regulatory authorities, the European Commission, the EMEA and marketing authorisation holders for human and veterinary medicinal products to communicate with each other, although the human and veterinary operations of the system are separate. Thus, industry may submit PSURs and adverse reaction reports to the system and these become available to EU regulatory authorities. EU member states can use the system to submit adverse reaction reports for centrally authorised products, initially submitted to them, to the EMEA. A marketing authorisation holder can access its information (but not those of other companies) to cross check data against its own records (for example). The system can also be used to submit adverse reaction reports from third countries.

At the core of EudraVigilance is the Database Management System (DBMS). This allows integrated input, use and query functions, on a strict permissions basis, to registered users and it allows a data tracking function within the system. It incorporates a veterinary pharmacovigilance database which employs information derived from a number of sources, including adverse drug reactions entered into the system, data supplied by national authorities and information regarding authorisations within the EU. The system allows the European Commission, the EMEA and national authorities to access and to manipulate pharmacovigilance data for veterinary medicinal products authorised in the EU. As mentioned above, veterinary pharmaceutical companies can access their own data and records to check for validity and accuracy.

Data on all aspects of veterinary pharmacovigilance (adverse drug reactions, lack of efficacy, adverse reactions following off-label use, violations of MRLs, adverse environmental effects and suspected transmission of any infectious agents via a veterinary medicinal product) can be placed on the system.

Veterinary professionals and others may report adverse reactions to the marketing authorisation holder, to the regulatory authority or to both. The regulatory authority can then input the data into the EudraVigilance system. The marketing authorisation holder is required to report all suspected serious adverse reactions and all adverse reactions in humans to veterinary medicinal products in an expedited manner through the dedicated and secure Gateway, via a web tool (EVWEB) or by using a simplified electronic reporting form. The majority of EU regulatory authorities are registered with the system. More information is available at the EudraVigilance website mentioned above. Finally, it should be emphasised that the system permits the electronic reporting of pharmacovigilance data, as required by EU legislation.

Finally, the issue of sanctions arises; what should be done if pharmacovigilance data suggest that some regulatory action might be necessary? This is addressed in Article 78 of the original Directive. If the member state considers that a marketing authorisation should be suspended, withdrawn or varied to alter the terms of the original authorisation, it should inform the EMEA, the other member states and the company. Furthermore, if a member state considers that any action is a matter of urgency, it should notify the European Commission, the EMEA and the other member states by the following working day at the latest.

There is specific guidance on products that have been authorised through the mutual recognition procedure provided in Volume 9B (originally available as EMEA/CVMP/143/99-Rev. 1). This provides more detailed advice on several aspects of pharmacovigilance including PSURs and the benefit:risk balance for individual products. The CVMP has also elaborated guidelines on action to be taken when urgent measures need to be taken on an authorised product to safeguard human or animal health. Such actions

might include the recall of a product or a batch of product. Among other things, this requires a rapid alert transmission from the competent authority where the adverse event has occurred to the other member states involved if this is a mutual recognition procedure product, and to the EMEA and European Commission. In the case of products authorised through the centralised procedure, the rapporteur should also be notified. In all cases, the Chairman of the CVMP should be notified. It is the intention of these provisions that rapid and concerted regulatory action can be taken, across the EU if necessary, if the adverse events that have occurred are considered to be very serious, and these are addressed in the EMEA's Crisis Management plan for pharmacovigilance and other safety issues related to centrally authorised products (*Table 2.4*).

One of the important issues addressed in Volume 9B is the question of causality. This is accomplished using the ABON system (Woodward and Gray, 1994):

Category A: probable
Category B: possible
Category O: unclassified (insufficient data to draw conclusion)
Category N: unlikely to be related to the medicine in question

The new draft of Volume 9 envisages subdividing Category O into O (unclassifiable/unassessable; insufficient data to assess causality) and O1 (cases where other factors prevented a conclusion being reached, but a product association could not be discounted).

The issues surrounding causality are discussed in Chapter 27 and so are not dealt with further here (Woodward, 2005b). However, it should be noted that it may be difficult to assess causality from a single case report, or indeed from a small number of case reports, and in those cases it may be more practical to make assessments of causality by the analysis and evaluation of a series of reports, where trends can be identified and generalities and comparisons made (Meyboom *et al*., 1997; Jones, 2000; Keck and Ibrahim, 2001) in conjunction with the use of data from PSURs.

Regulation 2309/93

Effectively, this Regulation extended the legal provisions for pharmacovigilance for national and mutual recognition products laid out in Directive 2001/82/EC to those authorised through the centralised procedure through the EMEA, the CVMP and the European Commission. Not surprisingly therefore, the Regulation did not reiterate the definitions set out initially in Directive 81/851/EEC and repeated in Directive 2001/82/EC, it merely cross referred to them in Article 41. A separate Regulation, Regulation (EC) No. 540/95, made provisions for dealing with non-serious adverse reactions arising in the EU and in third countries.

Article 42 made it clear that the EMEA had to cooperate with national authorities in the member states in dealing with pharmacovigilance issues, and that it had to receive all 'relevant information about suspected adverse reactions' arising from products authorised in accordance with the Regulation, i.e. products authorised through the centralised procedure. This Article also stipulated that the marketing authorisation holder and the member states had to inform the EMEA about any suspected adverse reactions arising from centrally authorised products.

There was also a requirement for a Qualified Person. This was described in Article 43 and the text there was similar to that of the corresponding text in the Directive, although here, of course, it focused on the duties with respect to products authorised through the centralised procedure. The requirements that applied to serious adverse reactions in the Directive also applied to serious adverse reactions for centrally authorised products. This was described in some detail in Article 44. Again, these had to be reported to the member states immediately, and no later than 15 calendar days after the information had been received.

A similar requirement applied to serious adverse reactions occurring in third countries to

products authorised through the centralised procedure in the EU. Curiously, there was no mention here of adverse reactions in humans following exposure to veterinary medicinal products, unlike the text in the Directive. Article 45 placed the onus on the member states to inform the EMEA and the marketing authorisation holder of all suspected serious adverse reactions to centrally authorised products that had been reported to them.

The requirement to draw up guidance given in the Directive was repeated in Article 46. Article 47 was somewhat vague as it entreated the EMEA to 'cooperate with international organisations concerned with pharmacovigilance'. Nevertheless, as VICH (see later) is almost the only international organisation involved with veterinary pharmacovigilance, the options were perhaps limited, and it did allow scope for any that might become 'concerned' in the future. However, the EMEA has a formal confidentiality agreement with the FDA in the United States and it engages in informal discussions with other international agencies.

At this point, it would be educative to consider examples of adverse events from the mutual recognition procedure. However, at the moment no such publicly available examples exist and it is not clear if and when such information will become generally accessible, although Directive 2004/28/EC and Regulation (EC) No. 726/2004 make allowances for public disclosure. What is clear is that there will be electronic exchange of information between the European regulatory authorities and the EMEA using the EudraVigilance electronic systems available to them (Wood, 1998b). In 2004 the EMEA published a *Public Bulletin for 2003 on Veterinary Pharmacovigilance* which noted, *inter alia*, that most adverse reactions reported for centralised products were for companion animals and that the CVMP had made a number of recommendations, such as warnings for corticosteroid products to reduce the incidence of adverse reactions in dogs. Once EudraVigilance is operating fully, the EMEA will be better placed to analyse the data for centralised procedure adverse reactions and it is intended that the findings will be published (on the EMEA website).

2004 and onwards

As described earlier and reflected in *Figure 2.1*, the European Commission has been in the process of revising the veterinary legislation in the EU and this exercise extended to Directive 2001/82/EC and Regulation 2309/93 (Clayton and Zanker, 2000a–e). This revision was required because the legislation introducing the centralised procedure and the EMEA carried with it a necessity to examine the functioning of law and its procedures in the light of working experience. The changes initially proposed for the veterinary legislation ranged from the trivial to the far reaching. For example, it was proposed to amend Regulation 2309/93 to make the EMEA formally responsible for pharmacovigilance and to change the name of the EMEA. Major changes proposed for the requirements of those aspects of Directive 2001/82/EC related to pharmacovigilance included a requirement that the Qualified Person be resident in the EU, and that adverse reactions occurring in third countries, that is, outside of the EU, be notified to EU competent authorities within 15 days.

There was also a proposal that in the future both the Qualified Person and the adverse reaction records should be subjected to inspection by the competent authorities (Clayton *et al*., 2001). These proposals have taken some considerable time to materialise as they were subject to extensive consultation with the industry, with other interested parties and with national governments and ministers from EU member states, and were subject to political debate within the European Parliament and its committees. Changes to pharmacovigilance aspects of the legislation were expected as the European Commission recognised shortcomings in the operation of pharmacovigilance in the EU's member states (European Commission, 2001b), and owing to the widespread recognition that the system whereby marketing authorisations were reviewed every 5

years was resulting in considerable difficulties, particularly in the provision of data, and that this might be replaced, or replaced in part, by a more robust system of pharmacovigilance (Clayton, 2001; Clayton and Zanker, 2001e).

The review process was eventually finalised on 31 March 2004 when Directive 2004/27/EC was published to amend the human pharmaceuticals legislation (Directive 2001/83/EC) and Directive 2004/28/EC was published to amend the veterinary legislation (Directive 2001/82/EC). On the same date, Regulation (EC) No. 726/2004, governing the operation of the centralised procedure and the EMEA, finally emerged to replace and repeal Regulation (EC) No. 2309/93.

As expected, the review has resulted in some relatively minor amendments. For example, under Regulation (EC) No. 726/2004, the Committee for Veterinary Medicinal Products now becomes the Committee for Medicinal Products for Veterinary Use, although it retains the CVMP acronym, while its counterpart, the Committee for Proprietary Medicinal Products becomes the Committee for Medicinal Products for Human Use (CHMP). The European Medicines Evaluation Agency enjoys a change of name to the European Medicines Agency to reflect its wider remit and activities over and above evaluating data and dossiers for marketing authorisation applications and MRLs, in line with earlier proposals, although it will retain its logo and the letters EMEA. Many of the requirements of regulation (EC) No. 2309/93 are retained in Regulation (EC) No. 726/2004, albeit in revised form. However, many of the changes to the legislation are more substantive. For example, applications for marketing authorisations must now be in accordance with the Annex to Directive 2001/82/EC as amended by Directive 2004/28/EC.

There are a number of changes to pharmacovigilance imposed by amending Directive 2004/28/EC. Of these, the most important are:

Article 72: The original requirement for member states to encourage the reporting of adverse reactions is replaced with a more overt and specific requirement on veterinary practitioners and other health care workers to report, although this is limited to suspected serious or unexpected reactions and to adverse reactions in humans.

Article 73: The requirement to establish pharmacovigilance systems is replaced by a requirement to administer them, presumably on the assumption that they have now established them. More significantly, member states who have 'collected' information on adverse reactions are required to communicate this to all other member states and to the EMEA, and this information must be made permanently available, without delay, through the EU's database to all member states *and* to the public.

Article 74: The qualified person for pharmacovigilance must now reside in the EU.

Article 75: Again, some tenses have changed from the original legal texts to reflect requirements that by now should be implemented. A major requirement now is that all suspected adverse reactions occurring in the EU and third countries must be reported electronically to the authorities, except in exceptional circumstances.

Furthermore, in addition to the reporting of 'conventional' adverse reactions, marketing authorisation holders are now required to notify any suspected transmission through a veterinary medicinal product of any infectious agent occurring on the territory of a third country. Clearly, this would include any transmissible spongioform encephalopathy, passed on by contaminated material of biological origin, as well as any other infectious disease.

The amended Article 75 also changes the periodicity of PSURs. The original requirement was for the submission of these reports at 6-monthly intervals for the first 2 years after marketing commenced, then at annual intervals until 5 years was reached, and then at 5-yearly intervals after that. However, the revised legislation has also dispensed with the 5-yearly renewal cycle and replaced it with a single renewal 5 years after authorisation, but with

the addition of the more frequent PSURs, presumably to give an enhanced degree of comfort to make up for the lost data submission at the now defunct renewals. Consequently, the PSUR cycle becomes every 6 months for 2 years, then annually for 2 years, and then at 3-yearly intervals rather then 5. However, Article 75.6 allows for a further reduction in periodicity depending on market field experience.

This Article also introduces a prohibition on the marketing authorisation holder from communicating pharmacovigilance findings with the general public without prior or simultaneous notification to the EU authorities.

Article 77: This now introduces a firm requirement for the marketing authorisation holder to use 'internationally agreed veterinary medical terminology' for the transmission of reports on adverse reactions. This presumably means both spontaneous reports and PSURs. This is a clear reference to a requirement to use the VEDDRA system of terminology.

Article 78: The original legislation allowed member states to suspend a marketing authorisation in urgent cases. The amended legislation makes it clear that the urgency applies to the protection of human or animal health. However, now the EMEA must give an opinion on any such actions, through the CVMP, and provide its opinion to the European Commission. This then allows the Commission to extend the suspension or whatever preventative action has been taken in the affected member state to all other member states.

The wording in Regulation (EC) No. 726/2004 has also changed. Whereas the outgoing regulation made several cross references to Articles in the contemporaneous Directive, the new regulation repeats much of what is written in the Directive, thus giving it more of a stand-alone appearance, and emphasising the role and responsibilities of the CVMP and EMEA. Again, there is a new emphasis in Articles 46 to 54 of the new regulation on the transmission of pharmacovigilance data between member states, and between member states and the EMEA. Article 52 of the regulations requires the EMEA to cooperate with international bodies concerned with pharmacovigilance, while Article 53 requires the EMEA and member states to work together in pharmacovigilance activities for all veterinary medicinal products *regardless* of their route of authorisation. The latter is perhaps an encouragement for member states to pay more attention to older products authorised under national legislation, and to treat these with the same standards as those authorised through mutual recognition or by way of the centralised procedure.

Outside of the Articles referring specifically to pharmacovigilance, there are other pertinent references. For example, in the section entitled *Tasks of the Agency*, Article 55 specifically states that its purposes are 'for the evaluation, supervision and pharmacovigilance of medicinal products', while Article 57.1(i) lists as one of its functions 'coordinating the verification of compliance with the principles of good manufacturing practice, good laboratory practice, good clinical practice and the *verification of compliance with pharmacovigilance obligations*'. Both the Directive and the Regulation now make reference to 'necessary measures' against marketing authorisation holders who fail to comply with pharmacovigilance requirements and these should be 'effective, proportionate and dissuasive penalties'. Importantly, under Article 57(f), the EMEA is charged with the task of making pharmacovigilance data available to the public.

The Directive required transposition by 30 October 2005. The UK chose to do this, not by introducing further Statutory Instruments under the Medicines Act 1968, but instead by disapplying this Act and introducing legislation under the European Communities Act 1972. This had the added benefit of repealing some 50 older Statutory Instruments and removing the requirements of the Medicines Act itself, and replacing these with a single Statutory Instrument which covers all aspects of veterinary medicines legislation in the UK, thus simplifying the UK's own legal framework (Dean, 2005a, b). The Regulation,

which took immediate effect and required no transposition into national legislation, came into effect 20 days after its publication. It contained some relatively minor changes such as the CVMP's and EMEA's changes of names. The majority of the substantive changes in Titles I, II and III did not apply until 20 November 2005, while some parts of the Annex to the Regulation, which sets out which types of products are either compulsorily subject to the centralised procedure or put through on a voluntary basis, did not come into effect until 20 May 2008.

VICH

VICH is the abbreviation for the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products, and it followed a pattern set for human medicines by the International Conference on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH) founded in 1990 (Idänpään-Heikkilä, 1992; Talbot, 2000). It can trace its origins to a number of earlier initiatives, including the International Technical Consultation of Veterinary Drug Registration (ITCVDR) and in particular to the 7th ITCVDR meeting held in Buenos Aires in 1992. In 1994, two resolutions were adopted by the ITCVDR and the OIE[1] (Office International des Epizooties) and these eventually led to the establishment of the VICH in 1996 (Clayton and Zanker, 2000f; Zanker, 2003a, b). Behind both initiatives are the simplification and rationalisation of regulatory requirements and the facilitation of international trade (Anonymous, 2000; Ozawa, 2000; Holmes and Hill, 2007).

ICH provides an international forum for discussions and exchanges of views on all aspects relating to the safety, quality and efficacy of human medicines, including toxicity testing, studies in human subjects and the harmonisation of regulatory requirements, and notably guidelines (Diggle, 2000). It is a multiparty organisation involving the pharmaceutical industry, the EMEA, regulators from the EU countries, the US and Japan as well as several observer representatives and the World Health Organisation. One of the major areas addressed by ICH has been the harmonisation of requirements for pharmacovigilance (Bahri and Tsintis, 2005, 2007).

VICH attempts to achieve similar aims to ICH, and like ICH it has representatives of the animal health industry and regulatory authorities from Japan, the USA and the EU. The EU is represented by the EMEA and the European Commission. The OIE is an observer (Verschueren, 1999) as are Australia, New Zealand and Canada (Clayton and Zanker, 2000f; Roth, 2004; Vannier, 2004). VICH has now elaborated and agreed a number of guidelines and 33 of these, relating to topics as diverse as stability testing of new drugs, environmental impact assessment, and efficacy, genotoxicity and carcinogenicity testing, are in their final forms. A further seven guidelines are in draft stages (Clayton and Zanker, 2000f; Vercruysse *et al*., 2001, 2002; Zanker, 2003a, b; Hennessy *et al*., 2006; http://vich.eudra.org/htm/guidelines). Of these seven draft guidelines, three are related to pharmacovigilance:

- Pharmacovigilance of Veterinary Medicinal Products: Management of Adverse Event Reports (AERs) – VICH GL 24 (VICH, 2000).
- Pharmacovigilance of Veterinary Medicinal Products: Management of Periodic Summary Update Reports (PSURs) – VICH GL 29 (VICH, 2001a).
- Pharmacovigilance of Veterinary Medicinal Products: Controlled List of Terms – VICH GL 30 (VICH, 2001b).

The area is complex for a number of reasons. In the EU, vaccines, pharmaceuticals and ecto-parasiticides such as sheep dips and spot-on formulations are all regarded as veterinary medicinal products and in most member states are regu-

[1] The OIE performs a similar function to the World Health Organisation, in the animal health context. It was formed in 1924 and is based in Paris. In 2003, the OIE had 165 member countries, and these countries are usually represented by their Chief Veterinary Officers or equivalent (http://www.oie.int/).

lated by a single veterinary agency or by the EMEA and CVMP. However, and, for example, in the USA, pharmaceuticals are handled by the FDA's Center for Veterinary Medicine, vaccines by the Department of Agriculture's Center for Veterinary Biologics and ectoparasiticides by the Environmental Protection Agency. Hence, VICH has to gain agreement from several regulatory agencies and in some areas, including pharmacovigilance, this has proved to be difficult and these activities have been halted or significantly delayed (Zanker, 2003a). However, at the present time, there does appear to be a strong desire to make progress by all the parties involved, and not only in the pharmacovigilance area.

Good Pharmacovigilance Practices (GPPs)

There is no absolute definition of Good Pharmacovigilance Practices (or Practice; GPP). In general terms, it can be regarded as the means and routines put in place to ensure compliance with pharmacovigilance requirements. Hence, it may differ slightly from one country to another depending on the legislation in place. However, in general, there are some overarching principles that apply to all aspects, wherever they may exist. These can cover good reporting practice, development of reports and case series, investigation of signal development, triage of workflow, statistical analyses, organisation of workflow, communication of data and conduct of pharmacoepidemiology studies (Anonymous, 1995; Nelson *et al*., 2002; International Society for Pharmacoepidemiology, 2004; Food and Drug Administration, 2005; Epstein, 2008). The concepts can be extended to pharmacoepidemiology.

In this respect, the animal pharmaceutical industry is endeavouring to make veterinary pharmacovigilance a success. Recently, the European representative body for the industry, IFAH-Europe, published a guideline on GPP (IFAH Europe, 2004). This not only clearly summarises the relevant EU legislation and requirements, partly through a question and answer approach, but also describes best practices and provides guidance on causality assessment. In this way, it aims for the industry to achieve compliance with the legislative requirements and so assists in achieving regulatory compliance. It can be read along with other reports (e.g. Koster *et al*., 2000; Nelson *et al*., 2002) to provide a basis for best practices and for success. With the spectre of pharmacovigilance inspections in Europe (Bleumink *et al*., 2001; Koster and van den Oetelaar, 2005) now a reality, this booklet is a timely and helpful guide to what has become a very complex subject. Certainly in the EU, pharmacovigilance can be expected to be pursued with some vigour, as made clear in the EMEA's Road Map proposals and Work Programme for 2004 (EMEA, 2004a, b). Guidelines and advice on good pharmacovigilance practice are being developed by some EU authorities. For example, the Medicines and Health Products Regulatory Agency (MHRA), the UK competent authority for human pharmaceuticals, is developing guidance on good pharmacovigilance practices for human medicinal products (Jack and O'Mahony, 2008). The booklet covers:

- The legal framework in the EU
- The scope and description of adverse reactions (serious, expected) and recording/reporting
- Causality coding
- Third country reporting
- Periodic safety update reports
- Sales figures and incidence calculation
- Adverse reactions in clinical trials
- Tools (Qualified Person for Pharmacovigilance and databases)
- Communications with regulatory authorities

In addition, it provides a useful decision tree for the reporting and analysis of adverse drug reactions. Following the principles and advice set out in this booklet should allow drug sponsors to comply fully with the requirements of EU pharmacovigilance legislation and guidelines for veterinary medicinal products, and, moreover, its recommendations are more widely applicable to pharmacovigilance activities in other, non-EU regions.

Discussion

There is quite clearly a considerable body of EU legislation governing the authorisation of veterinary medicinal products and their uses in the Community. This is supported by a large number of guidelines and guidance documents. Pharmacovigilance forms an integral part of the post-marketing surveillance of these products in the EU (and elsewhere) and this is directed at all aspects of safety, including adverse reactions in the treated animal patient, in exposed humans, adverse environmental events and those resulting in violations of maximum residue limits, possibly through the invalidity of withdrawal periods. This can only serve to enhance the benefits of pharmacovigilance by affording better protection to humans, animals and the environment and by providing more detailed information on the adverse effects of individual veterinary medicines or specific classes of veterinary drug. These efforts will be enhanced through the sharing of pharmacovigilance data across the European Union (Dean, 2005c).

To support pharmacovigilance activities by marketing authorisation holders and by regulatory authorities, there is an impressive array of general and specific guidelines covering all areas of the endeavour, from the relatively simple guidance given in Volume 9/9B to the more complex information provided on electronic reporting and its associated areas such as the preferred terminology.

As a result of this constantly growing and increasingly complex legislation, the veterinary pharmaceutical industry has had to adapt accordingly and change its practices to suit an increasingly demanding regulatory environment. The recent review of EU legislation described in this chapter will add to concerns that EU pharmacovigilance initiatives are becoming overly complex and potentially burdensome, and possibly out of all proportion to any problems that might occur – perhaps because they are developed in parallel to those for human medicinal products where adverse reactions are clearly a major public health issue. Indeed, in the EU there are increasing concerns that the regulatory burden, including pharmacovigilance requirements, might affect global competitiveness (Clayton, 2005). On the other hand, the increasing requirements for pharmacovigilance are offset by the partial abolition of renewals. The electronic transmission of data and the associated requirements for approved terminology, approved names for specific species and breeds, not to mention the added requirements for computer hardware and software, with the associated problems associated with validation of computer systems (Hoffmann *et al.*, 1998), will only add to the complexities to be faced.

In 1994 Professor Michael Rawlins posed the question with respect to pharmacovigilance of human medicinal products in an article entitled 'Pharmacovigilance: paradise lost, regained or postponed?' (Rawlins, 1995). He concluded that it was neither lost nor regained but rather it was a continuing story of endeavour by all those involved, and so its position was postponed. This is also true for veterinary pharmacovigilance, certainly in the EU, but in the continuing work in which all parties are engaged, it is important not to lose sight of the ultimate goal of pharmacovigilance – to protect animal patients, exposed humans and the environment from any potentially harmful effects of veterinary medicinal products, and where these do occur, to balance these against the benefits before taking any precipitant actions. In fact, striking the correct balance between benefits and risks will be one of the major challenges to be faced in the implementation of the reviewed EU legislation (Clayton, 2004). In human pharmacovigilance, the European Commission has recently revealed plans to make 'improvements' to the legislation (European Commission, 2007; Arlett, 2008; Waller *et al.*, 2008). It is highly doubtful if many (or any) of these improvements will be worthwhile in the veterinary sector and, in the interests of proportionality, their extension to the veterinary legislation is not on the whole to be welcomed. However, it may be short sighted to assume that any such extension will not occur.

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3 Pharmacovigilance and the European Medicines Agency: conduct of pharmacovigilance activities

K. Grein

Introduction

The European Medicines Agency (EMEA) is the European Union (EU) body responsible for coordinating the existing scientific resources put at its disposal by member states for the evaluation, supervision and pharmacovigilance of medicinal products (EMEA, 2006). The marketing authorisation and supervision, including pharmacovigilance, of veterinary medicinal products in the EU is regulated by Directive 2001/82/EC as amended by Directive 2004/28/EC (referred to as Directive 2001/82/EC) and Regulation (EC) No. 726/2004 (European Parliament and Council, 2001, 2004). Common principles govern the conduct of pharmacovigilance of veterinary medicinal products in the EU independent of the marketing authorisation procedure (national, mutual recognition, decentralised or centralised). However, the processes and responsible bodies differ according to the marketing authorisation procedure.

The role of the EMEA within the EU regulatory network

The EMEA provides the member states and the institutions of the EU with the best-possible scientific advice on any question relating to the evaluation of the quality, safety and efficacy of medicinal products for human or veterinary use referred to it in accordance with the provisions of EU legislation relating to medicinal products (EMEA, 2006). The role of the EMEA secretariat is primarily one of the coordination of all the activities that the Agency as a whole, including its scientific committees, is responsible for and of communicating the opinions and recommendations of the committees to the partners and stakeholders concerned. The committees are responsible for the scientific evaluations and for formulating opinions and recommendations. The EMEA and its Committee for Medicinal Products for Veterinary Use (CVMP) have a key role to play in the scientific evaluation and supervision of veterinary medicinal products in the EU, particularly for centrally authorised veterinary medicinal products.

The body that is responsible for the authorisation procedure for a veterinary medicinal product is either the EMEA together with the European Commission, or national competent authorities in EU member states, depending on the procedure chosen for the marketing authorisation application. In the centralised procedure, which is optional for new chemical entities and

innovative products, and mandatory for products derived from biotechnological processes, the application for a marketing authorisation is submitted to the EMEA, and the CVMP carries out the scientific evaluation. Following the evaluation, and on the basis of a positive opinion reached by the CVMP, the European Commission issues a marketing authorisation. Such a centralised marketing authorisation is binding in all EU member states. The other procedures are the mutual recognition procedure and the decentralised procedure, where the scientific evaluation is carried out by the competent authorities of the member states in which the product is intended to be marketed, with one country, the reference member state, taking the lead. The aim is to agree on the assessment of the benefits and risks of the product and to derive identical conditions for the marketing authorisation in all the countries involved, i.e. the Summary of Product Characteristics (SPC), including the target species, indications, contra-indications, and any specific advice, restrictions and risk mitigation measures. The Coordination Group for Mutual Recognition and Decentralised Procedures (veterinary) (CVM(v)) has been established to co-ordinate and facilitate the functioning of the mutual recognition and decentralised procedures. If no agreement can be reached on the assessment or the conditions of the marketing authorisation and a serious risk is considered possible by one or more member states, the matter of concern is referred to the CVMP for arbitration (Article 33 of Directive 2201/82/EC). The CVMP then gives an opinion on the matter of concern that will be the basis for a subsequent Commission decision, which is binding for the resulting marketing authorisations. The marketing authorisations from the mutual recognition and decentralised procedures are national authorisations issued by the member states concerned. National marketing authorisations, i.e. individual marketing authorisations issued by member states, exist for veterinary medicinal products that were on the market in the EU before the system described above was introduced in the legislation in 1995, and can be issued today, if a product is intended for one single EU member state only.

In addition to its role in the evaluation of centralised marketing authorisation applications and arbitrations arising from mutual recognition and decentralised procedures, the CVMP is also responsible for issuing scientific opinions on referrals made in order to achieve the harmonisation of decisions on marketing authorisations (Article 34 of Directive 2001/82/EC), in cases where there is a Community interest or other safety-related issues (Article 35 of Directive 2001/82/EC and Article 40 for variations). In the case of a regulatory action taken as a result of the evaluation of pharmacovigilance data by a member state, the CVMP is required to issue an opinion (Article 78 of Directive 2001/82).

The CVMP is also responsible for issuing scientific advice, the evaluation of dossiers submitted for the establishment of maximum residue limits in food of animal origin, and developing scientific and regulatory guidance, providing a harmonised approach to the evaluation and supervision of veterinary medicines throughout the EU. In providing scientific guidance, the CVMP is supported by its Working Parties and Scientific Advisory Groups. For further details see the EMEA website (http://www.emea.europa.eu/htms/general/contacts/CVMP/CVMP.html).

Member states and the EMEA have been given distinct roles in the legislation for supporting the pharmacovigilance and monitoring system of veterinary medicines in the management and/or assessment of individual adverse events, periodic safety update reports, results from post-marketing studies and other pharmacovigilance data. The legislation provides for a co-ordinating role for the EMEA, and in particular regarding the common European pharmacovigilance database and information exchange system. In order to foster harmonisation and to maximise the efficient use of resources, the CVMP Pharmacovigilance Working Party (PhVWP-V) supports the CVMP in respect to both advice regarding the safety of centralised products and the development of scientific and regulatory guidelines, and also as a forum for discussions and the provision

of advice relating to pharmacovigilance issues concerning nationally authorised products with reporting lines both to the EU member states and to the CVMP.

The PhVWP-V provides the expertise for drafting guidance on the conduct of pharmacovigilance in the EU in support of the legislation in order to assist the CVMP and the European Commission which are ultimately responsible for the final documents. This guidance includes procedural details and clarification on the roles and responsibilities of the applicants/marketing authorisation holders, the member states, the EMEA and the European Commission. It also covers the different elements of assessment of pharmacovigilance data. Any such guidance is, unless otherwise specified, applicable to all veterinary medicinal products, independent of their marketing authorisation procedure. Comprehensive guidance on pharmacovigilance comprising guidelines for marketing authorisation holders, guidelines for competent authorities and the EMEA and guidelines on the electronic exchange of pharmacovigilance information is compiled in Volume 9B of the *Rules Governing Medicinal Products in the EU*, which is published by the European Commission (European Commission, 2007a). Further specific guidelines and Standard Operating Procedures (SOPs) are published by the EMEA (http://www.emea.eu/index/indexv1.htm).

Furthermore, the EMEA has a key role in the communication of pharmacovigilance information to all partners and stakeholders concerned as well as in fostering pharmacovigilance throughout the EU. Of particular importance is the communication and co-operation with EU member states, the European Commission and with manufacturers of veterinary medicines, which occur on a routine basis in relation to all procedures and processes that are under the CVMP's responsibility, as well as communication on safety issues with veterinarians. The EMEA also holds communications on pharmacovigilance issues with other regulatory authorities outside the EU, and in particular with VICH (see Chapter 2) partners and with international organisations responsible for public and animal health.

The CVMP remains responsible for the evaluation of all centrally authorised products throughout their whole life cycle, i.e. during the post-authorisation phase including pharmacovigilance. For veterinary medicinal products authorised nationally (mutual recognition, decentralised or purely national marketing authorisations) the responsibilities for the conduct of pharmacovigilance lie with the national competent authority or authorities that issued the marketing authorisation. The CVMP becomes involved in the assessment of pharmacovigilance issues concerning these products when safety matters arising from pharmacovigilance information are referred to the CVMP.

The CVMP is also responsible for assessing pharmacovigilance data concerning classes or groups of products independent of their authorisation procedure. This may become necessary, for example in regard to a class of active ingredients or issues related to specific types of formulation(s). In such cases the CVMP establishes its scientific position and provides advice to regulatory authorities, marketing authorisation holders or veterinarians and to the general public, where appropriate.

Principles of pharmacovigilance evaluation and safety-related regulatory action

A marketing authorisation for a veterinary medicinal product is based on the outcome of the benefit : risk assessment that pertained at the time of the authorisation. The benefit : risk balance is considered positive on the basis of the information available for the specified indication(s) and conditions of use. It considers the benefits and risks for the target species, the person administering the medicine or coming into contact with it or the treated animal, the environment and, with food-producing animals, the consumer of food of animal origin. At the time of the initial authorisation, only limited safety data are available and the benefit : risk balance is therefore

reviewed throughout the life cycle of a product in light of new data, including pharmacovigilance data.

Key elements of pharmacovigilance are the detection of new safety signals in relation to the use of veterinary medicinal products and the assessment of these signals. The regular review and analysis of reports on adverse reactions is necessary for signal detection. This review may occur routinely on the basis of data submitted under the spontaneous reporting system or of the contents of periodic safety update reports (PSURs). Potential signals may be identified, e.g. when an increase in the number of adverse reactions is observed, a particular clinical sign is observed more frequently than expected, or new, previously unidentified clinical signs are reported. Emerging safety signals need to be analysed and assessed.

If safety concerns have been confirmed following the safety assessment and the benefit : risk balance has changed, risk management options will need to be considered. The appropriate action then required will depend on the severity and frequency of the adverse reactions observed, on whether more information is needed to reach a conclusion and if appropriate risk mitigation measures already exist.

Possible risk management options include:

- intensified pharmacovigilance surveillance, e.g. the request for more frequent periodic safety update reports;
- requirement for post-authorisation surveillance studies;
- changes in the marketing authorisation conditions, e.g. changes to the SPC to include additional or modified precautionary measures and contra-indications through a variation application or via an interim change imposed through urgent safety restrictions;
- direct provision of specific safety information to veterinarians and other health-care professionals and animal owners;
- suspension or withdrawal of the marketing authorisation if the benefit : risk balance is considered unfavourable and no adequate risk mitigation measures are available.

Further details on the procedures to be followed and responsibilities are described in Volume 9B (European Commission, 2007a).

The role of the EMEA for centrally authorised products

The EMEA is responsible for all centrally authorised products throughout their whole life cycle. This includes the conduct of pharmacovigilance. The legal provisions for pharmacovigilance for centralised veterinary medicinal products are laid down in Regulation (EC) 726/2004, as well as in Commission Regulation (EC) No. 540/95 (European Commission, 1995) and, for variations and urgent safety restrictions, in Commission Regulation (EC) No. 1085/2003 (European Commission, 2003).

The first routine involvement of the EMEA with respect to pharmacovigilance during the life cycle of a centralised product is at the time of the initial validation of a marketing authorisation dossier and application. At this point, the applicant for the marketing authorisation presents the detailed description of the pharmacovigilance system and, if appropriate, risk management systems that may be required. The applicant also provides proof of the availability of the services of a Qualified Person for Pharmacovigilance, which is examined as part of the validation process. For further details see Volume 9B. To ensure that applicants for marketing authorisations and marketing authorisation holders comply with their pharmacovigilance obligations and to facilitate compliance, pharmacovigilance inspections will be carried out by the national competent authorities (see Chapter 9).

Once the product is authorised the EMEA's role lies in the administration, management and evaluation of emerging pharmacovigilance data from the spontaneous reporting system and PSURs. It is also responsible for post-marketing surveillance data for centralised products. If necessary, and as a result of the CVMP evaluation of these data, the EMEA initiates and recommends appropriate follow-up measures and monitors

compliance with the legislative requirements. For details regarding the nature and content of reports, see Volume 9B and Chapter 11. The specific responsibilities of the marketing authorisation holders, member states and the EMEA for the submission and management of pharmacovigilance data are described in detail in Volume 9B. The principles of the electronic submission of data, the maintenance of the EudraVigilance Veterinary database and use of these data are described under the EudraVigilance Veterinary heading.

Following the issuing of a marketing authorisation for a centralised veterinary medicinal product by the European Commission, the Data Lock Points are determined, and these are used as the basis for the submission of PSURs and the periodicity of the PSUR cycle (see Volume 9B). The established PSUR cycle may be reset following a variation or extension that may have potential safety implications or if, due to emerging safety concerns, more frequent PSURs are considered necessary.

Following receipt by the EMEA of a PSUR and a review to establish its completeness and compliance with regulatory requirements, the rapporteur assesses the PSUR and presents the conclusions to the CVMP for endorsement. The assessment focuses on whether the benefit : risk assessment remains positive and whether risk management actions may be necessary.

The CVMP regularly assesses spontaneous adverse event reports submitted for centralised veterinary medicinal products for new and/or unexpected effects, the occurrence of more known adverse reactions which are more frequent than expected or other safety signals that could change the benefit : risk balance evaluation of the product.

The benefit : risk balance is also reviewed when the application for the renewal of the marketing authorisation of a product after 5 years is considered. Particular importance is given to assessment of the pharmacovigilance data to evaluate whether the benefit : risk balance of the product remains positive. The revised legislation foresees that any marketing authorisation, once renewed after the initial 5-year period, is then valid for an unlimited period. On justified pharmacovigilance grounds one additional renewal may be requested after a further 5-year period. At the time of writing in 2008, guidance for the criteria governing the requirement for a second renewal is being developed by the EMEA in co-operation with the European Commission.

The CVMP may also be required to review pharmacovigilance data for a centrally authorised veterinary medicinal product if a member state suspends the use of the product on its own territory in accordance with Article 45(4) of Regulation (EC) 726/2004, and if this action was triggered by pharmacovigilance data. In the case of such a suspension, the matter is then referred to the EMEA for the CVMP to consider the data and to prepare an opinion.

The EMEA has established a Crisis Management Plan, which allows rapid and efficient handling of crisis situations arising from safety issues involving a veterinary centrally authorised product. A crisis is defined as an event that occurs when new information that could have a serious impact on animal and/or public health is received for a veterinary centrally authorised product, and which requires immediate action. The Crisis Management Plan defines and implements a strategy for the rapid and efficient handling of crisis situations by the EMEA secretariat in liaison with the CVMP, the Rapporteur, the member states, the European Commission and the marketing authorisation holder (http://www.emea.europa.eu/index/indexv1.htm).

In cases where the CVMP considers regulatory risk management action necessary as a result of any of the above scenarios, and where the safety of the product has been re-evaluated in light of the new data, the EMEA communicates and co-operates with its partners, in particular the marketing authorisation holders, member states and the European Commission (for details see European Parliament and Council, 2004; European Commission, 2007a; http://www.emea.europa.eu/index/indexv1.htm). Veterinarians and other health care professionals, as well as the general public and international partners are informed,

where appropriate, of any significant safety issues.

The role of the EMEA in products authorised nationally

For veterinary medicinal products authorised through the mutual recognition or the decentralised procedure, as well as for purely national authorisations, the responsibilities for the conduct of pharmacovigilance lie with the national competent authority or authorities that issued the marketing authorisation. The CVMP becomes involved in the assessment of pharmacovigilance issues for these products when safety matters arising from pharmacovigilance information are referred to the CVMP.

This can be through a safety referral based on pharmacovigilance reasons under Articles 35 (or 40) of Directive 2001/82/EC, or when a member state considers that regulatory action on a marketing authorisation is necessary as a result of the evaluation of pharmacovigilance data in accordance with Article 78 of Directive 2001/82/EC.

A referral under Article 35 can be triggered by EU member states, by the European Commission or by applicants or marketing authorisation holders. The aim is to assess the specific safety issues addressed in the referral and to recommend conditions that apply to all veterinary medicinal products in the EU falling under the scope of the referral. Based on the CVMP's opinion, the European Commission then prepares a Commission Decision, which, once adopted, is binding for all marketing authorisations and member states concerned. Details on the procedure are described in the following references: European Parliament and Council (2001) and European Commission (2007b).

Article 78 of Directive 2001/82/EC establishes a procedure concerning the urgent measures required for nationally authorised veterinary medicinal products as a result of pharmacovigilance activities. When a member state considers, as a result of the evaluation of pharmacovigilance data, that a marketing authorisation should be suspended, withdrawn or varied according to the definitions specified in the legislation, the EMEA must be informed. The CVMP then considers the matter and the accompanying data, and issues an opinion. Specific guidance on the implementation of these provisions and the procedures for the CVMP is being prepared by the EMEA in co-operation with the Commission.

With its mandate to provide support to both the CVMP and national authorities, the PhVWP-V provides advice on the safety of veterinary medicinal products authorised nationally in order to enable effective and harmonised risk identification, assessment and management.

EudraVigilance Veterinary

Directive 2001/82/EC and Regulation (EC) No. 726/2004 lay down the requirements for the electronic reporting obligations of adverse events on an expedited and periodic basis, save in exceptional circumstances. Electronic reporting and data exchange are requested from the national competent authorities and from all marketing authorisation holders for all veterinary medicinal products authorised in the EU.

The EMEA, in collaboration with the European Commission and member states, established EudraVigilance Veterinary, the common European pharmacovigilance database and data-processing network for the exchange, processing and evaluation of adverse event reports, in accordance with Article 76 of Directive 2001/82/EC and Article 51 of Regulation (EC) 726/2004. The establishment of EudraVigilance Veterinary serves the following main objectives:

- It assists the rapid and secure transmission of adverse events reports between all partners by providing the necessary technical tools.
- It assists the administration and management of adverse events reports.
- It provides signal detection functionalities and supports scientific evaluation of adverse events reports.

- It establishes a central repository of highest quality data.
- It fully complies with the respective EU guidelines and international standards.

Two different routes have been established for registered users to report to EudraVigilance Veterinary: reporting via a Gateway or using the EudraVigilance Veterinary Web Reporting Module (EVWEB). These tools provide for the secure exchange of safety messages and data between pharmacovigilance parties. Using the EudraVigilance Gateway provides a fully automated way to exchange safety messages, e.g. between the locally established pharmacovigilance system of a marketing authorisation holder and the pharmacovigilance system of a partner of the EudraVigilance Veterinary community. The EVWEB is a web-based module and allows secure exchange of safety messages in a semi-automatic way to registered parties that do not have their own gateway established. Further information on EudraVigilance Veterinary and details on registration and tutorials are provided on the EudraVigilance Veterinary web page (EMEA, 2008).

A simplified electronic reporting form has been developed for use by marketing authorisation holders with limited experience of direct reporting of adverse reaction reports using the EVWEB. This takes into account that at present, and for many products, safety reports are sent only occasionally or intermittently and the marketing authorisation holders concerned are unlikely to become sufficiently familiar with EVWEB to ensure consistent data input. Such marketing authorisation holders may, in agreement with the national competent authority, use this simplified web-based form. To do so, no prior registration is required. The data created through this form are attached to an e-mail message from the user to the chosen national competent authority, which will upload the information directly into the EudraVigilance Veterinary database. For further details and a tutorial see the EudraVigilance Veterinary web page (EMEA, 2008).

Several member states have additional electronic reporting tools available for local marketing authorisation holders. These member states will ensure that the information collected through these systems is transferred to the Eudra Vigilance Veterinary database.

In order to facilitate reporting by veterinarians, an electronic form similar to the simplified electronic form for marketing authorisation holders is being developed. This form will also facilitate the transfer of the information to EudraVigilance Veterinary. The EudraVigilance Veterinary system allows that follow-up reports can be added to the initial reports, once additional information on the reported case becomes available.

Standard terminology has been agreed and established (e.g. specifically developed clinical terminology for the electronic reporting of adverse reactions to veterinary medicines, VEDDRA, lists of species and breeds or country codes) for the coding of most of the information submitted through EudraVigilance Veterinary (EMEA, 2008). It is recommended that for the non-coded information, in particular for that included in the narrative sections, English language should be used. Detailed advice on the transmission of electronic reports, the processing of electronic safety messages and processes for quality assurance, such as handling of duplicate reports, is described in European Commission (2007a).

Access to EudraVigilance Veterinary is as follows: only registered users may send and/or have direct access, with Regulatory authorities' users having read and write access to all reports in EudraVigilance Veterinary and all other registered users having restricted access to the data that they have submitted. Further specific guidance on access to EudraVigilance Veterinary data for regulatory authorities, marketing authorisation holders and the general public implementing the legal provisions of Article 57(1)(d) of Regulation (EC) 726/2004 is being developed.

In order to allow EudraVigilance Veterinary users to analyse safety data collected in the database, a data analysis system, the Eudra Data Warehouse, has been designed. The aim is to

reinforce the detection, evaluation and tracking of potential safety issues, thus allowing better-informed evaluations and decisions about the safety profile of particular veterinary medicinal products. The Eudra Data Warehouse provides for a range of general query tools for descriptive analysis of the scientific as well as the administrative data and more specific tools for signal detection (European Commission, 2007a; EMEA, 2008).

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4 Veterinary pharmacovigilance in France

G. Keck and X. Pineau

Introduction

The legal basis of French veterinary pharmacovigilance was established in 1999, and the pharmacovigilance scheme has been fully operational since January 2002. The National Agency for Veterinary Medicinal Products (Agence Nationale du Médicament Vétérinaire, ANMV) is responsible for organising and managing the pharmacovigilance scheme under the framework of the Food Safety Agency, the Agence Française de Sécurité Sanitaire des Aliments (AFSSA).

The basis of the French scheme lies in the existence of pharmacovigilance centres and a pharmacovigilance committee. The French decree was based on the transposition of European legislation, combined with an adaptation of the human pharmacovigilance scheme (30 regional pharmacovigilance centres located in teaching hospitals, with its own pharmacovigilance committee).

In France, the pharmacovigilance system is an 'interactive' concept: two centres of pharmacovigilance, located in the veterinary schools of Lyon and Nantes, are accessible directly by veterinary practitioners who wish to notify a suspected adverse reaction, usually by telephone, enabling a detailed description of the case to be obtained as well as allowing an immediate answer to be given to questions raised by the caller concerning the possible imputation of the suspected product(s) and any steps to take (additional tests, treatment, etc.). All information is recorded in a standard computerised form (Keck, 1992).

Pharmacovigilance data are regularly evaluated by a National Veterinary Pharmacovigilance Committee, which recommends to the authorities the steps to be taken in order to minimise the risks of adverse effects.

Veterinary pharmacovigilance centres (VPCs)

According to the French decrees, health professionals (veterinarians, pharmacists, poison control centres) must report serious suspected adverse reactions in animals or suspected adverse reactions in humans to one of the two pharmacovigilance centres (VPCs). Health professionals are also encouraged to report non-serious adverse reactions and other incidents (lack of expected efficacy, MRL violations/withdrawal period issues and environmental problems).

The VPCs are intermediate bodies at the interface between reporters and the ANMV and play a

key role in the so-called interactive system. Unique in Europe, the two VPCs (part of the Agency's pharmacovigilance department) are located in the veterinary schools of Lyon and Nantes, respectively. Most of the time (95%), reports are made by telephone. Information is exchanged directly between the reporter and trained pharmacovigilance personnel. The reporter obtains useful information for the management of the suspected adverse reaction (first evaluation of clinical plausibility of drug action, relevant therapy, prognosis), while the VPC collects initial data for construction of the case report. A report form is later sent to the reporter, for the subsequent provision of follow-up information.

The VPCs share their 'hotlines' with animal poison control centres that have now been operating for a considerable time (30 years for the Lyon's poison control centre). This permits a round-the-clock service (out-of hours, calls are dealt with by poison control centre personnel and the information collected is later transmitted to the VPC). The extensive experience gained in the management of pharmacovigilance cases has been beneficial to the VPCs since their launch and it explains the large number of cases (around 2,500 SARs per year).

The VPCs perform causality assessment, and ensure electronic transmission of the reports to the ANMV. They also provide the relevant information to the marketing authorisation holder. Serious suspected adverse reactions in animals or suspected adverse reactions in humans are transmitted in an expedited manner (within 15 days), while other cases (e.g. non-serious, lack of efficacy) are transmitted quarterly. Companies must also transmit their own cases to the ANMV at defined intervals (in an expedited manner or every 6 months to every 5 years, as appropriate, depending on the type of adverse reaction(s)).

Relationships with the veterinary pharmaceutical industry are permanent and operate at various levels, including a pharmacovigilance working group of the Syndicat de l'Industrie du Médicament Vétérinaire (SIMV) (Veterinary Pharmaceutical Industry Syndicate) which has collaborated for several years particularly with regard to specific and mutual technical procedures, including the development of data entry forms, the specific computer software 'Sentinel-Vet', good pharmacovigilance practice, regular two-way exchanges of information about unexpected adverse effects, and agreements to evaluate the incidence of adverse drug reactions for new veterinary medicinal products.

Information is also exchanged with the European Medicines Agency (EMEA) and with veterinary pharmacovigilance operations in agencies in other EU member states. The pharmacovigilance system in France, with its interrelationships, is summarised in *Figure 4.1*.

Causality assessment or 'imputability'

In France, the Sentinel-Vet programme mentioned above, which is a specific software package for veterinary pharmacovigilance, uses the ABON classification discussed elsewhere in this book (see the Introduction and Chapters 2 and 27). This programme is used by the two VPCs, the ANMV and some pharmaceutical companies. The programme has an internal causality algorithm, adapted from the one created by the French human pharmacovigilance system which combines what is known as intrinsic and extrinsic imputability (or causality) of the suspected adverse effect (Pineau, 1997).

Intrinsic imputability considers:

- Chronological data: time to onset of signs, effect of dechallenge (when drug treatment is stopped) or rechallenge (when the drug is re-administered).
- Semiological data: are the clinical signs suggestive of an effect of the drug or not? Have other causes been evaluated?

Extrinsic imputability considers the following:

- Is the adverse effect known, e.g. published in the basic literature or mentioned in the SPC?
- The dose: the numbers of animals reacting/animals treated may also be taken into account.

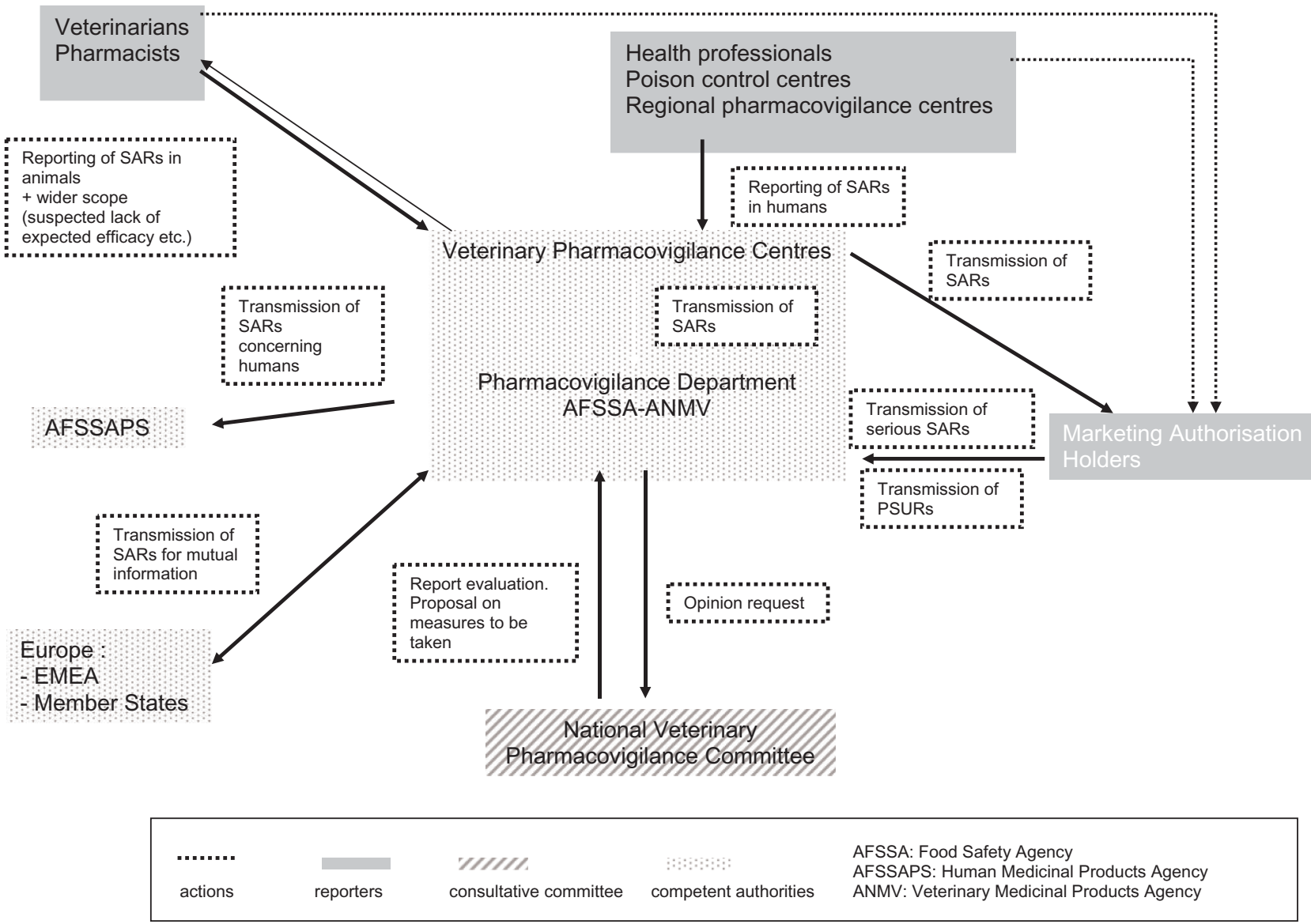

Fig. 4.1 The veterinary pharmacovigilance centres in France and their interrelationships with other organisations involved.

Table 4.1 Selected list of opinions provided by the French Veterinary Pharmacovigilance Committee (http://www.anmv.afssa.fr/pharmacovigilance/) (original documents in French).

Opinion CNPV-20 (13 June 2006) regarding measures to be taken to prevent adverse effects after exposure to deltamethrin-based antiparasitic collars
Opinion CNPV-18 (13 June 2006) regarding measures to be taken to prevent adverse effects after exposure to amitraz-based solution for cattle, sheep, goat and pig
Opinion CNPV-14 (6 December 2005) regarding measures to be taken to prevent adverse effects after use of imidacloprid in humans and carnivores
Opinion CNPV-13 (6 December 2005) regarding measures to be taken to prevent adverse effects after administration of drying-off intramammary suspensions in cattle
Opinion CNPV-06 (29 June 2005) regarding measures to be taken to prevent adverse effects after administration of vitamin E-selenium-based products in ruminants
Opinion CNPV-04 (15 June 2004) regarding measures to be taken to prevent adverse effects after off-label use of fipronil in rabbits

Each criterion has a specific weighting which is used automatically by the algorithm on a specific scale to classify the case in the ABON categories.

Unexpected adverse drug reactions (ADRs), which usually belong to the category 'O', give rise to an 'automatic alert' for the product in question:

- First level: highlights cases previously assessed as O or N, which may be re-examined.
- Second level: highlights cases previously assessed as O or N and associated with the same type of symptoms or pathological disorders, e.g. inflammation of the digestive tract. This provides stronger supporting evidence as well as an indication of the possible mechanism of action underlying the ADR.

The national veterinary pharmacovigilance committee

The National Veterinary Pharmacovigilance Committee is composed of an expert panel from the pharmacovigilance centres, the ANMV, the industry, the veterinary profession (practitioners and teachers), pharmacists and members of the Human Pharmacovigilance Scheme. This Committee provides the ANMV with evaluation reports and opinions on a specific product or therapeutic group. It proposes measures to be taken, if necessary, to ensure minimisation of risk of adverse reactions. The ANMV has the responsibility of taking adequate measures, if necessary (e.g. modifications of product literature, authorisation suspension or withdrawal).

According to the transparency policy of the ANMV, the evaluation reports and opinions are made available to the public on the ANMV's website. Some selected opinions are shown in *Table 4.1*. The Committee also publishes a pharmacovigilance newsletter twice a year.

Veterinarians and other health professionals receive several levels of feedback on pharmacovigilance data:

- Each reporter receives information during a telephone exchange, as explained above.
- Report forms (spontaneously sent or following an initial phone call) are acknowledged, and reporters receive a letter including causality assessment and comment.
- Committee newsletters are sent to all veterinarians (and pharmacists), with reference to opinion and evaluation reports.
- Members of the Committee participate in major veterinary conference events or continuing education sessions.

- Veterinary students are sensitised to their future reporting duty (regulatory basis and 'real-life' examples are provided by the VPC, which may stimulate their interest in the topic).

The place of veterinary pharmacovigilance in the initial and postgraduate training of veterinarians has been considerably developed: students participate actively in the functioning of the VPC. Pharmacovigilance surveys have been published as reports, as have theses, for example on immunological reactions after warble fly treatments, shock following sulphonamide-trimethoprim injections in horses and microbiological incidents after intramammary treatment of dry cows (Raguet *et al.*, 1995).

Collaborations with human pharmacovigilance and toxicovigilance centres have been developed in various areas, including methods of collecting and assessing data and the risks and effects of veterinary product residues in animal food (e.g. clenbuterol residues in calf liver, which have led to several cases of cardiotoxicity in humans in 1993 in France (Pulce *et al.*, 1991).

Key figures for 2006

By way of illustration, the major findings for 2006 are shown below. In that year, the ANMV received more than 6,500 reports:

- 50% were spontaneous case reports that occurred in France and were mostly transmitted by the two VPCs.
- 15% were reports from other countries.
- 36% were reports from PSURs.

When the 3,190 spontaneous case records that occurred in France are analysed, the trends are consistent with the previously observed data in France and, except for the origin of the reports via the VPC, in European countries (Keck and Ibrahim, 2001). The great majority (86%) of reports came from the VPC, 14% from marketing authorisation holders.

Primary reporters were veterinarians (88% of cases reported to the VPC) followed by animal owners (5%) (*Figure 4.2*). Veterinary schools reported only a few cases, despite the fact that they are regularly encouraged to report. Pharmacists reported very few cases. Human poison control centres reported cases concerning human exposure to veterinary drugs.

The species involved are mostly companion animals (42% dogs and 38% cats; *Figure 4.3*). Reports concerning 'new companion animals' such as dwarf rabbits and snakes are increasing. Far fewer reports involve large animals and animals that are intensively farmed (poultry, pigs). However, despite the regular decreases in percentage due to the higher proportion of companion animals, the number of calls is relatively constant from one year to the next. Besides animal health considerations, there are often concerns about chemical residues in animal products (milk, meat, eggs).

These differences are observed also in toxicovigilance and are probably linked to the higher status of pets and socio-economic considerations (Keck *et al.*, 2004). Special susceptibility to toxicity is an important aspect of the findings, as demonstrated by the numerous cases of permethrin intoxications in cats or fipronil toxicity in dwarf rabbits.

Calls concerning humans are relatively numerous (around 5%). They frequently involve exposure to antiparasitic drugs or the accidental self-injection of vaccines.

The seriousness of the cases reported is shown in *Figure 4.4*. About 23% of reports were considered as suspected serious adverse reactions (fatal or life threatening) in animals. In humans (5%), all reports are considered as serious, whatever the severity of the case. The remaining reactions were considered as non-serious. The high percentage (11%) of cases of asymptomatic exposure was associated with queries from veterinarians regarding (for example) suspected over-dosages.

The therapeutic groups involved are shown in *Figure 4.5*. A high number (43%) of reports concern antiparasitic products (mostly ectoparasiticides),

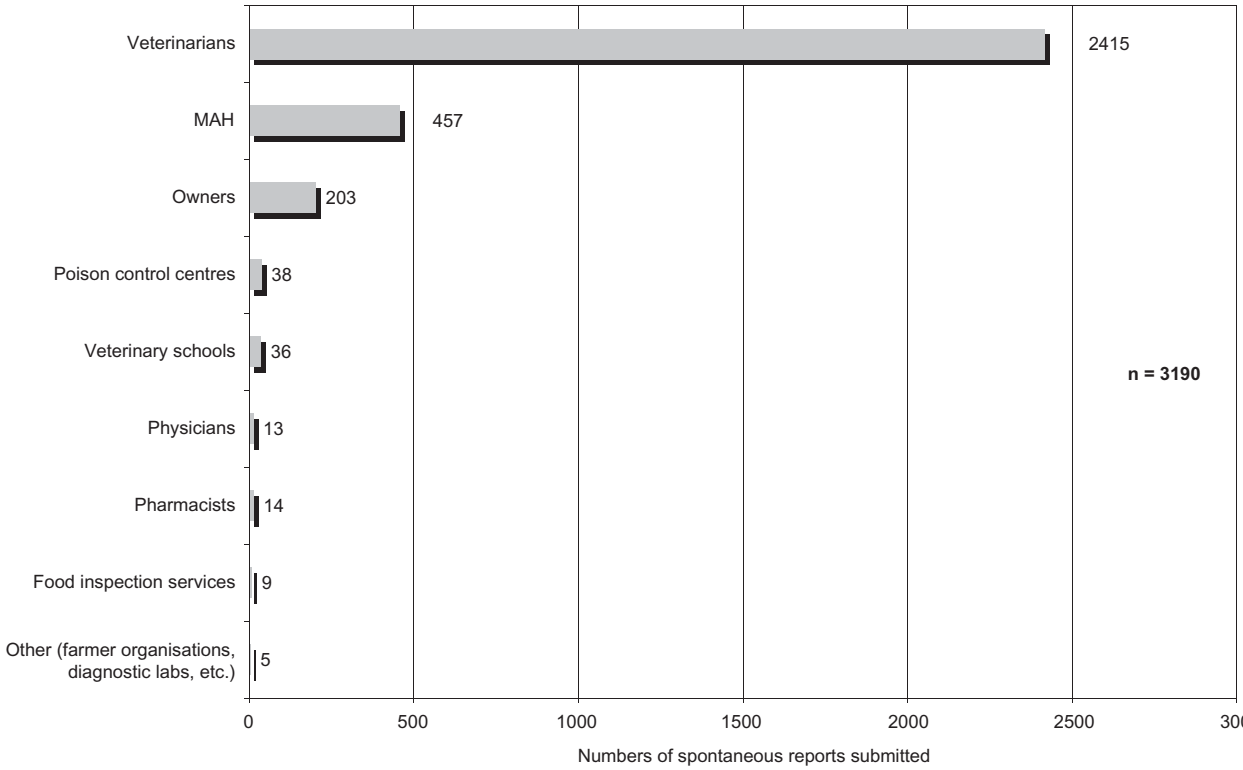

Fig. 4.2 Categories of reporter. MAH – marketing authorisation holder; note, there are no details regarding the primary reporter in MAH cases. These values are for spontaneous reports only: serious cases from MAHs (excluding PSURs) and serious/non-serious cases from VPCs. (Source: AFSSA Annual Report 2006.)

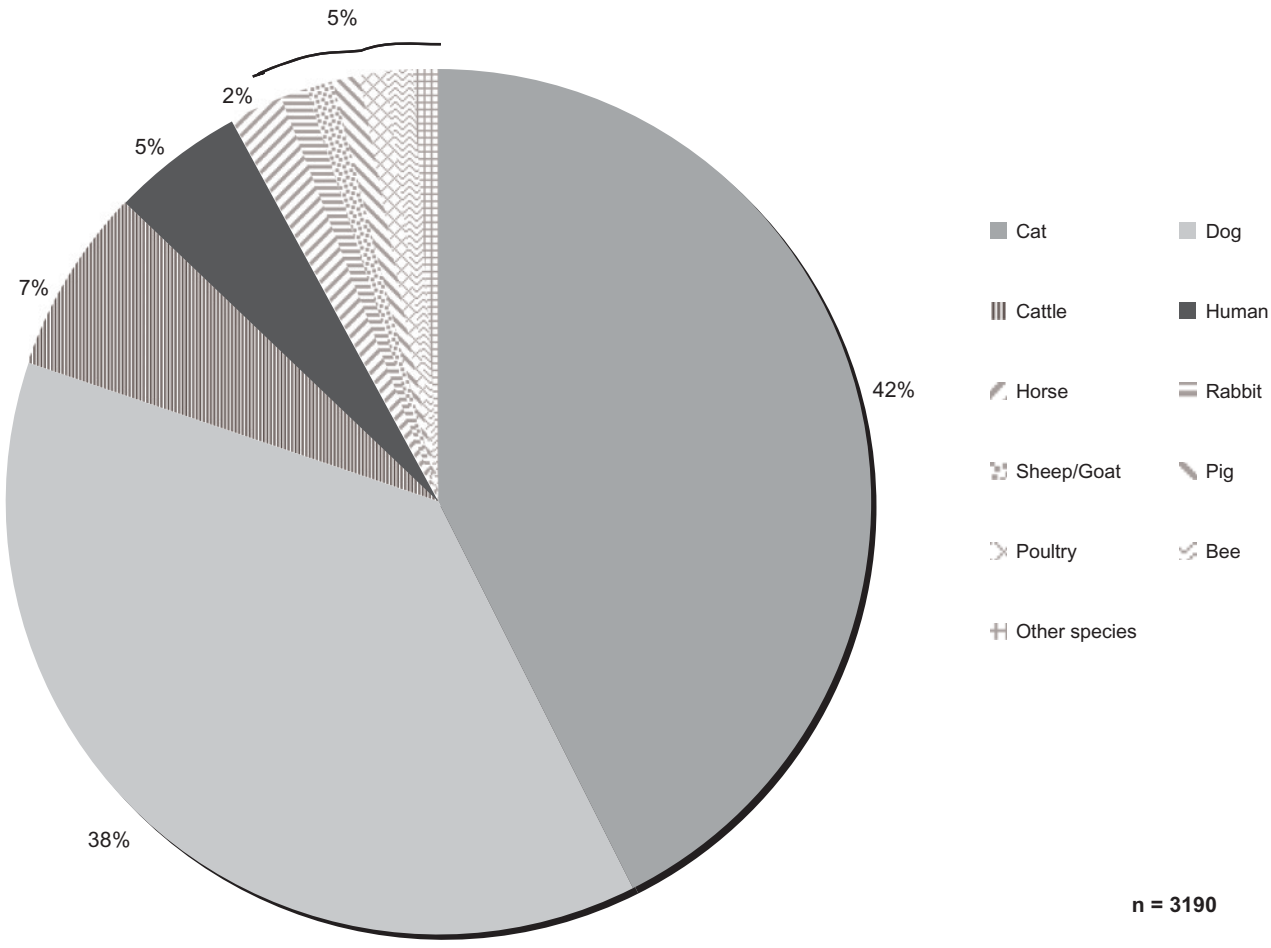

Fig. 4.3 Species involved. (Source AFSSA Annual Report 2006.)

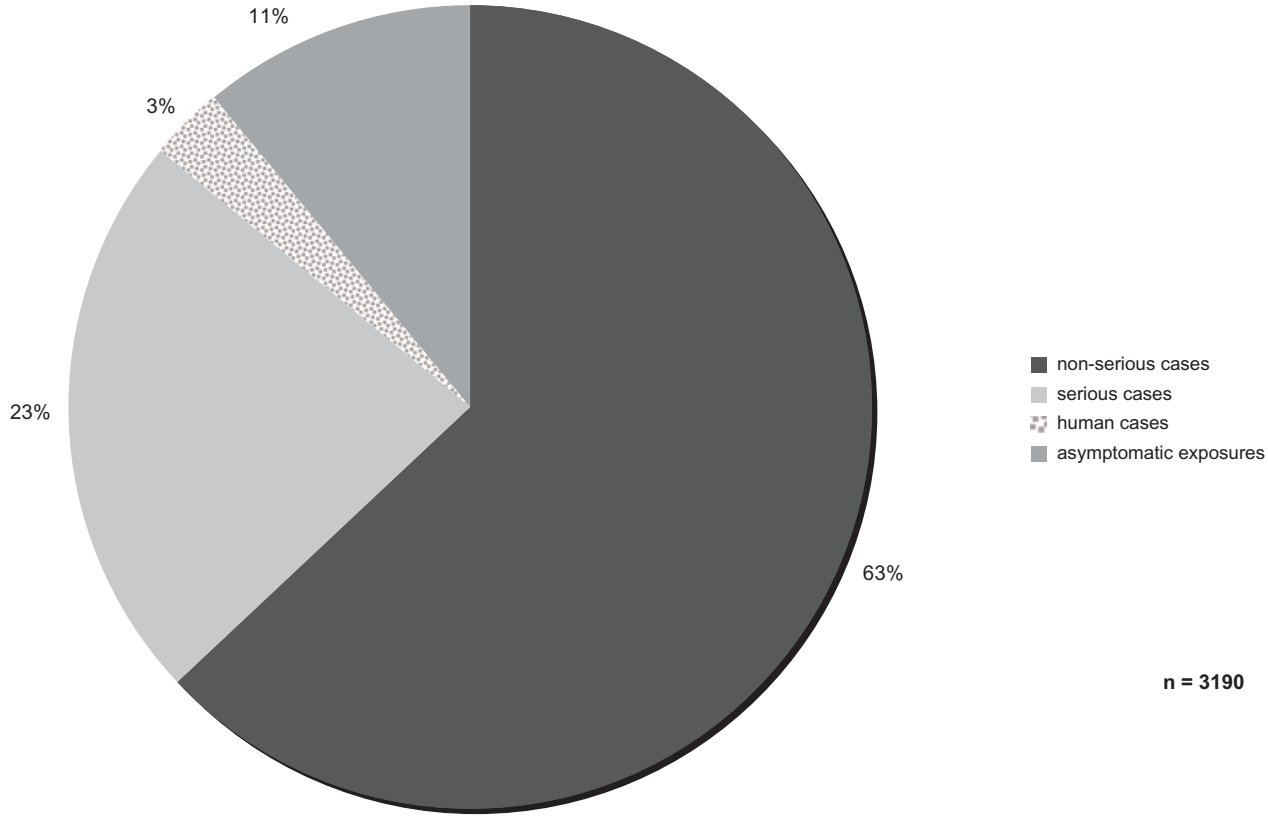

Fig. 4.4 Seriousness of cases reported. Note: PSUR cases not included; only non-serious spontaneous reports transmitted by VPCs. (Source: AFSSA Annual Report 2006.)

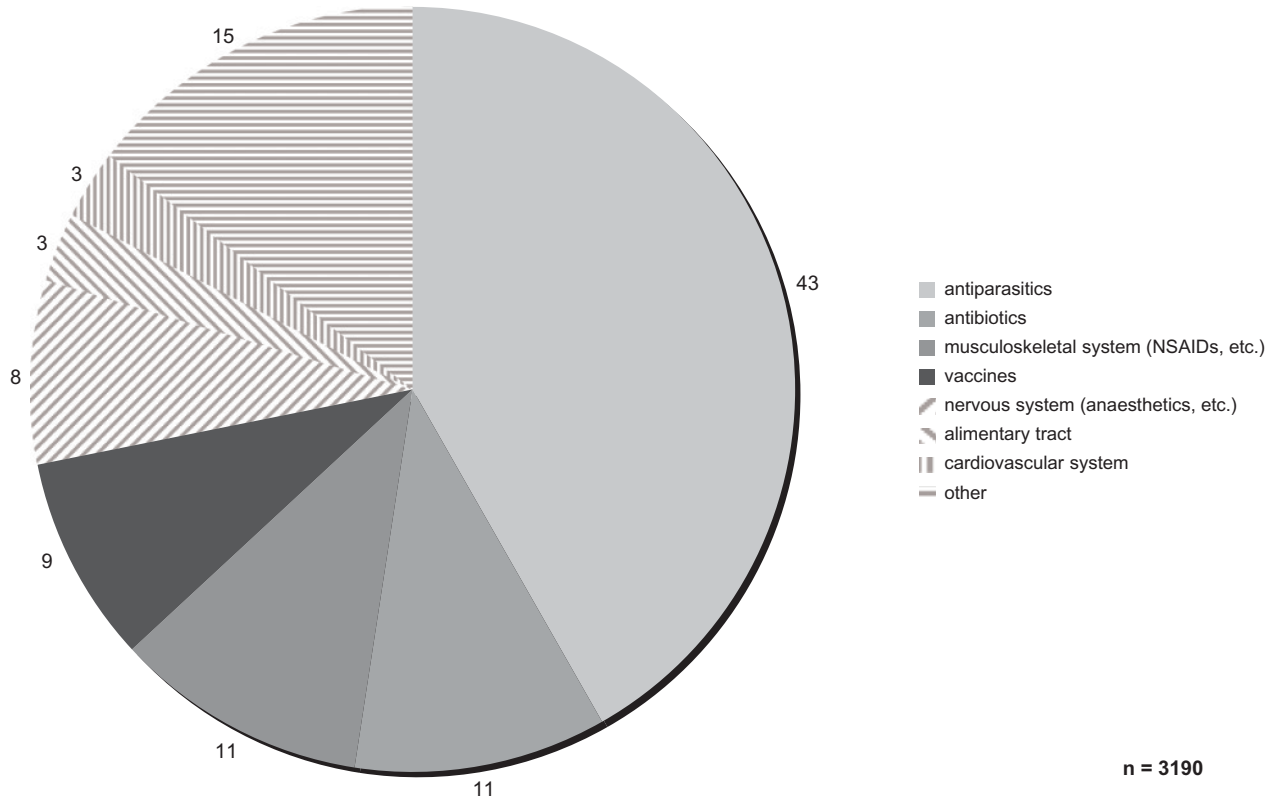

Fig. 4.5 Therapeutic groups using ATCvet code nomenclature. Note: total is more than 100%, as one report may involve two or more therapeutic groups. (Source: AFSSA Annual Report 2006.)

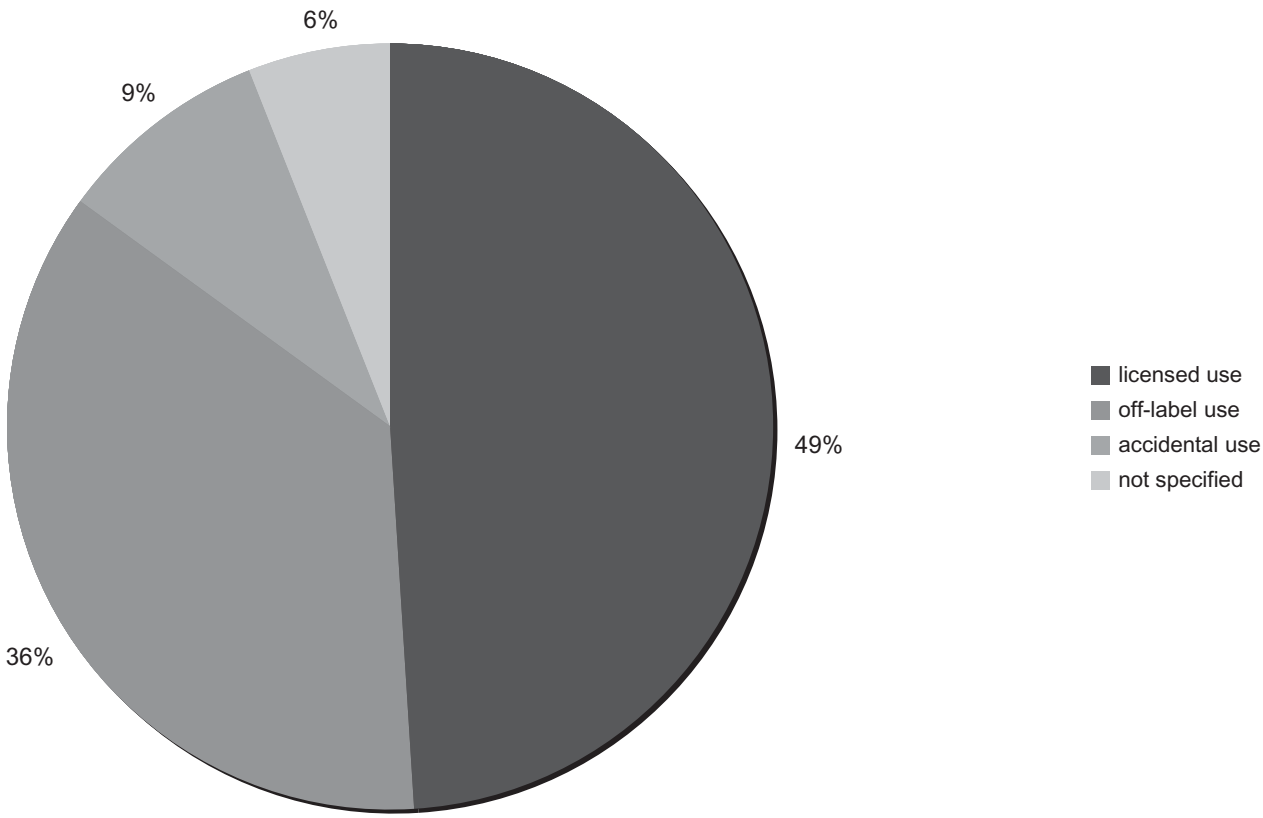

Fig. 4.6 Types of use categories. Note: the number of cases for 2006 was less than for 2005 as 2005 also included values for third country reports. (Source: AFSSA Annual Report 2006.)

followed by antibiotics (11%), drugs intended for the treatment of musculoskeletal system disorders (mainly anti-inflammatories) (11%), vaccines (9%) and drugs acting on the central nervous system (mainly anaesthetics) (8%).

Suspected lack of efficacy involved antimicrobials, antiparasitics and vaccines. Spontaneous individual reports provided further information in this field. However, the system is not suitable for detailed epidemio-surveillance for resistance, which is better dealt with by networks employing detailed bacteriological analyses.

The type of drug use categories are described in *Figure 4.6*. About half (49%) of reports occurred following licensed use of the products, while the other half (51%) were off-label use, accidental use or not specified.

Causality assessment (*Figure 4.7*) produced the following distribution: about half (49%) the reports were assigned causality A (probable) or B (possible), 35% were assessed as O (unclassified) and only 4% as N (unlikely).

Other considerations

Residues and violation of withdrawal periods were recently included in the field of veterinary pharmacovigilance. Spontaneous individual reports of antibiotic residues in milk, for example, may suggest an insufficient withdrawal period, maybe due to biological variations in the treated animals. In most cases, they are associated with non-respect of the conditions of use, as shown by surveys of the Groupements Techniques Vétérinaires in France (Raguet *et al.*, 1995). They may sometimes concern the illegal use of veterinary products such as clenbuterol, for which our centre was involved in the detection of human adverse

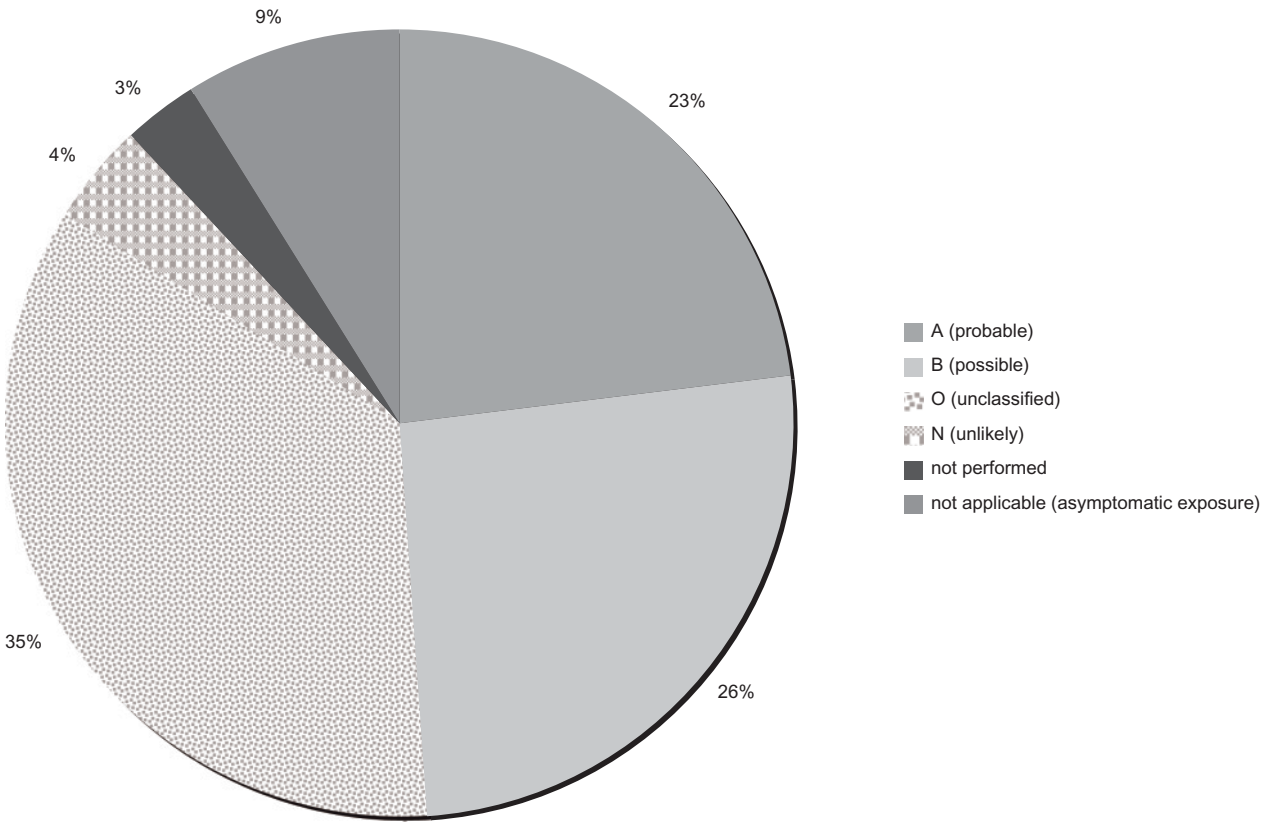

Fig. 4.7 Causality assessment. Note: in 2005, third country reports from MAHs were included; in 2006, only cases that occurred in France were taken into account. This explains why the number of cases for 2005 was more than for 2006. (Source: AFSSA Annual Report 2006.)

effects after the consumption of calf liver (Pulce *et al*., 1991).

Ecotoxicology and environmental issues which are now intensively considered in the pre-registration stage have been included in the field of post-marketing surveillance. This is pertinent in some cases such as aquaculture treatments or endectocides residues in faeces of cattle or horses, but probably needs additional expertise in the field to engender spontaneous reports, which could, however, constitute an important signal.

Conclusion

Veterinary pharmacovigilance has shown remarkable development in recent years. As with every new branch of science, there is still much to be achieved, especially concerning the scientific aspects: the regulatory framework has probably grown more rapidly than the involvement of veterinary research and teaching institutions, which could be more active in the field of ADR notifications as a source of clinical observations but also as structures of veterinary pharmacovigilance, associated with the regulatory authorities.

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5 Pharmacovigilance in Germany

C. Ibrahim and A. Wilke

Introduction

Following the granting of a marketing authorisation, and even though the requirements for authorisation relating to quality, efficacy and safety of the products are already very demanding, veterinary medicines must be continuously evaluated, to monitor the balance between their benefits and risks and to ensure that the benefit : risk balance remains positive.

For this objective, pharmacovigilance of veterinary drugs should improve the knowledge and prevention of adverse drug reactions (ADRs) in target animals, and improve the surveillance of possible harmful effects to humans as users or handlers of veterinary medicinal products or persons who come into contact with treated animals. It should also assist in the post-marketing surveillance of adverse environmental effects.

The wide scope of veterinary pharmacovigilance, in addition to clinical adverse reactions, also includes lack of expected efficacy, adverse reactions after extra-label use and suspected inadequate withdrawal periods after the use of products in food-producing animals (Keck and Ibrahim, 2001).

In Germany, two main national regulatory authorities are responsible for the authorisation of veterinary medicinal products and thus also for post-marketing activities, including pharmacovigilance. The Federal Office for Consumer Protection and Food Safety (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, BVL) in Berlin is responsible for all chemically defined pharmaceuticals, homeopathics, herbal drugs, drugs used in alternative medicine and medical devices. In future it will also have responsibility for the authorisation of veterinary blood products and haemovigilance. The Paul-Ehrlich-Institute (PEI) in Langen is the regulatory authority for veterinary vaccines and sera. In addition, the Friedrich-Loeffler Institute (FLI) at Insel Riems is the competent authority for the authorisation of exotic vaccines which are administered in government-initiated vaccination schemes against diseases such as blue tongue, avian influenza and swine fever. The FLI is also responsible for post-marketing surveillance of these products, and for veterinary diagnostics.

Due to the decentralised federal system in Germany, the Federal Regional Authorities (Bundesländer) play a role in the monitoring of the safety of medicinal products, mainly in surveillance of compliance with marketing authorisation conditions, by conducting pre- and post-approval inspections of Good Manufacturing Practice (GMP) and Good Clinical Practice

(GCP). However, they are not responsible for pharmacovigilance inspections, as the latter are a new task for the BVL. Pharmacovigilance inspections for vaccines have not yet been transposed into national law.

The overall tasks involved in veterinary pharmacovigilance are essentially the same for pharmaceuticals and biological products. The legislative framework is derived from EU Regulation (EC) No. 726/2004 (European Parliament, 2004b) and Directive 2001/82/EC, as amended by Directive 2004/28/EC (European Parliament, 2004a), which have been transposed into German national law and also the Guidelines contained in Volume 9 (European Commission, 2004) and subsequently Volume 9B of the *Rules Governing Medicinal Products* in the EU. This legislation and guidance does not differentiate between pharmaceuticals and vaccines. However, at national level there is a basic difference as these are regulated by different agencies. Veterinary pharmaceuticals are regulated identically to human medicinal products in accordance with the Medicines Act (Arzneimittelgesetz, AMG) (Anonymous, 2005a) under supervision of the Ministry of Health, whereas veterinary vaccines are subject to the regulation for animal vaccines (Tierimpfstoffverordnung) (Anonymous, 2006), thus giving a slightly different legal base, under the supervision of the Ministry of Food, Agriculture and Consumer Protection. The following description of the German system will concentrate mainly on the veterinary pharmaceuticals under the responsibility of the BVL.

The spontaneous reporting system was established on the veterinary side as early as 1980 in what at that time was the responsible regulatory authority, namely the Federal Office of Health (Bundesgesundheitsamt). Since 1978 the national Medicines Act has given an official mandate to the regulatory authorities for human and veterinary medicines, to collect and evaluate ADRs and implement regulatory measures for risk management if necessary.

For the first 10 years, the spontaneous system on the veterinary side only existed at a very basic level, with an average of 10–15 reports a year. At that time there was only one veterinarian working on pharmacovigilance, among many other tasks. The cases were first collected on paper sheets and then in a simple Microsoft Access® database without standard terminologies. The system did not allow for specific queries. The pharmacovigilance system has since made significant progress.

Operationally, it became more successful in the 1990s, especially after 1995 when pharmacovigilance activities at the EU level also increased with the establishment of the European Medicines Agency (EMEA). This was due partly to better resources and more scientific and technical-administrative staff, but also to new ways of promotion of the pharmacovigilance system, especially by providing better feedback of information on adverse reactions to veterinary practitioners. The current structure of the Department for Veterinary Medicinal Products within the BVL consists of five units and a total of 85 staff members. Of these, 44 are scientists including veterinarians, pharmacists, biologists, chemists and biochemists. The interdisciplinary qualifications of the staff are useful as they cover the whole range of aspects and tasks for the authorisation of veterinary medicinal products. The non-scientific staff such as project managers provide administrative support. For post-marketing issues there is a special unit called 'Support and Surveillance after Authorisation' with 14 veterinarians and two biologists. Three project managers complete the staff.

This unit deals with pharmacovigilance, such as spontaneous ADR reports and periodic safety updates (PSURs), risk assessment, risk management, risk communication, renewals and safety-related variations, evaluation of pharmacovigilance system descriptions in authorisation dossiers and pharmacovigilance inspections. This separation into pre- and post-marketing units has proven quite useful, as it gives a good overview of interrelated and interacting issues.

In Germany there are currently 2,033 veterinary products on the market. They can be split according to the procedures used to obtain the marketing authorisations:

Table 5.1 Companion animals in Germany (Industrieverband Heimtierbedarf e.V., 2007).

Companion animal or related	*Numbers (million)*
Aquariums	1.95
Terrariums	0.42
Garden ponds with fish	1.4
Cats	7.8
Dogs	5.3
Rodents	6.3
Birds	3.8

- 102 centrally authorised;
- 265 mutual recognition and decentralised products;
- 1,666 nationally authorised.

The farm animal population in Germany comprises 12.6 million cattle, 27.1 million pigs, 2.4 million sheep and 120.6 million poultry of which 107.3 million are chickens (50.6 million laying hens and 56.8 million broilers), 10.6 million turkeys, 2.4 million ducks and 0.3 million geese (Statistisches Bundesamt, 2008). About 1 million horses (Deutsche Reiterliche Vereinigung e.V., 2002), 5.3 million dogs and 7.8 million cats are kept in Germany. The majority of these products are authorised for these species, although some like sheep, turkeys, ducks and geese have a 'minor species' status, meaning that few products have an explicit authorisation for them as the 'target species'. Companion animal numbers are shown in *Table 5.1*.

Pet and companion animals such as ferrets, hamsters, guinea pigs, dwarf rabbits, gerbils, exotic birds, fish and reptiles also have a 'minor species' status with regard to products authorised for them, meaning that extra-label use frequently occurs because very few or no products are specifically authorised. Thus 'extra-label use' cases are the rule for these species and they represent a significant number of ADR reports on the whole.

A glance at the animal health products market in Germany (Bundesverband für Tiergesundheit e.V. (BfT), 2006[1]) shows that parasiticides (ecto- and endoparasiticides) and antibiotics (antiparasitics and anti-infectives, respectively, in the table) represent the largest part of the market at 49%. All other pharmaceutical specialities account for 27%, followed by vaccines (biologicals) with 24% of the whole market share (*Table 5.2*).

Table 5.2 Market for veterinary medicinal products in Germany (Bundesverband für Tiergesundheit e.V., 2006).

Veterinary medicinal product	*Value to market (€ million); market share*
Biologicals	138; 24%
Anti-infectives	184; 32%
Antiparasitics	100; 17%
Pharmaceutical specialities	155; 27%

In terms of animal species, the products intended for pet animal products are increasing and currently represent more than 50% of the market, whereas the products for food-producing animals are decreasing. This also reflects the situation of the German agriculture business, where economic pressures and challenges reign. A survey conducted between May and November 2007 showed a decrease in the cattle and pig population of 0.6%, while the prices for piglets and pork meat are responsible for the temporary downwards trend in pig production. There are also regional differences in Germany and recently the increase in prices for milk and milk products has led to a slight increase in dairy cattle numbers in northern Germany, whereas in southern Bundesländer, such as Baden Württemberg or Hessen, cattle numbers are decreasing.

Reporting routes and players in the spontaneous reporting scheme

Under German national law, the Medicines Act makes it mandatory for marketing authorisation

[1] Aennchenplatz 6, 53173, Bonn, Germany, http://www.bft-online.org/.

holders (MAHs) to report all ADRs coming to their knowledge to the regulatory authority (RA), serious ones within the expedited 15-day time frame and non-serious ones included in the PSUR within the given frequency. Most reports (about 80% of all reports) that the BVL receives come via the MAHs. Compliance with pharmacovigilance obligations has increased especially during the last 2 years, one reason being the introduction of new EU legislation with the requirement to establish and describe the company pharmacovigilance system in the marketing authorisation application dossier. Perhaps the main reason is the introduction of pharmacovigilance inspections by the BVL in 2006. These new legal requirements have indeed increased awareness and the importance of pharmacovigilance amongst pharmaceutical companies.

The majority of cases reported, around two thirds, come from third countries, particularly the USA, Canada, Brazil, Japan and other EU member states. Numbers from Germany, which are clearly the special focus for the BVL, still remain comparatively low. In this chapter the focus is only on the cases occurring in Germany.

The primary reporting source is the veterinary practitioner. For veterinarians the reporting of ADRs is not mandatory, but there is an ethical obligation laid down in the professional code for veterinarians (Tierärztekammern). This relates to a veterinarian's responsibility for public and animal health and animal welfare. The veterinarian has the option to report either directly to the regulatory authority (BVL or PEI), or to the Drug Commission of the Veterinary Medical Association or to the MAH. According to the percentage of reports coming via the MAHs, this is apparently the preferred route of reporting. The reasons may be (1) that veterinarians wish to consult the MAH on its product's pharmacological characteristics and (2) because of insurance and indemnity issues. The veterinarian is most certainly the key player in the functioning of the spontaneous reporting system. Very often he or she is the direct eye-witness to the adverse reaction and has the most detailed background information on the patient, including anamnesis, co-medication, previous treatments, rechallenge and dechallenge. Therefore, good participation by veterinarians is crucial.

A significant effort has been invested by BVL staff in recent years in promoting the system and motivating veterinarians to make use of it. The most important way to motivate veterinarians is to provide feedback by publishing the findings and analyses of the cases in professional journals (Simon, 1999). In addition, a simple guide explaining the pharmacovigilance (PHV) system, which is based on an EU document designed and created by the CVMP Pharmacovigilance Working Party and has been translated into all the national languages of the member states, was distributed by the official veterinary journal *Deutsches Tierärzteblatt* in September 2007 to all 35,000 veterinarians in Germany (EMEA, 2005c). Although under-reporting is still a problem, especially relating to non-serious and expected reactions, numbers of reports have increased continuously over the last few years, as can easily be seen in *Figure 5.1*.

The increasing number of reports received between 2006 and 2007 (in fact double) does not signify an increased risk, but only an increased awareness of and better compliance with pharmacovigilance obligations (Wilke and Ibrahim, 2007). For industry, one of the triggering factors can be attributed to the introduction of pharmacovigilance inspections, as already mentioned. However, a 50% increase in reports from veterinarians was also noted. In this latter case, this may be due to the efforts involved in promoting the reporting system, especially through publications and by participation in talks given by BVL staff members at veterinary conferences and seminars. The inclusion of pharmacovigilance issues in lectures at universities may also explain the better use of the system by younger vets.

As veterinarians are allowed to dispense and sell veterinary medicinal products directly to animal owners, only a small proportion of non-prescription products is sold through pharmacists. Thus the number of reports received by pharmacists or their professional association is relatively small. Nevertheless members of BVL

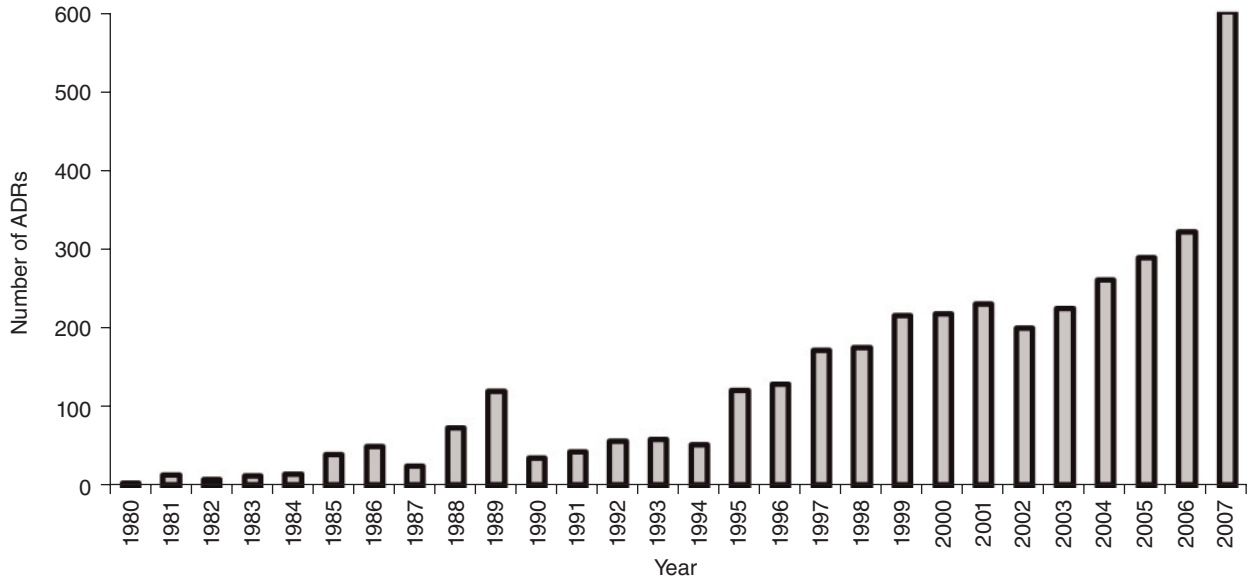

Fig. 5.1 Number of national ADRs per year reported to the BVL (without counting duplicates and follow-ups).

staff also give talks to pharmacists in the framework of their professional continuous learning, to increase awareness of potential risks related to the use of veterinary products and to enable them to give better advice to animal owners.

A small fraction of reports are received from the general public, including farmers and other animal owners. The BVL accepts these reports, although the route via the veterinarian is considered the best and preferred one. The veterinarian has the appropriate qualifications and expertise, resulting in better and more professional reports.

Whichever way of reporting is chosen and whoever the report comes from, the national regulatory authorities, the BVL and the PEI, are the final and only institutions where the ADR reports are collected and evaluated in specific central databases. ADR reports obtained from the published literature, peer-reviewed or not, are also taken into consideration by the regulatory authorities, either as received from the MAH or through regular monitoring of databases by BVL staff.

The nature of ADR reports

Most reports from the spontaneous reporting system are serious ADRs in animals (50%) or ADRs related to human beings (8%) (see *Figure 5.2*). These serious reports in animals can be split further into ADRs with (36%) and without (24%) fatalities. About 32% of the reports are related to non-serious ADRs in animals.

The majority of the reports are related to companion animals, especially dogs and cats (*Figure 5.3*). For horses – which for pharmacovigilance purposes are defined as companion animals as well – the numbers of reports are quite low. For farm animals such as cattle and pigs, the total number of reports is even low. However, the numbers of animals concerned in these ADRs are higher than in the companion animal group. This is related to the fact that farm animals are usually medicated in herds or flocks. Individual treatments are done only in exceptional circumstances. Companion animals are typically treated individually and the related figures show that there are only a few animals affected in each report. The ADRs received for 'minor species' are summarised under 'Other' (see *Figure 5.3*). This group includes dwarf rabbits, guinea pigs, ferrets, birds, bees and turtles.

All products containing the same active substance in the same pharmaceutical formulation are given the same Anatomical Therapeutic Chemical classification system for veterinary

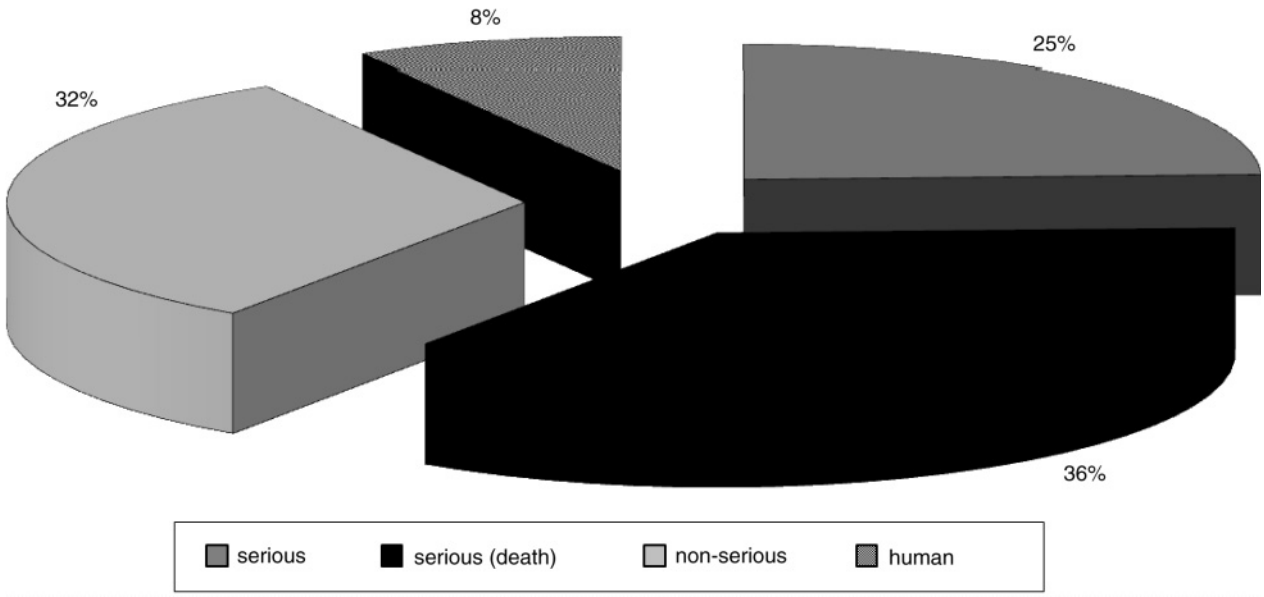

Fig. 5.2 ADRs occurring in Germany in 2007.

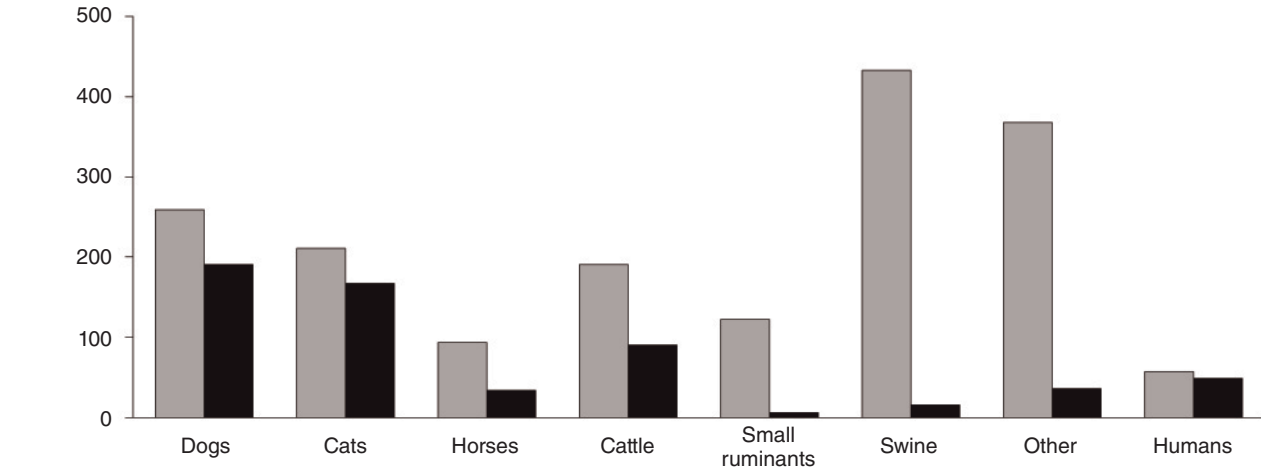

Fig. 5.3 ADRs that occurred in Germany in 2007, stratified by species with number of reacting individuals.

medicines (ATCvet code categories) (Dahlin *et al.*, 2001). *Figure 5.4* shows the veterinary medicinal products involved stratified by their main ATCvet code. Most of the reports are related to antiparasitic products, insecticides and repellents (QP) or anti-infectives for systemic use (QJ). These products are most frequently used in veterinary medicines, and therefore higher numbers of ADRs are expected. It by no means suggests a higher risk for these products.

Drugs acting on the nervous system (QN) are mainly anaesthetics or anxiolytics. The alimentary tract and metabolism (QA) group primarily consists of drugs such as antidiarrhoeals and spasmolytics. However, some vitamin supplements are also included. Some of these injectable

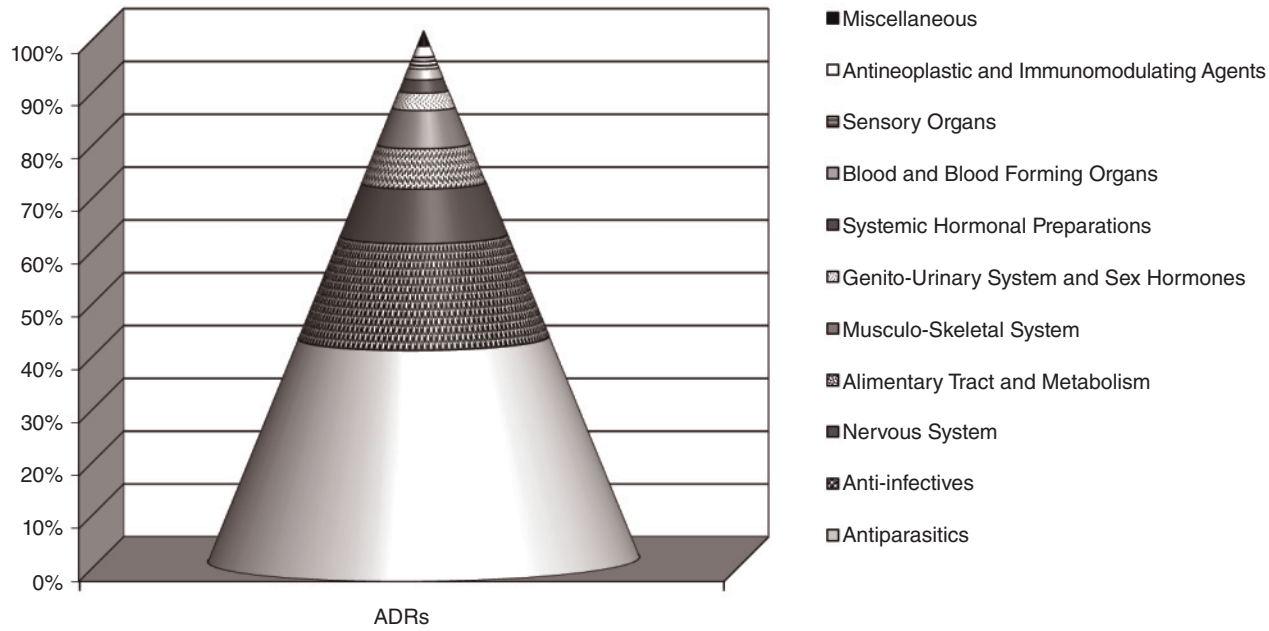

Fig. 5.4 ADRs that occurred in Germany in 2007, stratified by main ATCvet code of the products involved.

supplements contain Cremophor as an adjuvant, and this is known to induce anaphylactic reactions in animals. This issue was covered by a Graduated Plan several years ago and resulted in warnings within the Summary of Product Characteristics (SPC) about these anaphylactic reactions. Although the general number of ADRs decreased, there are still a certain number of anaphylactic reactions each year.

Typical reactions such as emesis and diarrhoea are frequently related to non-steroidal anti-inflammatory drugs (NSAIDs), which represent the majority of the ATC subgroup the musculo-skeletal system (QM). Of the genito-urinary system and sex hormones class (QG) these are mostly oxytocin or gonadotropin-releasing hormones (GnrH). For the systemic hormonal preparations (excluding sex hormones, QH) steroidal anti-inflammatory drugs (SAIDs), insulin or thyroid hormones are frequently mentioned in relation to adverse drug reactions.

Vitamin K, iron substitutes, anti-anaemics (vitamin B_{12} combinations) and solutions for infusion are covered by the blood and blood-forming organs group (QB). A particular problem associated with a solution for infusion is a known side effect in cows with milk fever: here, infusion with calcium and/or magnesium, which is administered to treat hypocalcaemia and hypomagnesia, can lead to cardiac arrest if the dosage is too high.

Eye drops and ear drops administered for the treatment of local inflammation are covered by sensory organs (QS). The ototoxic antimicrobial substance gentamicin is frequently reported in relation to deafness in dogs.

Antineoplastic and immunomodulating agents (QL) are known for their potential to cause ADRs and they must be used with a carefully considered risk : benefit evaluation by the veterinarian.

Management of ADRs and the German database

As required by EU legislation, Competent Authorities must take appropriate measures to encourage the reporting of suspected adverse reactions. Competent Authorities must also ensure that they establish a pharmacovigilance system, used to collect information useful in the

surveillance of veterinary medicinal products, and to scientifically evaluate the collected data (European Commission, 2008).

The heart of the pharmacovigilance system is the database

Since electronic reporting became mandatory in accordance with EU legislation transposed into the Medicines Act (Arzneimittelgesetz) (Anonymous, 2005a), a transition phase commenced. The BVL's former Microsoft Access® database, which included approximately 10,000 records since its inception in 1980, was no longer adequate. A new pharmacovigilance database for the two Competent Authorities dealing with veterinary pharmacovigilance became essential.

This new German database has been developed on the basis of software used for human medicinal products at the PEI and the Federal Institute for Drugs and Medical Devices (Bundesinstitut für Arzneimittel und Medizinprodukte). These databases have been specifically designed for use by these regulatory authorities and they have been employed successfully since their implementation in 2005. The modification work for veterinary use was done in close cooperation with BVL's and PEI's scientists and with IT experts from the software company. As a result, the product was not only a pure pharmacovigilance database, but also a complete system for the management of ADRs.

To fulfil the legal obligation for electronic reporting while the new database was under development, electronic reporting via EudraVigilance was initiated from 1 January 2005. At first, only serious ADRs were transmitted via EudraVigilance, while in parallel data were entered into the former Microsoft Access® database (until the end of 2005). In 2006, all reports were submitted through EudraVigilance. However, only the serious ADRs were submitted, while the non-serious ones were stored locally. In 2007, all files were submitted. All XML files produced through EudraVigilance have now been imported into the new German database. So at the time of going into service with the new system at the beginning of 2008, there were more than 1,000 national ADRs available.

Basic principles of the German pharmacovigilance database

The system operates on a Master–Slave–Follow-up model which aggregates reports from multiple sources to a single source object (*Figure 5.5*). The complete information for each ADR is co-located in one Master file. Each source of the information related to one ADR is defined as a Slave. All Follow-ups are related to Slaves. Thus, a sender-based hierarchy is implemented. The advantage of the system is that all relevant information is saved within one master file without the loss of any sender details.

All steps like data entry, data review and coding, duplicate detection and matching, causality assessment, analysis and data exchange, including the relevant business logic, are implemented in one configurable solution. Controlled vocabulary dictionaries such as VedDRA (EMEA, 2007a, b) and the List of Species and Breeds (EMEA, 2007c) are an integrated part of the database. Additional controlled terminology according to dosage forms, administration routes, units or strengths, which were agreed within the EU, are included using drop-down lists (EMEA, 2006b).

Direct data views such as HTML-preview of the data are implemented, so it is possible to see the content of a case without opening the file. This is both convenient and time-saving. Delegation via email and communication on the cases via 'To Dos' are important features for the smooth process of work. The system has a complete audit trail and case tracking. The database structure is extensible and further adaptations of new features are possible.

The database is connected via the Gateway to permit electronic reporting obligations. It follows the *Guideline on Data Elements for the Electronic Submission of Adverse Reaction Reports related to Veterinary Medicinal Products Authorised in the*

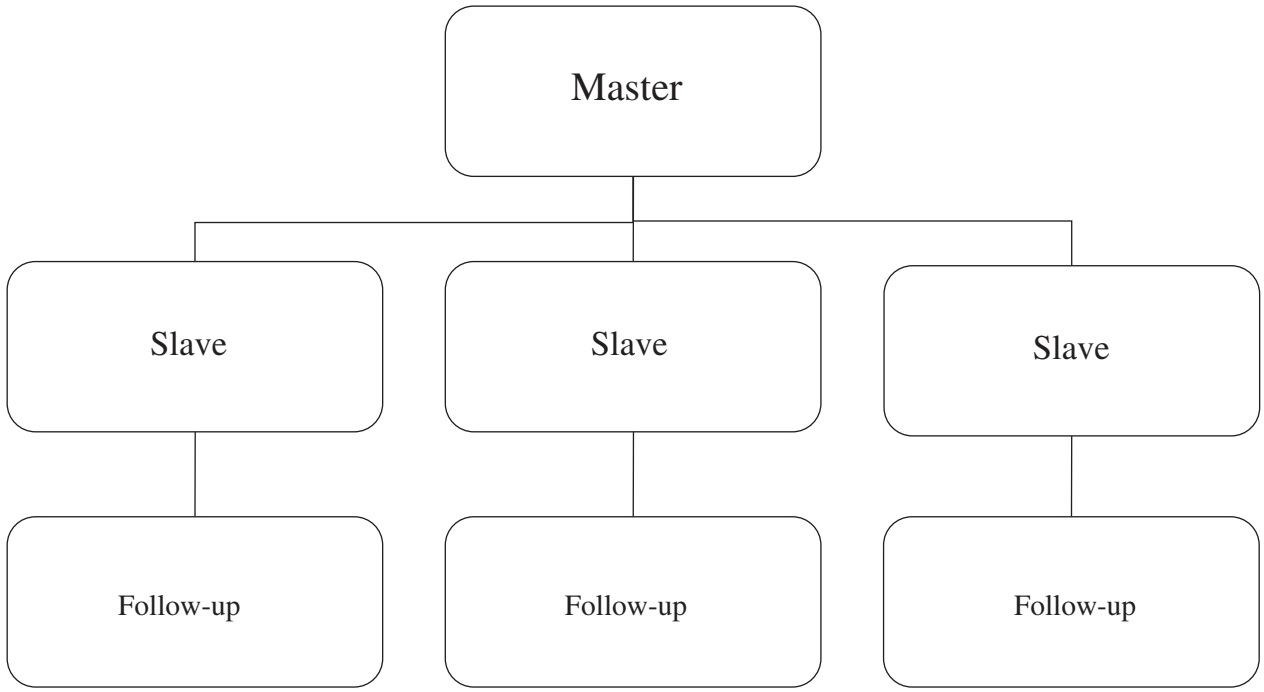

Fig. 5.5 The Master – Slave – Follow-up model.

European Economic Area (EEA), including message and transmission specifications corresponding to XSD (EMEA, 2005a).

The database is connected via a software package (Gateway) complying with the *Electronic Standards for the Transfer of Regulatory Information (ESTRI)* to the European pharmacovigilance database. Compliance with electronic reporting obligations can be achieved by direct transmission of XML documents. XML files created by the German database meet all the requirements of the *Guideline on EudraVigilance Veterinary XML-Schema Definition (XSD) Version 2.2.0* and *Veterinary Acknowledgement XSD, Version 2.2.0* (EMEA, 2005b).

Management of ADRs – the workflow

The database supports all the necessary work steps within a customisable workflow when working on adverse event reports:

1. Registration of ADRs
2. Scientific Data Review/Entry and Causality Assessment
3. Correspondence
4. Review/Control
5. Archive

At (1), the first step is a basic registration of either the electronic-based (from MAHs or veterinarians) or paper-based (from veterinarians who still prefer the traditional way of reporting) incoming reports. Then, the product(s) involved are allocated into the system and the relevant data such as active substances and other critical information, received from the authorities' drug dictionary, are completed. The pharmacovigilance system has an interface with the national drug database (AMIS), so information can easily be transferred, ensuring that it is always up to date. If the case is received as a paper report, the name and address of the sender is completed and entered into the database's address book. All paperwork is scanned and added electronically to the ADR file.

Step (2) includes the review of electronically received ADRs or the data entry of ADRs received on paper, respectively. A duplicate check must be done by query through the case browser. Criteria for these queries are brand names, species and

date of onset of reaction. All work on data related to the animal and the adverse reaction is done by Scientific Officers. At this stage of the workflow the correct allocation of the products involved is checked. The coding of the reactions and relevant medical history data are based on VEDDRA. Coding can be done either by using the search function for special terms or by browsing through the VEDDRA hierarchy. A detailed description of the causality assessment is given below.

After a positive validation of the report, a scientific officer is responsible for the transfer of the report to EudraVigilance. To ensure optimal compliance for expedited reports, a traffic-light system is implemented within the case list.

At (3), nearly all correspondence is done by technical assistants referred to as project managers. If the case comes in as a paper report, a standard letter of receipt is sent to the reporter. An individual letter of receipt can also be tailored by the scientific officers. It includes comments on the ADR or any further advice for the reporter.

In ADRs with more than one suspect drug involved the additional MAHs concerned are informed by sending an anonymous copy of the report.

Before the ADR is sent to the archive, it is reviewed at (4) by the scientist working on it before. Correctness and completeness are checked again. If some additional information is still missing, reminder-status is added to the case. So it appears on a reminder list, which is reviewed by the project managers at intervals. At (5), the ADR is then archived.

Causality assessment

Causality assessments are performed in accordance with the requirements of the *Guideline on Harmonising the Approach to Causality Assessment for Adverse Reactions to Veterinary Medicinal Products* (EMEA, 2004). For a substantive evaluation there are several routine steps to consider. First, there must be a check on whether an adverse reaction has been reported before (usually combined with duplicate check queries; see above) or if it is already mentioned within the SPC (expected). The actual SPC of the product is stored in the BVL's product database or in the Online Drug Database for Veterinary Practitioners (Vetidata®) respectively.

It has to be considered if the adverse reaction is in accordance with the known pharmacological or toxicological profile of the drug. For further research, there are several relevant veterinary online journals and an academic library available for BVL's scientists to use. The correct use of the product and its administration or application to the animal must be reviewed. As a result, label use or off-label use is determined. This includes checking the administered dosage and comparing this with the recommended dosage and the route of administration described in the SPC.

For the scientific evaluation of the ADR, an electronic algorithm (Algorithm for Causality Assessment for Adverse Reactions to Veterinary Medicinal Products, Microsoft Excel® Sheet, Xavier Pineau, Veterinary Pharmacovigilance Centre of Lyon, France) is used. It is considered very helpful to perform standardised, reliable and continual assessments. Within the weekly routine meetings of the BVL's Pharmacovigilance Unit there are discussions on actual ADRs in order to reach a common approach to assessment.

Archiving

The original ADR report is stored on paper within the product dossier. These documents are managed within the professional BVL archive.

Working copies of the ADRs are held in folders which are managed by the project managers. They are organised chronologically on a product basic. This working archive was completed in 1980. Due to its close connection with the Pharmacovigilance Unit, it is easy for each employee to access.

In addition, electronic copies of the ADRs are stored in the Microsoft Access® database or in the new German database. These databases are run by BVL's IT staff. A daily back-up avoids data

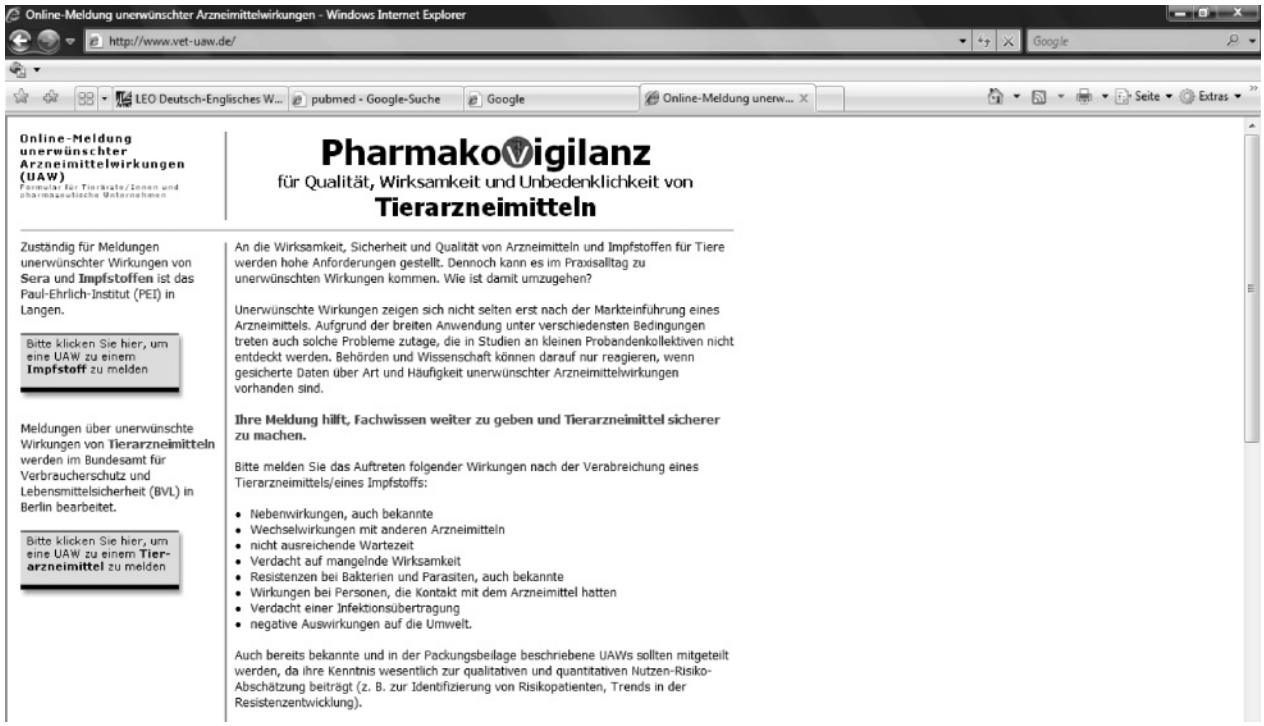

Fig. 5.6 Start page of the online form.

loss. The server for these files is located remote from the paper archive and within another building, so disaster recovery is guaranteed.

Reporting routes and electronic reporting

With a view to using resources efficiently and avoiding delays in access to relevant information, electronic reporting is the appropriate modality for transmitting ADR reports. A major step to achieve this was taken in 2005 when the legal obligation for electronic reporting was established. While nearly all EU Competent Authorities are registered as users of the electronic data transfer system EudraVigilance Veterinary, there are delays within the animal health industry in implementing this (in February 2008 there were 22 companies registered). On 15 March 2008, a BVL Bulletin came into effect to enforce electronic reporting. In doing so, this enacted the Verordnung über die elektronische Anzeige von Nebenwirkungen bei Arzneimitteln (Anonymous, 2005b).

www.vet-uaw.de

As a special service for veterinarians and smaller companies, a new Online Reporting Form has been developed (*Figure 5.6*). It was created in the interests of convenient handling. The Online Reporting Form is available via a common BVL and PEI website, which is hosted in cooperation with the German Veterinarians Association: www.vet-uaw.de (please note that UAW is the German abbreviation for ADR). It is available in German and English language versions.

On the home page of the website some general information relating to pharmacovigilance, ADRs and electronic reporting is provided. The user is advised that ADR reports related to immunologicals should be to sent to the PEI and those for pharmaceuticals to the BVL. After this, the navigation splits into the Online Forms for MAHs and for veterinarians (*Figure 5.7*). Both routes offer one form for reactions in humans and one in animals. While the form for MAHs requires a basic understanding of ADR reporting, including the use of standard list terms (VEDDRA, Lists of

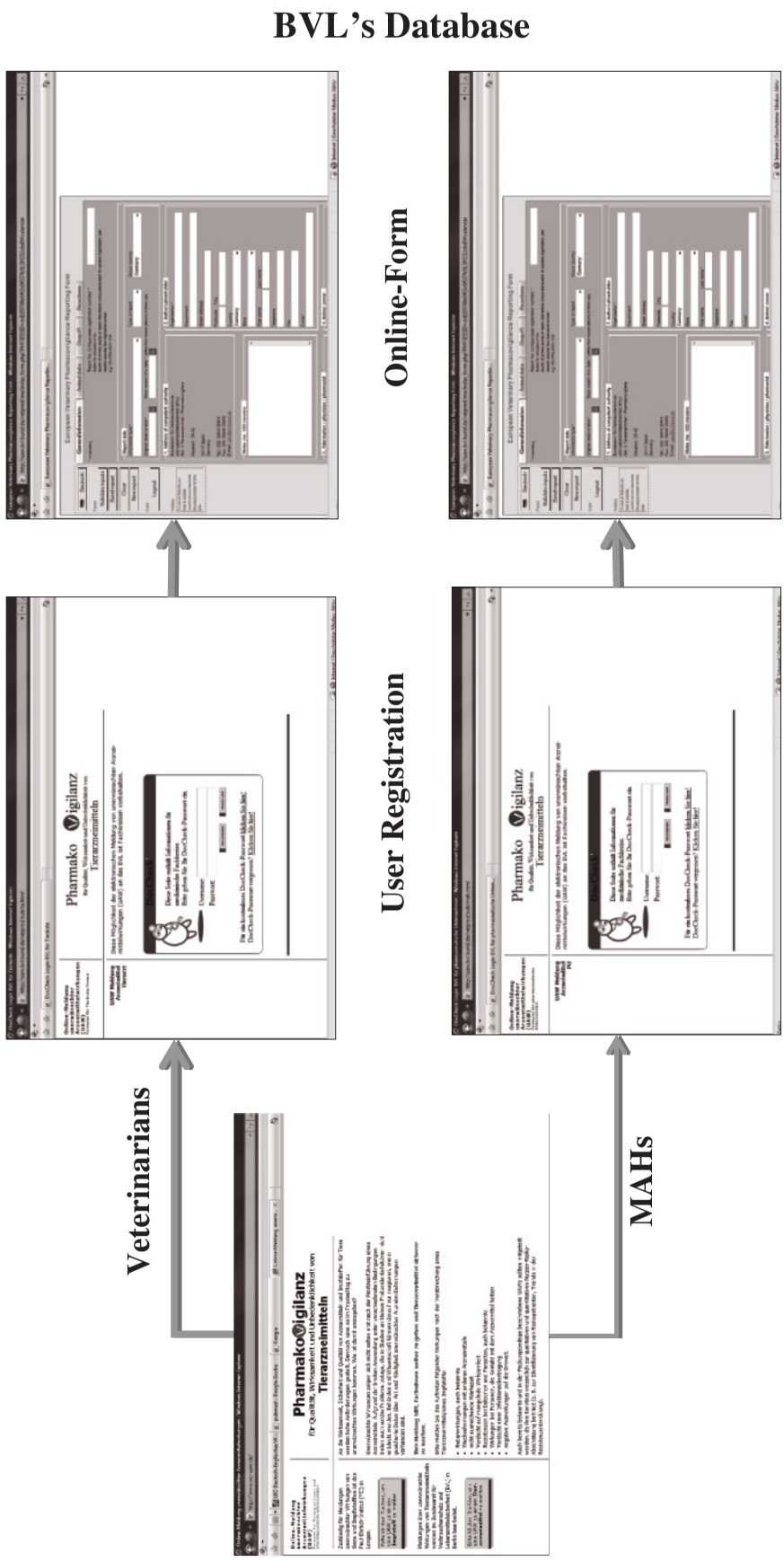

Fig. 5.7 Navigation to the Online Reporting Form. (Courtesy of Dr. Anke Finnah, BVL.)

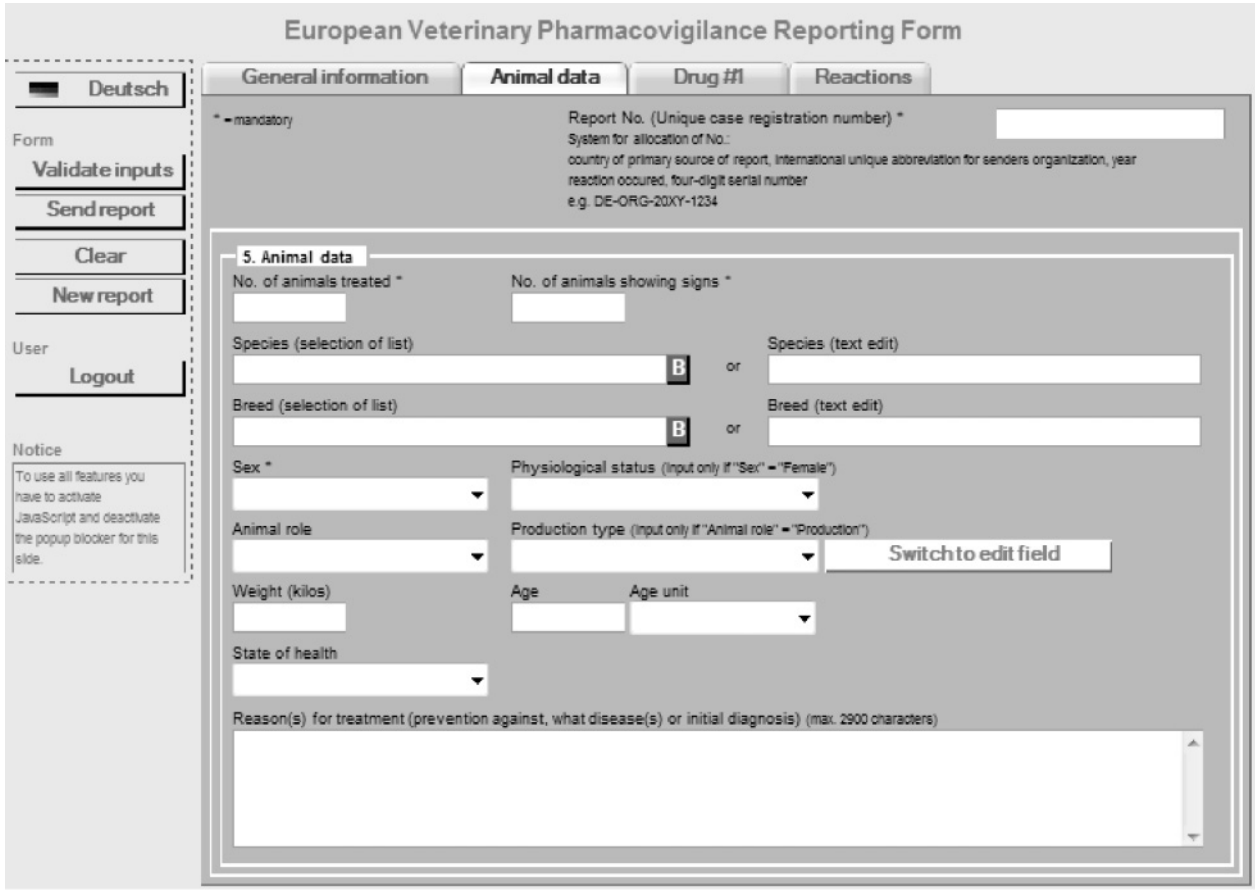

Fig. 5.8 The German Online Reporting Form.

Breeds and Species), the veterinarians' forms only contain user-defined text fields to ensure maximum user-friendliness.

The Online Reporting Form (*Figure 5.8*) contains four main areas:

- General information
- Animal data
- Drug(s)
- Reactions

These are reachable using tabs at the top. Mandatory fields are assigned an asterisk and all mandatory fields must be checked for data entry and plausibility of information before submission. Incomplete reports cannot be transmitted to the database. In the case of failures, an error message occurs.

After transmission of data, a PDF file appears, summarising all the data reported. This file can be printed and saved on the local computer. It is evidence of transmission of the contents. In addition, the MAH will receive an acknowledgement of having fulfilled the legal obligation to report adverse reactions.

Other routes of electronic reporting

The ideal situation is to run a local database with direct connection to EudraVigilance via a separate software package called the Gateway. In this case, the files are created, sent and stored within one system out of two components. Alternatively, the EMEA's Online Tool EVPost allows sending of reports created in a local database without the Gateway software.

In general there is no need to run a local database to create and send electronic reports. The EMEA's Online Tool EVWEB (some basic training is required before using) and the EMEA's

MAH Simple Form can be used for creating and sending reports. Some additional work is necessary for the administration of a non-database system, especially for archiving the copies of files sent and for producing an overview over the entire data transfer.

The EudraVigilance Veterinary Online Tools and further information are available on the EudraVigilance website, http://eudravigilance.emea.europa.eu/veterinary/index.asp.

Signal detection

The primary aim of spontaneous reporting systems is the early detection of a new potential risk related to an increasing frequency of serious adverse drug reactions under normal conditions of use (van Puijenbroek *et al.*, 2001). Nevertheless, detecting increasing frequencies of known side effects of products is also important, because this may indicate a quality defect.

Traditionally, signal detection is carried out by a systematic manual review of every ADR. These case-by-case analyses are today limited due to the fact that the data model is quite complex and the data extensive. The detailed information relating to animals (sex, reproductive status, breed, health status), drugs (ATCvet code, active ingredients, label or off-label use) and the symptoms occurring (VEDDRA) included in every single ADR is difficult to handle and manipulate without electronic equipment. However, the scientific impact of case-by-case studies is irreplaceable. Statistical analysis of the data from a spontaneous reporting system can provide additional information concerning a possible relationship between a drug and an ADR. An electronic tool is essential, particularly for the detection of rare and/or unexpected ADRs.

Signal detection aims to focus the attention of experts on drug–adverse event associations which are disproportionally present in the database. At the time a signal occurs, the evaluation of the signal itself is the most important aspect. The scientific assessor must be aware that the signal could be false positive or false negative. The consequences arising from the detection of signals must be determined very carefully.

Finally, quantitative signal generation can be used to study more complex relationships, such as drug–drug interactions and drug-related syndromes. The results of signal detection should be considered as an additional source of information, complementary to the traditional analysis. Techniques for the detection of drug interactions and syndromes offer a new challenge for pharmacovigilance.

Queries within the German database

Pharmacovigilance experts usually detect new ADRs by manually reviewing spontaneous reporting systems. In Germany, standard frequency analyses are in place, which are run at intervals. Combined with the experiences of the assessors during their daily work on reports, a sufficient and reliable system for detection of possible risks is in place.

The Query tool of the German database allows frequency analyses of ADR reports based on filters. A filter includes a minimum of one condition, but a combination of several conditions in one filter is also possible. The combination of filters with 'and', 'or' or 'not' is defined as a query.

Most of the fields in the *Guideline on Data Elements for the Electronic Submission of Adverse Reaction Reports Related to Veterinary Medicinal Products Authorised in the European Economic Area* (EMEA, 2005a) and some internal fields of the database are available as filters (*Figure 5.9*). Approximately 130 data fields are accessible for filtering cases.

A selection of frequently used template queries (combinations of filters and/or queries) is offered to every user, but the possibility of free combinations of new filters or queries is also available. An example of a standard query is the routine review of O-assessed cases to see whether a synopsis of data leads to another and different assessment of these reports.

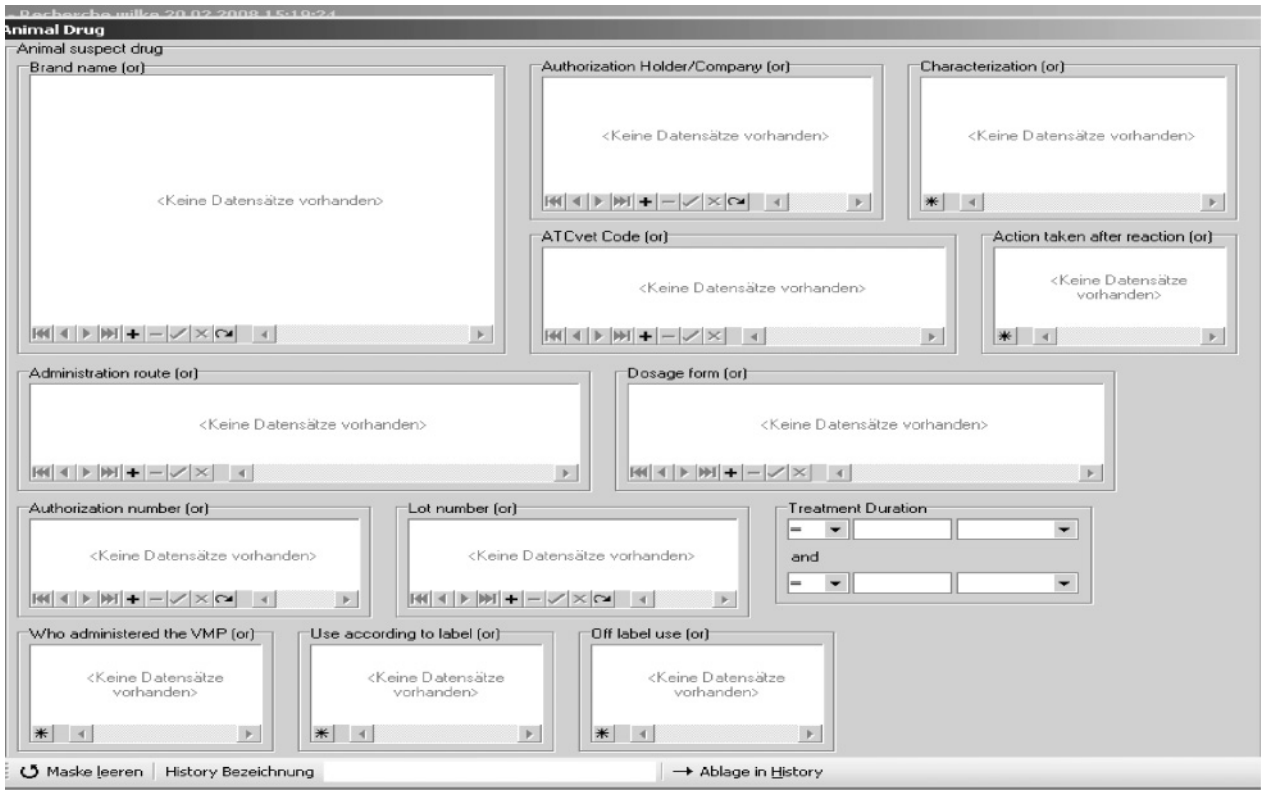

Fig. 5.9 Filter-entry masks.

As a result, for each filter the number of ADRs is shown and the cases are listed at the bottom of the search mask. It is possible to delve into cases for details or to create a Line Listing, including the main information, for a quick overview.

Output reports of results

Based on the results of the filters or queries, special Output Reports can be created. The information from the filtered ADRs can be presented either by Line Listing with report details or as overview tables for veterinary medicinal products (VMPs) or VEDDRA. The VMP Output Reports can be split further by ATC, Brand Names and Active Substances. The VedDRA Output Reports are available for each type of the hierarchy.

Within the Output Reports, the number of ADRs and the number of animals affected are highlighted. All reports can be stratified by species. In addition to that, the drug-related reports can be divided into 'reports with fatalities' and 'number of animals died'.

Quality checks

Through data quality checks, data are verified for required values and plausibility. Quality checks are performed to optimise and enhance data quality. They are essential because they increase the confidence in the data set and, in consequence, the detected relationships between VMPs and ADRs.

To apply data quality checks to the data in the system, data quality rules have to be defined first (as an example, for plausibility, the number of animals that reacted cannot be higher than the number of animals treated).

As mentioned above, there is a data check that occurs after the entry of data, which creates warnings at the time of saving the ADR to the system. Nevertheless it is possible to leave incomplete reports with warnings within the system; this is

necessary because some reports will have poor information. One important aspect of quality checks is to identify the number of reports with incomplete information.

Frequency analyses

Before scientific analyses can be applied, it is essential to have a fundamental knowledge of the information included within the database. Descriptive statistics with frequency analyses are necessary before any statistical method can be used in a meaningful way. Knowledge about rational stratifications is important for the correct use of these methods. Misconceiving can lead to erroneous signals later on.

As part of standard frequency analysis, the following parameters are run: numbers of ADRs by VMP, species, breed, country of occurrence, sex, seriousness, off-label use, exposed/affected number, VEDDRA, and other relevant information.

Data export and further analyses

In addition to the query tool integrated in the database, it is possible to export all existing tables via SQL statements for further statistical analysis. The tool used for these analyses is SAS® (version 9.1).

Standard epidemiologic measures

Applying quantitative signal detection methods to the database is necessary for the early detection and identification of drug safety alerts. Different techniques of data mining in pharmacovigilance databases are applicable (Wilson *et al.*, 2003).

The proportional reporting ratio (PRR) is the proportion of spontaneous reports for a given drug that are linked to a specific ADR, divided by the corresponding proportion for all or for several other drugs. PRR is frequently used for signal detection in pharmacovigilance.

PSUR assessment and management

Periodic safety update reports (PSURs) are intended to provide a picture of the worldwide safety situation for a veterinary medicinal product at defined time points after the authorisation. MAHs are expected to present summarised information on all adverse events, complete with a critical evaluation of the benefit : risk balance for the product. The main focus of a PSUR should be the presentation, analysis and evaluation of new or changed safety data relating to the period the PSUR covers. The contents of the PSUR should provide a basis by which the Competent Authority can decide whether the benefit : risk balance remains the same or whether further investigations or changes in the SPC are necessary.

Once a PSUR is received at the BVL and after it has been processed through administrative routes determined by a special Standard Operating Procedure (SOP), one of the scientific assessors will analyse it according to these standardised procedures based on the guidance in Volume 9B of the *Rules Governing Medicinal Products in the EU*. A set of templates is used to facilitate the different steps of the assessment. The ADRs in the Line Listings are compared with the ADRs already in the database which have been received by the spontaneous reporting route, for the product concerned. These data mainly concern the serious cases; they are checked in the database for double entries or for missing cases. The non-serious cases which have not been received previously are then added to the database. The reports are analysed according to species, indications and symptoms. The sales volume data are used to calculate incidences of ADRs. This can only be an estimate for products authorised for multiple species and for several indications. However, it is a useful tool to uncover trends and changes in frequencies of known ADRs. The benefit : risk assessment and the establishment of the

date for the next PSUR submission conclude the final assessment, which is sent to the MAH.

Whenever possible, the PSUR assessment is conducted together with the renewal procedure, if it coincides in time and a renewal is outstanding. Although the current legislation has now dissociated the two procedures, they are by their nature intimately linked and it is useful to evaluate them in parallel. The examination and analysis of PSUR data are in general an intrinsic part of the renewal assessment. A further renewal can only be requested on the basis of critical pharmacovigilance data, although this is rarely the case and any further request for a renewal requires a full justification.

Once the final 5-year renewal is passed, the requirements for PSURs remain an important tool to monitor drug safety, in addition to the operation of the spontaneous reporting system.

The periodicity of submission of PSURs is determined by EU and national legislation, but this permits Competent Authorities to change the time frame if the maximum submission time of 3 years is not achieved and extended.

Of course, any amendments to the PSUR submission frequency must be agreed beforehand with the Competent Authority. In Germany, due to its risk-based approach, there is some flexibility on the setting of PSUR frequencies at authorisation. For new active substances the strict time frame is adhered to, but for less innovative newly authorised generic products, a 3-year submission frequency can be given from the start on a case by case basis, especially for well-known substances with a low risk profile and a favourable analysis of ADR reports in the German database. If there are none or only a few well-known, non-serious ADRs already covered by risk minimisation measures in the SPC, the product would qualify for such a flexible approach.

The BVL also supports a policy of synchronisation of birth dates on request of the MAH. Usually this is done for products from the same company with the same active substances, existing in different strengths, for different species and with different indications. Separate PSURs per product will then be submitted, but at the same point in time. This saves resources for both the company and the regulatory authority.

However, PSUR synchronisation goes much further than this. In view of the scarce resources usually available, and in order to avoid duplicate work, BVL experts have contributed considerably to the initiative from the European Surveillance Strategy Group (ESS) of the Heads of Veterinary Medicines Agencies (HMAVet) in sharing work involved in the assessment of PSURs at the European level.

Surveys of EU pharmacovigilance resources conducted on the human and veterinary sides by the EU Heads of Agencies (human and veterinary) resulted in the identification for the need to make more efficient use of available resources and expertise in member states. The work-sharing concept for both human and veterinary pharmacovigilance is based on the following:

- synchronisation of the PSUR submission cycles across all EU national competent authorities (NCAs) to ensure that products with the same active substance follow the same PSUR submission in all NCAs;
- sharing the assessment made by one EU Competent Authority with other NCAs.

Both industry and the authorities stand to gain from a situation where products, whatever the authorisation procedures (MRP, DCP or purely national), would follow a pan-European PSUR submission cycle based on European Harmonised Birthdates (EU-HBD) and harmonised Data Lock Points (EU-HDLP) for PSUR data. PSURs for all types of formulations, presentations, routes of administration, indications and species should be provided at the same date for any one active ingredient. Using the EU-HBDs would enable work-sharing of the assessment of PSURs between NCAs and, based on this, subsequent harmonisation of any regulatory action, if required, in all affected member states. Industry would have to prepare and submit only one PSUR valid for all member states, instead of a range of PSURs, across the EU. The Heads of Agencies agreed on 27 April 2007 in Bonn, during the German EU presidency, to apply this approach to veterinary

medicinal products. It was also agreed to start an initial pilot phase during 2008.

Cooperation between the authorities and industry is paramount for the success of this initiative. Therefore a joint PSUR synchronisation group has been working since December 2007 under the auspices of ESS and the HMAVet to establish the organisational details for the pilot phase and to prepare for the ongoing initiative. On the veterinary side, and in contrast to human pharmacovigilance, vaccines will also be included in the project. This is mainly due to the fact that in the veterinary market, vaccines represent 20–25% of the products, whereas for human products it is only 5–7%. The importance of these products in veterinary medicine is thus much higher. Of course, the inclusion of vaccines is a particular challenge, as the criteria for grouping vaccines need to be different from those proposed for pharmaceuticals, due to variations in antigen and adjuvant composition and other differences such as inactivation or attenuation procedures. Therefore consensus was reached that for vaccines, PSURs would be retained at the individual product level. The advantage is still obvious: submission of one PSUR per product valid for all EU countries at the same point in time and one agency acting as PSUR Reference Member State.

The BVL and the PEI in Germany are among six EU member states regulatory authorities participating in the Pilot Project for this initiative in 2008.

The principles for this initiative are laid down on the HMAVet website at http://www.hma.eu/veterinary and on the BVL website at http://www.bvl.bund.de.

Benefit : risk assessment and risk management

A veterinary medicinal product is authorised on the basis that the balance of benefits and risks is considered positive under the conditions defined in the SPC and on the basis of the information available at the time of authorisation. However, it is recognised that at the time of authorisation, information on the safety of a veterinary medicinal product is relatively limited and not all actual or potential risks can be identified. This is due to many factors including the relatively small group of patients from a selected population of target animals treated in clinical trials, the restricted conditions of use of the product, restricted co-medication, and relatively short duration of treatment and follow up.

During the post-authorisation period the product will be used in a different setting from that used in clinical trials and in larger patient populations. Much new information will be generated which may impact on the benefit or risk of the product. Thus the post-authorisation phase provides possibilities to learn more about the safety profile of a product and if necessary to adapt the product's marketing conditions to make its use safer and more efficacious under actual, realistic field conditions. Therefore post-marketing safety data collection and risk management based on observational data are critical for evaluating and characterising a product's safety profile and for making informed decisions on risk management and risk minimisation.

The pharmacovigilance system provides the tools for a continuous benefit : risk assessment throughout the lifetime of a veterinary medicinal product. The knowledge accumulated about the drug in the post-marketing phase provides the possibilities to assess benefit and risk in a more precise way, although for reasons of consistency and transparency the same methodological approach as in the pre-authorisation phase should be taken.

The main instruments for surveillance of benefit : risk in the post-authorisation phase are as follows:

- The spontaneous reporting system for collecting adverse reactions, which is very important for signal generation and signal detection, and especially to detect severe but rare adverse reactions. The great strength of this system is that it operates for all veterinary medicinal products throughout their whole lifetime. The reports received from the field

comprise the most important source of information. Further information on how to handle adverse reactions is given for MAHs and Competent Authorities in the relevant Guidelines in Volume 9B of the *Rules Governing Medicinal Products in the EU*. Regular screening of the EudraVigilance and national database for signals is a prerequisite for surveillance (*Concept Paper on the Use of Data in Eudra Vigilance Veterinary* – EMEA, 2006a). If in the course of routine checking of reports unusual serious findings are made, one has to decide whether the evidence available is sufficient to suppose that a new risk is present. In such cases, more detailed research is needed to determine whether any suspicion can be confirmed. On the occurrence of serious adverse reactions, the benefit : risk balance of a veterinary medicinal product may be reassessed at any time in the product's life cycle.

- The Periodic Safety Update Report (PSUR) is a document allowing a cyclical, comprehensive assessment of the worldwide safety data of a marketed veterinary medicinal product (*Guideline on Management and Assessment of Periodic Safety Update Reports of Veterinary Medicinal Products* in consultation phase – EMEA, 2007d). The PSUR creates the opportunity for a periodic overall safety evaluation to show whether a product's safety profile has remained the same since it was first authorised. If new risks become evident through the submitted safety information, regulatory measures may be required, or must be initiated to optimise the safe use of the product. The PSUR includes, in addition to the collected adverse reaction reports, data on the use of the product under field conditions, including sales volume data. Thus an incidence calculation can be made and the benefit : risk balance can be reassessed in the light of a larger and more reliable dataset, taking into account additional information on risks unknown at the time of authorisation.

  The benefit : risk balance is also re-evaluated periodically through PSUR reports (Article 75, Directive 2004/28), but a PSUR can also be requested by a regulatory authority at any time if an obvious signal arises from the spontaneous system.

- Post-authorisation surveillance/safety studies allow a more systematic approach to clarify specific concerns and questions. Such studies can be a condition arising from the authorisation procedure (Article 26.3, Directive 2004/28). They can also be requested if safety concerns arise at any time in the product's life cycle (see the chapter on the conduct of post-authorisation safety studies in Volume 9B (currently available as EMEA/CVMP/PhVWP/430286/2007 draft 13, 2008). The studies can be undertaken to generate or test hypotheses that may arise from signal detection of spontaneous reports.

- The purpose of a risk management plan is not to replace but to complement procedures in place to detect safety signals. Such a plan would be needed in situations where potential or actual risks are identified that cannot be managed through routine pharmacovigilance. It would particularly apply to products authorised under exceptional circumstances and for products involving concepts completely new to veterinary medicines. More details will eventually be provided in Volume 9B.

The data collected by the various instruments of the pharmacovigilance system provide a more extensive and reliable base for adequate benefit : risk assessment than do the limited data available at the time of authorisation. If the benefit : risk assessment from the point of authorisation is not maintained, and if it shifts unfavourably, risk management measures and risk communication to the public and health professionals must be initiated, as laid down in the legal provisions.

With regard to renewals, Article 28 of Directive 2004/28/EC clearly states that the authorisation may be renewed after 5 years on the basis of re-evaluation of the risk : benefit balance. A further renewal should only be requested if problems on pharmacovigilance-based grounds arise.

The re-evaluation should be based on the initial benefit:risk evaluation at the time of authorisation and should take into account pharmacovigilance reports from spontaneous sources and particularly the overview of all new information summarised in the PSUR. Additional information from other sources such as the published literature should be included. The PSUR assessment is linked closely to the renewal procedure and, as the work in Germany is done in the same department, it is combined as often as possible.

Risk management measures: the Graduated Plan

The German Graduated Plan is a measure to contain, prevent or eliminate drug-associated risks for human and animal health which are related to the adverse effects of drugs, such as side effects, bacterial resistance towards antimicrobial agents, lack of therapeutic efficacy, drug interactions, and detrimental effects on the environment by veterinary medicinal products. It is used to manage other risks, e.g. drug counterfeits and quality defects, which in the following text are called suspected risks (Ibrahim and Reginka, 1998).

The Graduated Plan according to § 62 and the related general Administrative Regulation based on § 63 of the German Medicines Act is a regulatory tool allowing for a stepwise procedure relating to the seriousness of the risk involved and the data available to sustain a well-founded suspicion of risk. Measures that modify the marketing authorisation status must be based on objective, representative data that can justify the regulatory action, and it is the regulatory authority that must provide the proof of the risk involved. The Federal Office co-operates at the national level with the Bundesländer health and veterinary authorities, drug commissions for health care professions, pharmacovigilance representatives of the pharmaceutical industry and federal ministries. On a supra-national level, the Federal Office co-operates with the EMEA, EU Competent Authorities and third countries, with the WHO/FAO and with the Office International des Epizooties (OIE). The Federal Office is entitled to inform the public about drug risks and any intended counteractive measures.

The Federal Office becomes aware of suspected risks through new data in applications for marketing authorisations, variations and renewals of veterinary medicinal drugs, by veterinarians and other health care professionals who spontaneously report their negative experiences with drugs, by Länder authorities, and by the veterinary pharmaceutical industry.

If the suspicion of a risk arises, mainly based on frequent or particularly serious reports from the spontaneous system or more rarely by literature reports or PSUR assessment, a new benefit:risk evaluation of the product may be necessary.

The Graduated Plan is divided into two steps related to the seriousness of the risk. Step I can be initiated if a suspicion of an association arises and if suggestions of a 'possible' risk between an adverse reaction and the use of a medicinal product exist. Step I consists of:

- the exchange of written information with the MAH within a time frame set by the regulatory authorities.

The information should include:

- information on all ADRs – national and international;
- sales data;
- countries where the product is authorised;
- the current SPC;
- an assessment of the risk and a statement on whether voluntary measures by the veterinary pharmaceutical company are intended.

If the suspicion cannot be substantiated following the written hearing with the MAH, the procedure will be stopped at this level. This is also the case if the MAH initiates measures of risk minimisation by including new warnings or by amending the SPC in other relevant ways.

However, if the information exchange at Step I or information from the spontaneous system or

other sources gives reason for a firm suspicion of a 'probable' risk, then Step II is reached. Step II is not necessarily preceded by Step I. It is always used if the Regulatory Authority deems regulatory measures necessary to assure drug safety.

Step II comprises:

- a proposal by the regulatory authority for envisaged regulatory measures;
- a written or oral hearing with the pharmaceutical company(ies);
- on a case by case basis, consultation with external experts.

After evaluation of all available information the BVL decides on the measures needed to be implemented. The following measures are available for consideration:

- SPC changes such as warning statements, contra-indications or user advice;
- variations of indications, dosage or route(s) of administration;
- changes of composition and formulation;
- changes to prescription status or restriction of use, e.g. only by veterinarians;
- batch recall and warnings to the public;
- amendment of the classification for dispensing of veterinary medicinal products;
- increased surveillance of companies involved in the production and distribution of veterinary medicinal products;
- increased surveillance of imports of veterinary medicinal products;
- suspension or withdrawal of the authorisation.

Companies can also be requested to conduct additional post-marketing safety studies.

If urgent measures are needed or if there is considered to be an imminent risk, the immediate implementation of precautionary measures can be ordered. In exceptional circumstances this may even be done without a hearing in Step II of the graduated plan (Beckmann, 1996).

The BVL provides information at the national and international level to other regulatory authorities and institutions evaluating the risks of veterinary drugs. This also allows for rapid dissemination of information to local authorities which are responsible for the enforcement of the German Medicines Act.

Two times a year the Graduated Plan Routine Meeting takes place at the Federal Institute for Medicines and Medical Devices (BfArM), the regulatory authority for human drugs, in Bonn. Representatives from the human and veterinary regulatory authorities, for both pharmaceuticals and vaccines, from industry associations and the Bundesländer working in the field of pharmacovigilance, risk prevention and risk management meet and exchange information and coordinate regulatory measures.

Whilst the Graduated Plan used to be an instrument for regulatory measures at the national level, and still is, it is now also used as a tool to implement in Germany decisions taken at the EU level. These include decisions on CVMP referrals or Urgent Safety Restrictions as well as other recommendations of the CVMP.

Some recent examples of regulatory measures in Germany illustrate the large spectrum of possibilities that the Graduated Plan covers, and these are cited below.

A Graduated Plan Step I was initiated for dexamethasone and gentamicin-containing eye drops, as reports of ADRs in dogs, cats and rabbits were received showing reactions including severe conjunctivitis, keratitis and corneal ulcers. The indication of the product was restricted to patients where the cornea was intact and a shorter treatment duration was given in the SPC. As an additional precautionary measure, warming of the product to room temperature was recommended. Since these measures were implemented no more ADR reports have been received for this product.

For tetrachlorovinphos-containing antiparasitic collars, suspension of the marketing authorisation was ordered at Step II because in vitro studies gave rise to suspicions of mutagenic and carcinogenic effects in target animals and their human owners living closely together. Post-marketing in vivo mutagenicity studies conducted by the MAH gave negative responses. On this basis the marketing authorisation was reinstated.

During the last 10 years, various restrictive measures relating to the use of antibiotics have been applied, in particular to the fluoroquinolone and tetracycline drugs. For injectable tetracycline products, prophylactic use was revoked, indications were restricted and an antibiogram to determine antimicrobial susceptibility was imposed prior to use for suspected infections with *Escherichia coli* and *Salmonella typhimurium*. The measure at Step II was based on the monitoring of resistance data in Germany showing increasing resistance rates for these bacterial species (Trolldenier, 1996).

In the use of fluoroquinolone-containing products in poultry, the establishment of sensitivity prior to treatment was also imposed at Step II. In addition, the use was restricted to serious infections as a treatment of second choice, if other therapies were not efficacious.

For monitoring of antibiotic resistance, the BVL conducts its own representative antimicrobial resistance monitoring focusing on pathogenic bacteria in food-producing animal species. The future perspective includes a larger spectrum of bacteria and extension to companion animals. The aim is to have objective data as a basis for regulatory measures. The monitoring data are integrated into the authorisation procedures and are considered an additional management tool for post-marketing surveillance strategy (Ibrahim, 2006).

Pharmacovigilance inspections

The first pharmacovigilance inspections were carried out in 2007. They were performed under the scope of training for both the veterinary pharmaceutical industry and the Competent Authority's inspectors. The main issue was to conduct routine inspections; triggered or product-related inspections have yet to be undertaken. The inspections are systems-based, which means that the systems and procedures used by companies in order to comply with EU pharmacovigilance requirements will be examined.

The first experiences from the training phase have been positive. It became clear that it is useful to perform pharmacovigilance inspections under the scope of a dialogue between industry and Competent Authorities. As a result, a better understanding of the expectations from both sides can be stated. Compliance problems with pharmacovigilance regulations during daily work became clear, while excellent solutions for these problems were presented by some of the companies inspected.

The initial step is to inform the company that a routine inspection is planned and a date for the inspection is requested. It is a 1-day inspection. After the date is established, an agenda for the inspection is suggested by the BVL. At the same time, the BVL's questionnaire is sent to the company. The company is asked to return the questionnaire at least 2 weeks before the inspection.

As mentioned before, the PEI is responsible for the regulation of immunological products. An assessor from the PEI and an assessor from the German Federal Regional Authorities (as a representative for Good Manufacturing Practice) are invited to the inspection as concerned partners, with observer status.

The inspection usually starts with a brief introduction about its aim and scope. The company is asked to introduce itself. The structure of the company, its national and international sites and relations and its general scope are usually described as well as its ranking in the market and the main products held. A description of the company's pharmacovigilance unit, as a part of the organisational chart, should be provided. The following details of the company's pharmacovigilance operation are required: the identity of the Qualified Person for Pharmacovigilance and his/her replacement (24 hours availability), the structure of the pharmacovigilance unit, the numbers of staff, its responsibilities and education/training. The cross-links with other units of the company or external parties in view of pharmacovigilance activities such as PSUR preparation or other important issues are examined.

Subsequent to this, the details of the handling of ADRs are investigated. The procedures for processing case reports such as the reception of reports (by call centre or sales managers) are examined. The first contact and the further correspondence with the reporter are very important. Veterinarians play a key role in the reporting system: they are eye-witnesses to the ADRs, which are the basis of all pharmacovigilance activities. Therefore the awareness of staff working in close contact with veterinarians should be focused on the essential principles of ADR reporting. This should also be reflected in the staff training programme. The monitoring of the successful participation of all staff members has to be documented.

The numbers of serious or non-serious ADRs is examined and, as a control measure, a check in view of 'late cases' (cases that have to be submitted within 15 days to the Competent Authorities according to Arzneimittelgesetz) and of the timeliness of submissions is made. The number of late cases is discussed.

Electronic systems for ADRs should be demonstrated if applicable. Issues such as the quality of data entry, the use of standard terminologies (VEDDRA, List of Species and Breed, etc.) or translation of data into English are covered. Further important topics are the correspondence with reporters and Competent Authorities (follow-up measures), surveillance of time periods, and the replacement and sharing of staff responsibilities. The archiving of all original documents should be in a safe and fire-resistant area. The archived data should be regularly reviewed for possible risks. In the case of electronic databases, a signal detection strategy should be in place. The risk management procedures including the responsibilities of staff and decision-making rules are examined. The preparedness for batch recall due to quality defects or any other urgent safety restriction is a critical part of these measures. The logistics that are in place for the prompt dissemination of information to the public need to be examined as part of the inspection.

A quality management system covering pharmacovigilance issues should be in place. This includes Standard Operating Procedures for the main working areas as well as internal or external audits of functionality. The system should be reviewed within certain time limits.

At the end of the inspection there will be a final discussion, with a brief summary given by the inspectors. A written report with findings and critical points is prepared by the inspectors and sent to the company by the regulatory authority. The inspected company will then have an opportunity to review the inspection report and provide comments. The final report is sent to all participants in the inspection.

The initial experiences with findings or deficiencies revealed that in some cases the qualified person for pharmacovigilance is not optimally integrated into the overall company structures. For example, he or she may have limited access to the headquarter's pharmacovigilance database. In the case of the distributor chain, the responsibilities of partners may have been unclear as there were no written contracts on pharmacovigilance obligations available. Third country reports are often delayed or completely missing. It has to be recognised that in some cases, pieces of documentation were missing or the archiving of files was poorly handled.

At present there are about 120 MAHs with at least one product actually on the German market. This means that there are large companies which are global players as well as very small companies acting only at the national level; the latter generally have only a few employees and limited resources. For each of these MAHs, the same legal principles apply, even if widely different resources are involved. Inspections show that there are many different and varied ways for all to meet the legal obligations satisfactorily.

Future perspectives and challenges

Pharmacovigilance is situated at the cross-roads of science and regulatory affairs. In order to operate an efficient pharmacovigilance system, both aspects are needed. Plans exist for greater

cooperation between the BVL and the five veterinary faculties at German universities.

This would also be in line with the concepts and strategies of the European Surveillance Strategy Group (ESS) of the Heads of Veterinary Medicines Agencies (HMAVet) of EU member states. One of the priorities of the HMAVet Action Plan agreed in 2007 is better cooperation with universities to increase pharmacovigilance-related research.

The creation of regional pharmacovigilance centres at veterinary universities is envisaged, acting in a complementary fashion to the spontaneous reporting system running at the BVL. Such centres already exist by law (§ 62 of the German Medicines Act) for human medicines following the completion of a successful pilot phase in regional hospitals.

In the future, it is foreseen that PhD students or other specially trained students will be based in university veterinary clinics to engage in the intense surveillance and follow-up of patients that have sustained adverse reactions and have been hospitalised in clinics or transferred to them. Such centres can also be used to study specific problems and questions arising from spontaneous reports. Other specific issues may arise from signal generation in EudraVigilance or national databases, or from clinical subjects arising in the daily routine at university veterinary schools. The gap between under-reporting in the spontaneous system and the real incidence of adverse reactions in veterinary practice will be given a targeted approach. It is expected that the network of pharmacovigilance centres at universities in Germany will increase drug safety and promote the veterinary pharmacovigilance approach amongst students and clinicians.

The evaluation of ADR data is done at the BVL in cooperation with the universities. Data from the BVL database could also be used for PhD projects. This kind of cooperation would enable the BVL, as the Regulatory Authority, to maintain contact with current science and provide a more realistic view about the benefit : risk balance of veterinary medicinal products.

A further way of examining the actual use of veterinary products in animal therapy is through the planned monitoring of a representative number of veterinary practitioners within a given time frame via a monthly questionnaire which has to be completed. This is expected to provide more information about the real use, according to indications, dosages and the efficacy/lack of efficacy, of veterinary products. By assembling the user profiles of drugs, future increases in resistance for antibiotics and antiparasitics could be more easily predicted.

A very special cooperation will take place with the University of Leipzig, namely the VETIDATA project. VETIDATA is a veterinary information and advisory centre for veterinary pharmacology/toxicology, drug therapy and drug law, situated at the University of Leipzig (http://www.vetidata.de/). The centre has established itself in recent years as a well-accepted and frequently consulted service amongst German veterinary practitioners, students and veterinarians working for government institutions and in industry. The BVL was a major contributor in the establishment of the VETIDATA drug database, ensuring the availability of accurate data on the authorisation status of products. In future, there will be further cooperation projects in the veterinary pharmacovigilance sector. One of these projects relates to e-learning for continuous professional learning, development and specialisation for veterinarians and students, while the other is a pilot for integrating pharmacovigilance into the curriculum for veterinary professional education, first in Leipzig, then in other German faculties. This will then work to achieve this objective at the European level.

The cooperation with universities and the involvement of veterinary faculties in pharmacovigilance issues opens up perspectives for a more scientific approach and can contribute to a better acceptance and participation in the system by veterinary practitioners and others.

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6 Veterinary pharmacovigilance – the UK experience

K.N. Woodward

Introduction

Human medicines, prior to licensing, are investigated in clinical trials, largely to determine their therapeutic properties, but also to detect adverse reactions. However, at least in preliminary trials, the doses used tend to be subtherapeutic or at the intended therapeutic level and many adverse effects only become obvious once the drug has been marketed (Tilson, 2000). Veterinary medicines are also tested in clinical trials to determine their therapeutic efficacy, and adverse effects may be noted during the conduct of these studies. Veterinary medicines are also tested for safety in each intended species and, in contrast to human medicines, usually at several multiples (up to five times) of the intended therapeutic dose. As a result, potential adverse effects resulting from high doses may become evident at an early stage, and products with a poor or suspect safety profile may not be further developed, or intended therapeutic doses may be reduced or otherwise altered accordingly. These factors undoubtedly contribute to both the apparent safety of marketed veterinary medicines and the contraindications and warnings that appear in the product literature.

Adverse reactions not seen in clinical trials or in safety studies do become apparent when the veterinary drug is marketed as larger numbers of animals become exposed. These adverse reactions may be as a result of direct toxicity or pharmacology that might be predictable from preclinical studies or clinical trials, or they may be idiosyncratic reactions to the medicine. As a result, veterinary regulatory authorities have introduced spontaneous reporting systems for the collection and analysis of data on adverse reactions to veterinary medicinal products. One of the established examples of these is the UK's 'yellow card' reporting scheme.

The UK approach is a particularly good example for consideration because it has successfully operated for around 20 years and it has been reporting its findings since 1987.

The UK scheme

Suspected adverse reactions in animals

The UK scheme is operated by the Veterinary Medicines Directorate (VMD). The UK pharmacovigilance scheme is known as the Suspected Adverse Reaction Surveillance Scheme (SARSS), and all adverse reactions remain 'suspected' until proof or, more often, until the balance of

evidence, suggests otherwise. As will become apparent in Chapter 27, causality assessment takes into account known facts about the drug or product in question (e.g. information from pharmacology and toxicology studies, and clinical trial data), information from other sources, (e.g. adverse events in other species including humans), as well as data from other adverse event reporting schemes in other countries, from clinical trials or from targeted post-marketing surveillance. Information from the published literature, especially from sources dedicated to adverse event reporting or iatrogenic medicine can be valuable, but such data are usually restricted to adverse effects in humans arising from the use of human medicines.

The eventual aim of the analysis of spontaneous adverse reaction reports is to try to establish biological plausibility by association of the nature of the adverse event(s) with the physical, chemical and biological effects of the drug in association with the extent and timing of treatment, and the time elapsed since treatment. Hence, establishing biological and medical plausibility is an important contributing factor in assessing the nature of suspected adverse reactions and attempting to establish causality.

One of the intentions of European legislation on veterinary (and human) medicines is to ensure harmonisation of the regulation of these products. However, as in so many other respects, this has to some extent yet to be achieved and the harmonisation of veterinary pharmacovigilance systems in the EU is only partially complete. In the UK, SARSS has been operational in its modern form, i.e. computerised storage and retrieval of data, since 1986. The scheme is fully integrated with an authorisation system which in turn deals with animal pharmaceutical (including ectoparasiticides) and vaccine products. Hence, it is a helpful and practical model to consider (Woodward and Gray, 1994; Woodward, 1996).

Close association with the authorising system and the other parts of the authorising authority means that product data, including information on excipients (e.g. solvents, anticaking agents, antioxidants, adjuvants), can be quickly assessed and, where necessary, regulatory decisions taken. Therefore, responses to emergency situations, though they occur infrequently, can be rapid and effective. SARRS assessments of reports are priority coded by the VMD and where prioritisation is high, specific targets can be established to meet the needs of the situation.

Spontaneous reporting to the VMD is usually carried out on a specific 'yellow form', form MLA 252A (Rev. 8/01), or on the European Veterinary Pharmacovigilance Reporting Form, both available from the VMD (http://www.vmd.gov.uk), as are a comprehensive SARRS guidance document and a brochure describing the scheme. This reporting form is divided into sections to record the name and address of the person making the report and a section for details of the suspected adverse reactions (SARs) in animals. The UK scheme recognises the importance of garnering data on, and reacting to, adverse events in humans exposed to veterinary medicinal products and there is a section on the yellow form specifically dedicated to reporting SARs in humans.

Adverse effects in animals take many forms, but the vast majority reported fall into well-defined categories:

- toxic or exaggerated pharmacological effects in treated animals;
- lack of efficacy in the target animal patient;
- idiosyncratic reactions in animals;
- toxic, idiosyncratic or other reactions in humans (mainly users);
- drug–drug interactions;
- local reactions, e.g. at the site of topical application or at the injection site.

SARs may arise not only from the authorised use of veterinary medicines, but also from off-label uses, including permitted uses under the cascade[1], from illegal use and from abuse of

[1] Under Article 10 of Directive 2001/82/EC as amended by Directive 2004/28/EC, a member state may permit a veterinarian, where there is no authorised product available, to use another veterinary medicinal product authorised for use in another species, or an authorised human medicinal product, or a product authorised in that species in another member state, providing that for food animals, suitable withdrawal periods are applied.

Table 6.1 Product classes excluded from VMD SARRS reports.

Class excluded	*2000*	*2003*	*2004*	*2005*	*2006*	*2007*
Non-veterinary products or unauthorised products	55	38	42	26	41	41
Unidentified products	16	30	23	44	51	14
Unlikely to be due to the medicine	44	75	143	91	124	232
Lack of expected efficacy	119	181	265	151	302	508
Unauthorised use of a product	117	158	186	262	230	230
Insufficient information	367	397	544	527	546	645

authorised medicines. They may also arise from the misuse of unauthorised or prohibited medicines. For example, the use of performance-enhancing drugs as misused in race-horse doping would be included under this category (Macri and Marabelli, 1992).

In the UK scheme, several classes, including non-authorised uses, are excluded from the total of valid SARs. In fact a range of classes are omitted including lack of efficacy, a recognised element of EU pharmacovigilance. This can be exemplified by reference to the reports for 2000–2006 where a number of classes, as shown in *Table 6.1*, were excluded from the SAR total figures (Gray and Knivett, 2001; Dyer *et al*., 2004–2008).

There have been a number of adverse reactions in dogs and cats reported to the VMD involving the use of human medicines, including colchicine, chlorpheniramine, fentanyl, ranitidine, tetracaine and vincristine (Spagnuolo-Weaver, 2007).

Veterinary medicines are made available in the UK according to their legal category. Those requiring veterinary diagnosis and supervision, including all antimicrobial products, are designated as prescription-only medicines and are used or supplied by veterinarians. Others may be made available through agricultural merchants or sold directly to the public (Rutter, 2003). Hence, a wide range of individuals have access to veterinary medicinal products and to the treated animals. In the UK scheme, most SARs are reported by the attending veterinarian or by the animal health companies. However, there is no restriction on who might submit SARs and reports may be submitted by other qualified health professionals such as physicians (in the case of human SARs to veterinary medicinal products) and pharmacists, or by the animal owner, including farmers and members of the public. The aim is to encourage, in so far as it is possible, the widest reporting of any suspected adverse reactions to the VMD.

For most adverse events, the key interest is identifying developing trends. However, it is important to closely monitor SARs for a newly or recently authorised veterinary medicine, in order that very early signals can be detected and developing trends identified. Some trends are indeed evident. The VMD publishes the results of its surveillance regularly in the *Veterinary Record*. In 1990, the majority of adverse reactions were reported in dogs and cats, as shown in *Table 6.2*. SARs in large animals were somewhat rare (Gray, 1991). Similar trends have persisted (*Table 6.2*) (Gray, 1993, 1994a, b, 1996a, b, 1997a, 1998a, 1999; Gray and Knivett, 2000, 2001, 2002; Gray *et al*., 2003; Dyer *et al*., 2004–2008), although in 1989, adverse reactions to NSAIDs were rare (Gray *et al*., 1990).

The identification of significant trends may lead to regulatory action such as changes in labelling, particularly if this is supported by reports in the literature. However, literature reports are frequently unavailable and regulatory action is therefore usually taken on the basis of the pharmacovigilance data alone.

Companion animals

Within the dog category, the vast majority of SARs can be ascribed to live vaccines, ectoparasiticides, non-steroidal anti-inflammatory drugs

Table 6.2 Adverse reactions in animals for selected years 1991–2006 (Gray 1993, 1994a, 1996a, b, 1997a, 1998a, 1999; Gray and Knivett, 2000, 2001, 2002; Gray *et al.*, 2003; Dyer *et al.*, 2004–2007).

Year	*Species*						
	Dog	*Cat*	*Horse*	*Cattle*	*Sheep*	*Pig*	*Fish*
1991	110	54	32	40	—	7	12
1992	143	135	40	87	22	9	3
1993	170	122	96	43	23	14	—
1995	211	218	69	54	13	9	1
1996	219	234	58	43	21	7	7
1998	271	351	73	32	18	4	—
1999	333	384	74	39	14	2	1
2000	426	482	73	41	20	1	1
2001	301	280	54	23	6	7	—
2002	349	282	44	26	15	—	—
2003	346	273	54	55	15	—	—
2004	389	278	53	62	7	5	0
2005	393	325	65	63	5	5	4
2006	516	385	82	53	15	2	—

Table 6.3 Adverse reactions in dogs for selected years 1991–2006 (Gray, 1993, 1994a, b, 1996a, b, 1997a, 1998a, 1999; Gray and Knivett, 2000, 2001, 2002; Gray *et al.*, 2003; Dyer *et al.*, 2004–2007).

Year	*Therapeutic type*				
	Live vaccine	*Anti-inflammatory*	*Antimicrobial*	*Anaesthetic*	*Anthelmintic*
1991	32	41	17	11	9
1993	32	43	33	16	3
1995	34	38	39	6	15
1996	6	36	40	6	22
1999	53	40	34	12	21
2000	49	52	44	8	15
2001	65	47	33	—	8
2002	65	56	39	—	7
2003	78	4	22	—	13
2004	102	43	22	10	20
2005	72	43	40	22	13
2006	101	25	42	—	9

and antimicrobial agents (*Table 6.3*). Similarly, in cats, the majority of SARs appear to be due to anaesthetic agents, inactivated and live vaccines and ectoparasiticides (*Table 6.4*). These findings appear to be similar to those for earlier years where the information was provided in less detail (see, for example, Gray *et al*., 1988, 1989). Adverse reactions to mixed vaccines were not reported separately prior to 1998, but it is clear from *Tables 6.3* and *6.4* that a trend is emerging. However, one of the features of the VMD's reporting is that the identities of the individual products are not revealed in order to maintain confidentiality. The data are anonymous in nature and do not reveal the names of the products or manufacturers involved. Notwithstanding these remarks, it is

Table 6.4 Adverse reactions in cats for selected years 1991–2006 (Gray, 1993, 1994a, b, 1996a, b, 1997a, 1998a; Gray and Knivett, 2000, 2001, 2002; Gray *et al*., 2003; Dyer *et al*., 2004–2007).

Year	*Therapeutic type*				
	Live vaccine	*Ectoparasiticide*	*Antimicrobial*	*Anaesthetic*	*Anthelmintic*
1991	15	5	—	22	12
1993	14	26	4	27	12
1995	26	91	14	24	17
1996	49	104	14	17	9
1999	78	111	17	32	19
2000	75	91	19	31	13
2001	57	45	16	—*	16
2002	65	26	39	—	16
2003	78	29	22	—	22
2004	65	47	10	—	16
2005	73	34	16	—	11
2006	76	40	14	—	43

*Anaesthetics included in broader category of 'neurological' after this point.

possible to make some general comments on trends over the last 10 years.

The major adverse effect in dogs associated with non-steroidal anti-inflammatory drugs reported to the VMD was gastric ulceration. In fact, gastric ulceration is a common side effect of many drugs of this class in a number of species including humans.

The nature of the reactions reported varies from product to product, as would be expected, and differs between cats and dogs (*Tables 6.3* and *6.4*). Hypersensitivity reactions and anaphylaxis have been reported with vaccines in both cats and dogs. Hypersensitivity appears to be one of the main problems following the administration of antimicrobial agents to dogs. This is particularly true with sulphonamides and potentiated sulphonamides. The most striking signs are polydipsia, stiffness and pain in the joints and haematuria. Such reactions in a number of species have been reported in the literature (Ikezawa *et al*., 1982; Davis, 1984). Marked post-vaccinal lameness has been noted frequently in dogs following the administration of inactivated vaccines, while pyrexia and lameness have been reported in cats, particularly following the administration of live calicivirus products. Anaphylaxis has been reported in cats and dogs after the administration of various vaccines, although this is a relatively rare event. A recent extensive epidemiological study of vaccinated dogs, conducted through a questionnaire survey of owners, revealed no evidence of an increase in ill-health following vaccination, and in fact provided evidence for a reduction (Edwards *et al*., 2004).

As indicated in *Table 6.4*, cats appear very sensitive to some ectoparasiticides. No details are given in the VMD's reports, but cats, especially young animals less than 6 weeks of age, are known to be sensitive to the toxic effects of synthetic pyrethroids as they have low ability to conjugate these compounds through glucuronidation (Oehme and Rumbeiha, 2000). The products involved include shampoos, spot-on-type products and collars containing insecticidal ingredients.

Suspected adverse reactions to anaesthetics (and neurological agents) in cats feature in the VMD's reports, but no details are given.

Interestingly, the predominance of adverse reactions in cats and dogs has been reported in other countries such as Australia and France (Keck and Lorgue, 1990; Maddison, 1992, 1994, 1996). There were other similarities too; for example, the occurrence of pyrethroid toxicity arising from the use of ectoparasiticides in cats

and gastrointestinal effects resulting from the use of non-steroidal anti-inflammatory agents in dogs (Maddison, 1992).

Large animals

With larger animals, including food-producing species, the numbers are generally too small to identify specific hazards and associated risks in terms of product-types involved, and insufficient information is provided in the VMD's reports. However, the literature suggests that a range of adverse effects may be evoked in cattle, often because of interaction with the gastrointestinal tract and its resident microflora with antimicrobial substances (Aksenov, 1973; Gralla, 1975; Kovalev *et al*., 1980; Manten, 1981; Keen and Livingston, 1983; Rollin *et al*., 1986).

There have been a number of instances in VMD reports where young cattle in particular have collapsed after treatment with some corticosteroid and analgesic preparations, with or without fits and ataxia. The reasons for these effects are not fully evident (Gray, 1993). Several reports of unspecified adverse effects of non-steroidal inflammatory drugs in horses have been submitted to the VMD.

One interesting issue has emerged with horses. Horse meat is often sold as pet food or as food for other animals, after euthanasia. This does not pose problems if the animal has been shot or killed with a captive bolt. However, it can produce problems when horses are killed with chemical euthanasia agents. One incident involved a colony of otters that died of barbiturate poisoning after being fed horse meat from an animal euthanised with the drug (Gray, 1991). As a result of cases such as this, advice has been issued by the VMD to prevent knacker meat from chemically euthanised animals from being fed to companion or indeed other animals (Woodward and Gray, 1996).

The VMD's reports suggest that the incidence of adverse events following the use of anthelmintic drugs is relatively low when compared with their widespread use.

Fish

Fish are often treated for disease, and this is frequently bacterial disease, which is treated with antibiotic chemotherapy (Burka *et al*., 1997). However, farmed fish often suffer with ectoparasitic disease and this is specifically noteworthy with, but by no means restricted to, farmed Atlantic salmon (*Salmo salar*) which are vulnerable to infestation by sea lice. A number of chemotherapeutic agents are used to combat these ectoparasites, including products containing organophosphorus compounds, and a number of reports of organophosphorus toxicity have been reported to the VMD. Signs of organophosphorus toxicity, including torpor and ataxia, have been reported in treated salmon (Gray, 1991).

In the EU, vaccines, including fish vaccines, are subject to the same legislation and scrutiny as pharmaceutical products and are subjected to potency testing and batch safety testing prior to release (Lee, 1993; Tatner, 1993; Hendricksen, 1996; Roberts and Lucken, 1996; Roberts and Sanders, 1997; Cowan, 2002; McVey *et al*., 2003).

Veterinary vaccines are usually very safe and adverse reactions are generally restricted to lack of efficacy, as is evident from some of the VMD's reports mentioned here (but see Chapter 19 on vaccine adverse reactions). This lack of efficacy may be caused by reduced antigen titres or possibly by the use of products that have exceeded their shelf lives. Adverse reactions are often of the immune-mediated type such as anaphylaxis and are associated with foreign antigens (Lund, 1988; Hera, 1994; Reddy *et al*., 1994; Siev, 1999; Zimmel *et al*., 2000; Greenacre, 2003; Kohn *et al*., 2003). Occasional reversion to virulence of attenuated vaccines may occur (Francis, 1993).

Lack of efficacy

A significant number of reports to the VMD involved lack of expected efficacy. For example, in 2000, 119 suspected adverse reactions were associated with this, mainly in dogs, cats, cattle and sheep (Gray and Knivett, 2001; Dyer *et al*.,

Table 6.5 Patterns of SAR reporting to the VMD. (Numbers are in percent.)

Year	*Veterinarians*	*MA holders*	*Public*	*Other**
1999	65	33	2	~1
2000	67	29	3	~1
2001	62	34	2	~2
2002	60	38	2	~1
2003	52	46	2	~1
2004	53	40	2	~1
2005	58	40	2	~1
2006	49	48	2	~1
2007	43	55	2	—

*State Veterinary Service, farmers, Veterinary Investigation Service, pharmacists, Pesticides Safety Directorate (UK agency responsible for pesticide regulation).

2004–2007). In 2001, 92 reports concerned lack of efficacy, most of which involved inactivated vaccines (Gray and Knivett, 2002), while in 2002, some 113 reports were made, with over 50% involving vaccines (Gray *et al*., 2003). There were 265, 151 and 302 reports of lack of expected efficacy in 2004, 2005 and 2006 respectively (Dyer *et al*., 2005–2007).

Adverse reaction reporting statistics in the UK

Data show that the majority of UK reports of suspected adverse reactions are submitted by veterinarians (*Table 6.5*). In the period 1999–2003, around 55–65% of reports were from this source, and around 30–45% were from marketing authorisation (MA) holders. The percentage of reports submitted by the public was low (around 1–2%) and the remainder (~1%) came from a variety of sources, including the State Veterinary Service, farmers and pharmacists. However, in 2007, the majority of reports originated from marketing authorisation holders.

By contrast, the percentage of suspected adverse reactions classified as serious submitted by veterinarians was in the range 25–35% for this period, with 60–75% being submitted by the MA holders (*Table 6.6*). The remainder, ~1% were submitted from all other sources (data based on Dyer *et al*., 2004).

Table 6.6 Patterns of serious SAR reporting to the VMD. (Numbers are in percent.)

Year	*Veterinarians*	*MA holders*	*Other**
1999	26	73	~1
2000	34	65	~1
2001	31	67	~2
2002	36	62	~2
2003	24	75	~2
2004	20	78	~2
2005	20	78	~2
2006	15	84	~1
2007	13	86	~1

*State Veterinary Service, public, farmers, Veterinary Investigation Service, pharmacists, Pesticides Safety Directorate.

For animal suspected adverse reactions, the person submitting the adverse reaction may send these initially to a retail outlet, veterinarian or pharmacist, depending on where he/she obtained the product, or he/she may send them direct to either the VMD or the MA holder. The latter should supply these to the VMD as part of a periodic safety update report (PSUR), except in the case of those classed as 'serious' which must be notified to the regulatory authorities in an expedited manner.

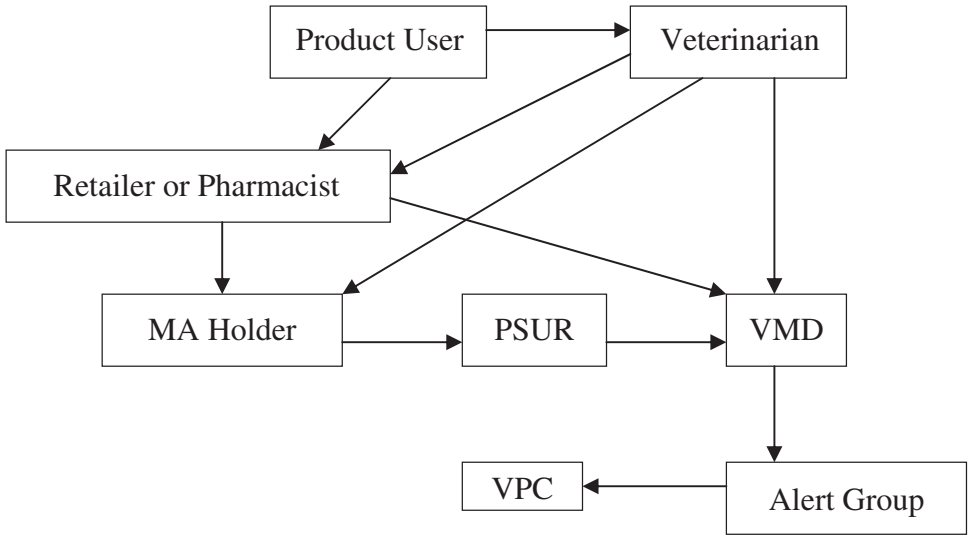

Fig. 6.1 Reporting routes for suspected adverse reactions in animals (MA – marketing authorisation; PSUR – periodic safety update report; VMD – Veterinary Medicines Directorate; VPC – Veterinary Products Committee). (Adapted from Veterinary Products Committee Working Group Report, 2004.)

Once suspected adverse reactions are submitted to the VMD, how they are handled depends on whether they were in animals or in humans. The Alert Group is an internal group of scientists within the VMD with representatives of all disciplines. It meets monthly to review all serious animal adverse reactions and all human adverse reactions and food safety or environmental incidents. Its conclusions are then referred to the Veterinary Products Committee (VPC). A quarterly summary of all suspected adverse reactions is considered by the VPC. However, suspected adverse reactions in humans are also considered by the Appraisal Panel for Human Suspected Adverse Reactions to Veterinary Medicines, usually referred to simply as the Appraisal Panel, an independent subcommittee of the VPC, prior to consideration by the VPC itself. The reporting structures and responsibilities are shown in *Figures 6.1* and *6.2*.

Regulatory activities

The importance of identifying trends is not merely the recognition that they may occur – it is more that they may eventually result in regulatory action that can help to prevent future occurrences or at least contribute to a reduction in their frequency. One example, that of feeding drug-contaminated knacker meat to other animals, has already been mentioned. The aim here was to advise the veterinary profession and others of the potential hazards associated with this use of food products obtained from chemically euthanised animals. Other examples have included advice on the treatment of dogs with avermectin compounds, recall of a product containing an organophosphorus compound (diazinon) where mortalities had occurred in cats and dogs, and the morbidity arising in dogs and cats (see *Tables 6.3* and *6.4*) following vaccination (Gray, 1997b–d, 1998b; Dean, 1998). The potential toxicity of permethrin in the cat and piperazine in kittens has also been publicised (Gray and Millar, 1987; Gray, 2000b). The recall of a sub-potent batch of kennel cough vaccine (to protect against infection with *Bordetella bronchiseptica* in dogs) was publicised (by the company, after discussions with the VMD) in 1997 (Smitherman, 1997).

An adverse reaction to the fluoroquinolone antimicrobial drug enrofloxacin has been reported in cats. This was initially described in the USA. The reaction involves diffuse retinal degeneration, particularly of the photoreceptor layer, and

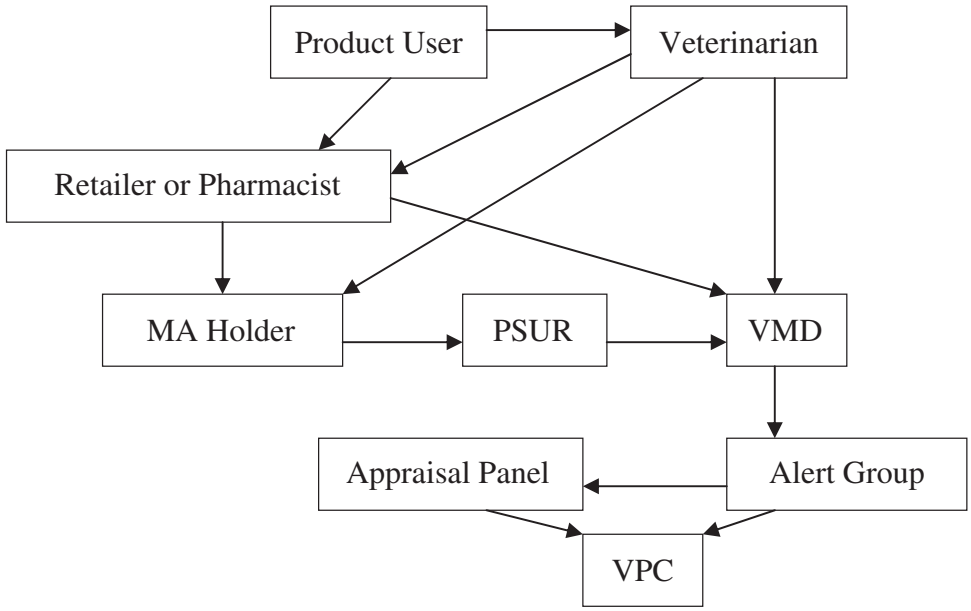

Fig. 6.2 Reporting routes for SARs in humans. (Abbreviations as for *Figure 6.1*.) (Adapted from VPC Working Group Report, 2004.)

the animals may become blind if treatment is not halted. The risk appeared to be most significant at doses above 5 mg/kg body weight each 24 hours (Gelatt *et al*., 2001). It has now been reported in cats in the UK (Crispin *et al*., 2002). Further research has revealed that the highest risk is in animals given doses exceeding 30 mg/kg body weight per day for up to 21 days (Watson, 2002). In 2006, 10 reports of blindness in cats following treatment with enrofloxacin were reported to the VMD. The majority of these animals appeared to have been overdosed with the drug (Dyer *et al*., 2007).

The VMD has also published a number of warnings regarding residues of antibiotics in milk, the safety of moxidectin in sheep, the ingestion of avermectins and moxidectin by dogs, scouring in lambs caused by diclazuril and the use of carprofen in cats on its website (http://www.vmd.gov.uk/).

The subject of vaccination in companion animals is topical as it has featured in elements of the media, and is currently the subject of interest from a number of pressure groups in Europe and elsewhere. Effects reported include hypersensitivity reactions, injection site reactions, respiratory effects, neurological and behavioural effects, and lack of efficacy. In addition, a specific group of signs including anorexia, malaise, pyrexia, stiffness, lethargy, depression, lameness and joint pains predominate in post-vaccination suspected adverse reactions (Gray, 1998b). It is difficult to identify the causative factors although immune determinants may be involved.

What is important is that the findings of all the suspected adverse reactions reported by the VMD need to be seen in context. They are often reported annually in terms of tens or very occasionally hundreds of events, whereas the products involved are generally sold in millions of doses. In terms of the vaccines, for example, the incidence of adverse reactions is approximately 0.004%. This must be seen against the alternative – non-vaccination and the likelihood of occurrence of potentially fatal conditions, not only in the individual animal, but also at the level of the population. Nevertheless, the apparent increased incidence of sarcomas at the injection site in cats, following the administration of vaccines, has caused concern (Dubielzig *et al*., 1993; Esplin *et al*., 1993; Kass *et al*., 1993; Hendrick *et al*., 1994; Doddy *et al*., 1996; Macy, 1999; Veterinary Products Committee, 2001; Gaskell *et al*., 2002; Gray and Knivett, 2002; Gray *et al*., 2003; Tjälve,

2003b; Dyer *et al*., 2004–2007; Vaccine-Associated Feline Sarcoma Task Force, 2005; see Chapter 19 on adverse reactions to vaccines). The CVMP has also issued its own advice to veterinary surgeons on this important issue (CVMP, 2003).

The VPC has called for additional warning statements and contraindications for a range of products. These include those containing organophosphorus compounds, corticosteroids, avermectins, antibiotics of all classes, and anthelmintic bolus products intended for use in cattle. Moreover, the marketing authorisations for products giving rise to significant concerns may be suspended and there are currently (early 2008) 18 products with suspended marketing authorisations, including the synthetic pyrethroids-containing ectoparasiticidal sheep dips where the marketing authorisations were suspended due to adverse environmental effects. Thus, the practical experience of a pharmacovigilance scheme can lead to regulatory action intended to ensure safer future use as well as providing advice to veterinarians and other users.

One of the major problems of any spontaneous reporting scheme is under-reporting (Moride *et al*., 1997; Alvarez-Requejo *et al*., 1998; van der Heijden *et al*., 2002), a problem recognised with the UK system as long ago as 1988 (Gray and Evans, 1988), and in recent years, more publicity has been provided in attempts to increase reporting (Gray and Knivett, 2002). In human medicine pharmacovigilance, reporting can be encouraged by feedback provided to the physician and other measures (Wallerstedt *et al*., 2007) and similar initiatives might be considered in the veterinary sector.

This chapter is primarily concerned with pharmacovigilance in animals following their treatment with veterinary medicines. However, as indicated earlier, the yellow card adverse reaction reporting scheme for veterinary medicines in the UK also allows for the reporting of adverse events in humans following exposure to veterinary medicines and this is an important aspect of veterinary pharmacovigilance. These yellow cards are made readily available to all veterinary surgeons, and the animal health industry representative body, the National Office of Animal Health (NOAH) regularly publishes a compendium of data sheets for veterinary medicinal products produced by its member companies (which comprise the vast majority of animal health companies in the UK and certainly all the major multinational and global companies) (Anonymous, 2006). This compendium is sent to all veterinary practices as a major source of information on products, their uses and associated warnings and contraindications. A copy of the yellow form for reporting suspected adverse reactions is bound into every copy of the compendium so that every surgery has not only a convenient source but also a reminder of its existence and that of the reporting scheme itself!

The VMD has also issued a warning of adverse effects related to the drug Econor (Gray, 2000a). This is notable if only because, to date, it is the only significant action taken on a veterinary product authorised through the centralised procedure. In March 1999 the European Commission issued a marketing authorisation for Econor following a positive opinion from the CVMP. Econor is a series of premix formulations containing varying amounts (1, 10 and 50%) of the antimicrobial drug valnemulin. It is used for the treatment and prevention of swine dysentery and enzootic pneumonia in pigs.

By the middle of September 2000, a total of 36 reports of suspected adverse reactions in treated animals had been reported to the national competent authorities and brought to the attention of the EMEA and CVMP. Of these, 34 were classified as 'serious' and involved lethargy, depression, erythema, oedema, pyrexia, ataxia, anorexia, pain and death (Macgregor, 2000). The product's marketing authorisation was suspended in Denmark and Sweden and later in Finland. The European Commission requested the advice of the CVMP and its Pharmacovigilance Working Party. Following the opinion of the CVMP, the marketing authorisation was suspended throughout the EU in late 2000. The CVMP proposed further investigations to be conducted by the marketing authorisation holder, which would be necessary before lifting of the suspension could be

considered (EMEA, 2000; Gray, 2000a). The 72nd Meeting of the CVMP in December 2001 adopted an opinion recommending termination of this suspension, presumably on the basis of favorable evidence provided by the marketing authorisation holder.

Environmental effects

Environmental risk assessments are an integral part of the assessment process in the granting of marketing authorisations for veterinary medicinal products (see Chapter 26). This process generally uses a two-pronged approach – a Phase I assessment which is largely, but not exclusively, a paper-based exercise using physico-chemical parameters such as solubility, volatility and pH, as well as biological properties including environmental fate, photostability and environmental persistence. As also described in Chapter 26, the results of the Phase I assessment may trigger the Phase II assessment where more detailed studies on environmental fate and ecotoxicology may be necessary.

Veterinary medicinal products may enter the environment in several ways. They may, for example, enter during manufacture. However, manufacturing plants are also subject to the provisions of Good Manufacturing Practice (GMP) and to legislation governing health and safety at work and environmental emissions and discharges. Consequently, contamination from this route is likely to be low and episodes infrequent. Indeed, a recent report sponsored by the Cranfield Centre for EcoChemistry, Cranfield University, reached that very conclusion (Boxall *et al*., 2002).

The other major source of potential environmental exposure is through farming, as the Cranfield report recognises. Exposure may occur through faeces and urine from topically treated animals, and be concentrated in farm slurry which either can lead to run-off from slurry pits or other storage sites, or is transferred largely to arable land as a fertilising agent. Contamination may be with the parent drug or as a combination of parent drug and its metabolites. Alternatively, medicines that are applied topically, like pour-on formulations or sheep dips, may drain from treated animals and thus contaminate land and water courses. These formulations often contain substances potentially damaging to the environment such as organophosphorus compounds and synthetic pyrethroids. Processing of wool from treated sheep may also lead to environmental contamination.

Contamination may also occur as a result of careless disposal of partly used containers and appliances (Boxall *et al*., 2003a). Some products are applied by shower rather than through dipping and as these become contaminated, bacteria present in them may cause infections in other animals treated subsequently (Watson *et al*., 2003; Shaw, 2004). In the USA, environmental warnings have been added to euthanasia products to prevent wildlife dying from barbiturate toxicity arising from disposal of unwanted product (Anonymous, 2003).

In addition to the contamination and effects of veterinary medicines in the environment, the potential contribution and adverse effects of human pharmaceuticals also need to be taken into account (Henschel *et al*., 1997; Christensen, 1998; Stuer-Lauridsen *et al*., 2000; van Wezel and Jager, 2002; Golet *et al*., 2003; McArdell *et al*., 2003; Swedish Medical Products Agency, 2004), and in the EU, the potential environmental risks associated with human pharmaceutical products must be assessed (Shaw, 2004). An unusual environmental issue has arisen in Pakistan. Here, there has been a dramatic decline in the numbers of Oriental white-backed vultures (*Gyps bengalensis*) and other vulture species. In one area, the decline in the Oriental white-backed vulture has been in the region of 95% since the 1990s (Prakash, 1999). The declines were matched by findings of renal failure and visceral gout in affected animals. This correlated with findings of high concentrations of the non-steroidal anti-inflammatory drug diclofenac, and the ability of diclofenac to reproduce the effects in the birds. It was hypothesised that the morbidity and mortality in the vultures was

due to the animals scavenging on dead livestock which had been treated with diclofenac prior to death. Diclofenac is available as an over-the-counter veterinary drug in Pakistan and is widely used (Oaks *et al*., 2004).

In 2003, a review concluded that for many veterinary medicines used in the UK, there were adequate data on environmental behaviour and effects. These included oxytetracycline, chlortetracycline, amoxicillin, diazinon, tylosin, dihydrostreptomycin, cypermethrin and sarafloxicin. Others, including trimethoprim, procaine penicillin, clavulanic acid, neomycin, fenbendazole, levamisole, ivermectin, lincomycin, enrofloxacin, deltamethrin and some immunological products were almost adequately characterised, while others such as progesterone, procaine hydrochloride and moxidectin suffered from larger gaps in the data set (Boxall *et al*., 2003b). However, this analysis was largely on the basis of publicly available data, and much of the information provided to regulatory authorities is confidential and so not necessarily included in the survey.

Environmental risk assessments for veterinary medicinal products in the EU have recently been criticised, largely on the basis of lack of transparency but also because the Phase I and Phase II risk assessments were said to be, inter alia, lacking in acceptability and harmonisation. Hence, the authors considered that the assessments lacked legitimacy (Montforts and de Knecht, 2002). However, representatives of the authorities responded robustly, pointing out that the environmental assessment of veterinary medicines is part of the regulatory and authorisation processes, and that harmonisation is being achieved through the VICH (see Woodward, 2005, and Chapter 2) in a process that is open to public consultation (Long and Crane, 2002).

Despite these considerations, frank effects of veterinary medicines on the environment in the UK would appear to be relatively infrequent. In 2001, the first year that they were mentioned in the VMD annual article, there were 34 reported incidents (24 of the 34 occurring in 2000) involving the death of fish or aquatic invertebrates. Many of these appeared to be due to 'inappropriate practice' during the use and disposal of sheep dip (probably illegal disposal of dip to water courses). There were no incidents involving other animals, including birds (Gray and Knivett, 2002). In 2002, six reports of environmental incidents were reported to the VMD. Of these, five involved sheep dips and one was a possible case of illegal poisoning.

In fact, this type of incident has been reported previously to the VMD. In 1998, there were 39 incidents involving sheep dips and these were largely attributable to cypermethrin-based products. Around a third of these were caused by operational failures. The incidents reported in 2002 were due to run-off and bad dip management, with at least one involving contamination of a water course (Gray *et al*., 2003), while in 2003, nine incidents were reported involving exposure or potential exposure of aquatic invertebrates to synthetic pyrethroids (eight cases) or an organophosphorus compound (one case). In six cases, there was sufficient information to conclude that the incidents had arisen from improper use (Dyer *et al*., 2004).

There were 11 incidents reported in 2004. Seven of these involved the incorrect disposal of sheep dips, mainly cypermethrin-based products. Others involved the poisoning of wild birds (Dyer *et al*., 2005). The numbers of environmental incidents reported to the VMD in 2005 rose dramatically to 81, possibly because a government body, the Environment Agency, submitted a number of reports. Many of these included reports of environmental contamination by sheep dips, largely with cypermethrin or diazinon-based formulations. The involvement of cypermethrin was confirmed analytically in 29 incidents, of which 20 were categorised as being major or serious (Dyer *et al*., 2006).

A total of 62 incidents were reported to the VMD in 2006. The majority of these (61) involved exposure or potential exposure of the aquatic environment. One of these was associated with the use of oxytetracycline, and for two others no product could be identified. The remaining 23 incidents involved cypermethrin and of these, seven were considered to be serious events.

A further 31 incidents were related to the use of diazinon, but none of these was considered as serious. Sheep dipping was the major activity associated with the cypermethrin- and diazinon-related incidents (Dyer *et al.*, 2007). As noted earlier, the marketing authorisations for the cypermethrin products were recently suspended by the VMD.

In 2007, 42 incidents were reported (Dyer *et al.*, 2008). All of these involved exposure of the aquatic environment. Of these, three incidents were classified as having a major impact on the environment, while eleven were classified as having a serious impact. In six of these reports, the ectoparasiticidal sheep dip active ingredients cypermethrin and diazinon were involved, and the majority of the incidents involved sheep dipping activities.

Adverse reaction reporting in other European Union countries

As all EU countries, or more precisely all European Economic Area (EEA) countries, which includes all 25 EU member states and Iceland, Norway and Liechtenstein, are applying the same European legislation, it might be expected that the schemes in operation in each would be similar. However, this is not the case as each country has introduced measures that, although in compliance with the Directives and other legislation, are not at face value similar. For example, although the VMD, the regulatory authority in the UK, is responsible for pharmacovigilance, including the collection and analysis of spontaneous reports, in France the centre is based in a university (the National Veterinary School of Lyon) but is officially linked to the regulatory authority. Like the UK, adverse reaction reports in Ireland are sent to the Irish Medicines Board. In Germany, the responsibility for authorisation of veterinary medicines is split between the Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL) in Bonn for pharmaceuticals and the Paul-Ehrlich-Institute (PEI) in Langen for vaccines; responsibilities for spontaneous adverse reaction reports are similarly split. In Sweden, the spontaneous reports are sent to the Department of Pharmacology/Toxicology at the veterinary school in Uppsala, while in The Netherlands they are dealt with by the Dutch Centre for Pharmacovigilance or BBD (Keck, 1992; Keck and Ibrahim 2001).

The Brussels-based International Federation of Animal Health (IFAH) or more specifically IFAH-Europe and its forerunner the International Federation of Animal Health (FEDESA), has conducted surveys of the implantation of veterinary pharmacovigilance across the 15 existing EU member states (i.e. prior to EU enlargement to 25 member states in May 2004). In February 2003, the IFAH survey revealed that apart from Austria where no information was forthcoming, all member states had implemented EU pharmacovigilance requirements and most were following the requirements of the former Volume 9 (see Chapter 2).

There were, however, some differences in local requirements. For example, although all required national reporting, some such as Germany, Denmark and Finland also demanded third country reporting, as required by Volume 9, while others like Ireland did not. Greece and Ireland did not require MRL violations to be reported, but most of the remainder did. Whereas most member states required environmental incidents to be reported, Greece, Ireland and Italy did not have this requirement. In most countries the national language was not essential for reporting or for PSURs. However, it was needed in Spain, Ireland, Italy, Portugal and the UK.

It therefore appears that there is still some progress needed to achieve full implementation of EU requirements across Europe. A former senior official of the European Commission who was later to become the first Director of the EMEA noted in 1992 that 'a better co-ordination of the assessment of adverse drug reactions (pharmacovigilance) should be achieved' (Sauer, 1992). Although said more in the context of the EMEA than of national procedures, on balance, in 2008, this goal is nearer to realisation, but more progress is still desirable.

Moreover, with the exception of the VMD in the UK, few European veterinary drug agencies publish the findings from their pharmacovigilance schemes or indeed from their residues surveillance schemes. Where results are available, they suggest that the overall profile is similar to that of the UK. For example, in Sweden, adverse reactions to vaccines in dogs were a relatively common adverse event, while in horses the majority of adverse reactions were to antimicrobial drugs; three horses died following treatment with trimethoprim-sulphadiazine products and seven died after administration of benzyl penicillin. Gastrointestinal symptoms, including gastric bleeding, were reported after dogs were treated with NSAIDs. Reactions to vaccines were also the major adverse events seen in cats (Tjälve, 1997; Tjälve, 2003a, b).

A number of suspected adverse drug reactions have been reported in Ireland for the 1998 period (Beechinor and Arthur, 1999). A relatively large number of these appeared to involve the administration of copper formulations in cattle, where a number of fatalities occurred after subcutaneous administration and injection site reactions after intramuscular dosing. Major adverse events were reported after administration of anthelmintic boluses. These are sizeable devices which are given using a specially designed dosing 'gun'. Over 30 reports, including oesophageal obstruction, trauma and death, were made to the Irish Medicines Board in the 1988 period and many of these involved the treatment of animals that were below the minimum age or weight for this type of treatment. Incidents involving the boluses were reported subsequently (Murphy and Arthur, 2000). These products are available across many parts of the world but without seemingly causing this type of problem and the reasons for the frequency of these reactions in Ireland remain obscure.

There were two reports of adverse drug reactions in humans. One of these occurred after exposure to the vapours from an iodine-containing cleansing agent and this was restricted to transitory eye irritation. The other involved accidental ocular exposure to a dog shampoo containing benzoyl peroxide. This resulted in some damage to the cornea, conjunctiva and tear duct.

In the period 2001–2002, 113 reports were received in Ireland. Again, there were several (14) relating to the administration of copper in cattle, with two fatalities. These appeared to be associated with overdose. There was a single fatality in a horse associated with five to six times overdose with oral moxidectin and another resulting from intravenous administration of oxytetracycline; the drug was not authorised for use in the horse and it had exceeded its expiry date by 12 months. A number of adverse reactions to vaccines were reported, including an anaphylactic-type reaction following administration of a vaccine for the prophylaxis of respiratory syncytial vaccine. Most notable were a total of 69 adverse events in pigs, including muscle tremors and convulsions, with a total of 14 fatalities after the administration of vaccines to prevent disease associated with *Mycoplasma hyopneumoniae* infections. The problems with anthelmintic boluses continued and were said to be the most commonly reported adverse reaction, although the numbers of reports were said to have declined (Murphy, 2003). In 2003, fatalities in sheep treated with nitroxynil were noted. There were three cases of accidental self-injection in humans, including one resulting in drowsiness and slurred speech where detomidine was involved (Murphy, 2004).

Many of the reactions in cats and dogs were due to hypersensitivity reactions. As in the UK, a number of reactions in horses arose from antimicrobial treatments and several animals died. Some of the incidents in goats involved off-label use of drugs, including ivermectin, while many of those in sheep involved ectoparasiticides. In one instance, over 35,000 sheep were treated with the endectocides ivermectin and over 600 died. It was found that incorrect administration was responsible and, as a result, severe damage to the throat occurred which led to the deaths observed. Incidents in cattle often involved ectoparasiticides or antimicrobial drugs. In one instance, topical application of synthetic pyrethroids resulted in skin irritation.

The reactions in humans tended to involve contamination with ectoparasiticides or accidental self-injection with vaccines. There was one fatality following exposure to an ectoparasiticide in 2000. A case of photodermatitis occurred after exposure to olaquindox, an antimicrobial drug used in pigs (Fewings and Horton, 1995).

Pharmacoepidemiology

In human pharmacoepidemiology, a number of techniques are employed to investigate adverse drug reactions (O'Neill, 1998; Strom, 2000a, b; Weed, 2000; Waller, 2001; Edwards *et al*., 2005). These include:

- case reports;
- case series;
- randomised clinical trials;
- cohort studies (prospective studies);
- case control studies (retrospective studies).

The majority of adverse reactions discussed here have involved spontaneous adverse reactions reported to regulatory authorities, while others fall into the case report or case series reports published in scientific journals. Case and case series studies, by their nature, make no use of controls, and while they may generate hypotheses (for example, a drug may cause a particular adverse reaction), they cannot be used to test hypotheses. Case series studies have been used to investigate the effects of vaccination in cats, dogs and ferrets, and the effects of potentiated sulphonamide therapy in dogs (Gaskell *et al*., 2002; Greenacre, 2003; Trepanier *et al*., 2003). Hypothesis testing can only be conducted with adequately controlled studies such as case control or cohort studies, or with randomised clinical trials (Strom, 2000b).

Both randomised clinical trials and cohort studies are expensive to conduct and this expense probably precludes their use for veterinary purposes. Case control studies, while still expensive, are nevertheless feasible and within the means available to veterinary practices of academic centres. They have been used occasionally, and some have been mentioned in the course of this chapter. The more notable examples are few in number and are illustrated by selected examples shown in *Table 6.7*, but there is a recognised need to develop and use epidemiological methods for monitoring product safety (Wood and Adams, 2006).

Other, more specialised approaches to pharmacoepidemiology are not employed in the veterinary area. For example, and as discussed earlier, in human medicine, prescription event monitoring is an important tool in post-marketing surveillance of pharmaceuticals. Prescription information is collected centrally and can be used to support investigations of specific adverse drug reactions in groups of patients (Mann *et al*., 1997; Mann, 1998, 2000). It is reliant on the central (or semi-central) collection of prescription data. In many countries, including the UK, prescriptions are dispensed by the veterinarians who write them, and in any event, they are not collected by any central organisation, thus making this approach unsuitable in veterinary post-marketing surveillance.

Consequently, it seems reasonable to assume that spontaneous reporting of adverse drug effects, supported by case reports, case series reports and occasionally by case control studies, will remain the mainstay of veterinary pharmacovigilance for the foreseeable future. Spontaneous reporting is almost certainly poorly subscribed to by veterinary professionals and hence there is probably considerable under-reporting (Bukowski and Wartenberg, 1996). Several measures have been suggested to improve this, including educational initiatives for veterinarians, use of diagnostic algorithms, allowing non-professionals to report adverse reactions (currently the case in the UK) (Bukowski and Wartenberg, 1996; Ibrahim, 2003) and the wider publication of adverse reaction data in specialist journals, particularly with more detailed analysis of the data than is currently the case (Keck, 2003). Interestingly, a paper published in 2003 on how veterinarians might respond to an adverse drug reaction included no advice on reporting the reaction to the authorities (Brumbaugh, 2003). Such advice is clearly needed.

Table 6.7 Selected case and case control studies and other studies in veterinary pharmacoepidemiology.

Objectives	*Findings*	*Reference*
Study of adverse effects of antibiotics in dogs	Epidemiological review of pet owners associated adverse effects with antibiotic therapy	Kunkle *et al*., 1995
Study of acute ibuprofen toxicity in dogs to determine risk factors for renal toxicity and gastrointestinal ulceration	Authors studied 116 cases and 93 controls. Risk of ulceration was lower for dogs where time from ingestion to intervention was known rather than unknown, and lower for Labrador breed. Risk was higher for prolonged time to intervention and for German Shepherd breed. Risk of renal failure was higher for prolonged time to intervention. Study suggests breed differences in susceptibility to gastric ulceration, but it failed to show a dose response	Poortinga and Hungerford, 1998
Study of diarrhoea associated with trimethoprim potentiated sulphonamides in horses and ponies	Study conducted in two parts. Part I was a case control study of 135 records over a 10.5-year period. Part II was an historical cohort study of 784 records for a 37-month period from 1 July 1990 to 31 July 1993 Diarrhoea occurrence was 21% and 3% in Parts I and II respectively. In Part I, significant factors were length of hospital stay and antimicrobial therapy (other than potentiated sulphonamides), while in Part II factors included other antimicrobials, penicillin therapy and combined penicillin and potentiated sulphonamide therapy. Prevalence of diarrhoea in horses receiving potentiated sulphonamides was not significantly different from those given penicillin	Wilson *et al*., 1996
Investigation of association between effects of salinomycin and polyneuropathy in cats	Underlined the fact that exposure to salinomycin is associated with occurrence of polyneuropathy in the cat	Van der Linde-Sipman *et al*., 1999
To determine whether foals with pneumonia treated with erythromycin, alone or in combination with rifampin (rifampicin) or gentamicin, had a higher risk of adverse reactions than controls treated with potentiated sulphonamides, penicillin G procaine or a combination of potentiated sulphonamide and penicillin G	Foals treated with erythromycin had an eight-fold higher risk of developing diarrhoea, and increased risks of developing hyperthermia and respiratory distress	Stratton-Phelps *et al*., 2000

Table 6.7 *Continued*

Objectives	*Findings*	*Reference*
To investigate adverse reactions in dogs to vaccination	Retrospective analysis of 311 cases reported to the Japanese Ministry of Agriculture, Forestry and Fisheries over 6 years from 1994 to 2000 Dermatologic signs were most frequent (53%) followed by gastrointestinal (16%) and cardiovascular/respiratory (14); 11 dogs (3.5%) died	Ohmori *et al.*, 2002
To investigate the adverse effects of distemper or rabies vaccinations in ferrets	A retrospective study of adverse reactions in 143 cases Results suggested a high incidence of anaphylactic reactions	Greenacre, 2003
To investigate whether vaccine brands, other injectable products, routine vaccination or various host factors were associated with the development of injection site sarcomas in cats	Multicentre, case control study of treated cats. No specific vaccine brand was associated with sarcoma development, nor was vaccination, specific antigens, reuse of syringes or history of trauma. Certain long-acting drugs, penicillin and methyl prednisolone may be associated with sarcoma development	Kass *et al.*, 1993, 2003
To investigate whether vaccination in dogs is associated with ill-health	Questionnaire study using 9055 postal questionnaires, of which 4040 were returned. No temporal association between vaccination and ill-health found after adjusting for confounding factors such as age; evidence that recent vaccination may improve health	Edwards *et al.*, 2004
To determine clinical signs and relationship to vaccination of polyarthritis in dogs	Retrospective study covering 39 cases between 1997 and 2002 Association between type I immune-mediated polyarthritis and vaccination, but no clear relationship	Clements *et al.*, 2004
To study adverse events in dogs vaccinated for rabies in the US	Retrospective study of adverse events associated with rabies vaccination in a study population of 257, 564 vaccinated dogs in 169 hospitals and 13 metropolitan areas during a 24-month period Study revealed a cluster of adverse events in one area only (Atlanta and Tampa/St Petersburg over a 4-month period	Moore *et al.*, 2005a
To study adverse effects of vaccination in dogs	Retrospective cohort study to examine adverse effects occurring within 3 days of vaccination in dogs Elevated risk of allergic reaction and other adverse reaction in vaccinated dogs. Risk decreased with increasing body weight; risk greater for neutered animals and older dogs (1–3 years vs 2–9 months)	Moore *et al.*, 2005b
Review of gastrointestinal effects of NSAIDs in dogs	Underlined association between NSAIDs and occurrence of adverse gastrointestinal effects in the dog	Lascelles *et al.*, 2005

Discussion

A good spontaneous reporting pharmacovigilance scheme for veterinary medicines is one that provides robust data on the occurrence of adverse reactions in treated animals and exposed human beings. Such data can be utilised to identify toxic or idiopathic reactions to medicines used in clinical veterinary practice, and ultimately can contribute to greater safety, or safer use, of the medicines involved. In Europe, the USA and in other parts of the world, relatively sophisticated schemes have evolved, albeit recently. These schemes and those in other countries should contribute to even safer and more effective use of veterinary medicinal products and more effective regulation in the future. The schemes should themselves become more effective and efficient as initiatives on international harmonisation take effect.

The UK's Suspected Adverse Reaction Surveillance Scheme has been an undoubted success and has contributed significantly to the safety of veterinary medicinal products on the UK market. It has achieved this either by providing reassurance for those products that have not given rise to concern because they do not feature in reports or because of additional labelling or other precautions and regulatory actions for those that do.

However, as with so many elements of the regulation of pharmaceutical products specifically and almost everything generally, the effects of European legislation and the demands of EU-wide implementation of requirements for pharmacovigilance, and further harmonisation will ultimately affect the workings of the UK scheme. Although the UK scheme is generally transparent, as is evident from the annual publications, some might argue that it is still not sufficiently transparent. The data are generally presented in an anonymous manner, as general categories of drugs or products – antimicrobials, anaesthetics or vaccines – and some might argue that the data would be improved if active ingredients were identified, even if it is not essential to identify actual products. Alternatively, major groups could be broken down into subgroups – β-lactams, macrolides, potentiated sulphonamides instead of antimicrobials, injectable or inhalation anaesthetics rather than simply anaesthetics (or worse 'neurological'), benzimidazoles and avermectins in place of anthelmintics, and specific vaccine products (rabies, leptospira, etc.) where practicable instead of inactivated or live vaccines.

Consequently, in the past there has been no way of knowing which specific veterinary medicines or active ingredients are associated with particular adverse reactions. This has only served to reduce the educational value of the information to clinicians and researchers who might otherwise benefit from the missing information. There is a need for reports of this type to evolve over the years, and those published by the VMD have certainly evolved. In the 2007 VMD report (Dyer *et al*., 2008), products are mentioned by name to give a degree of transparency which may not be welcomed by all.

Through its website, the VMD gives attention to topical issues. For example, it has drawn attention to the hazards involved in handling cytotoxic drugs in veterinary practice when treating companion animals with neoplastic disease. It recommends using disposable gloves and avoiding splitting tablets. There are no drugs licensed for the treatment of cancer in companion animals and veterinarians have to resort to the use of drugs intended for human use, which they may do in the EU under the cascade mentioned above. Veterinarians are unlikely to have facilities designed to cope with the use of such hazardous pharmaceuticals, and without adequate precautions human contamination could occur. This issue has been raised in the literature and recommendations have been made for both veterinarians and animal owners, including not breaking tablets, avoiding direct contact with the drug, removing the faeces and urine of treated animals and the use of gloves (Pellicaan *et al*., 2002).

It was announced in 2002 that a review of the UK scheme was planned by the VPC (Skilton, 2002). This aimed to examine the usefulness of the scheme to veterinary practitioners in terms of

feedback and reporting efficiency, and to examine the degree of publicity given to the scheme, and hence awareness in the minds of veterinarians when faced with an adverse event.

As previously mentioned, one of the drawbacks of spontaneous reporting is under–reporting, and as a counter to this in human medicine, other mechanisms have been introduced. Of these, perhaps the most successful is prescription-event monitoring (Mann *et al*., 1997; Mann, 1998, 2000). This relates adverse events to prescriptions written and dispensed. It relies on the fact that once dispensed prescriptions are collected, collated and analysed by a central repository, it is possible to compare these with national statistics on disease prevalence and adverse reactions. Unfortunately, there is no central repository for prescriptions for veterinary medicines, and no national registers for animals and so this would only be possible at the local level, e.g. within a veterinary practice. It seems that this approach, despite its advantages, would be impractical for veterinary medicines.

Other approaches in human medicines include monitoring average daily quantities of medicines prescribed or used and comparing the data thus generated with adverse reaction frequencies (Walley and Roberts, 1999) or conducting pharmacovigilance by monitoring over-the-counter products (Layton *et al*., 2002). Again, these methodologies do not lend themselves easily to veterinary drugs. In the case of the former, the actual amount of drugs dispensed is difficult to quantify as there is no central co-ordinating body (such as the national health service providers), while in the latter case, veterinary medicines are distributed by veterinary wholesalers and agricultural merchants and dispensed by veterinary surgeries, none of whom has the resources to monitor the medicines provided against any adverse reactions noted. For the foreseeable future it seems that veterinary pharmacovigilance will be heavily reliant on spontaneous reporting schemes.

The report arising from the VPC's review mentioned above was made publically available. The Committee, through the work of a subgroup, made a total of 29 recommendations for improving the UK scheme. Among them were the following (Veterinary Products Committee Working Group, 2004):

- improvement of PSURs (national UK) to include assessments of seriousness;
- the VMD to pursue further funding of pharmacovigilance;
- under-reporting of adverse drug reactions, especially by the pig, poultry and aquaculture industries, to be pursued, as well as the apparent under-reporting of needle stick injuries in humans (to be investigated by the Health and Safety Executive);
- establishment of a formal pharmacovigilance section in the VMD;
- persons reporting human adverse reactions to veterinary medicines to be encouraged to provide contact information for medical follow-up;
- attempts to be made to assess causality for individual human adverse reactions;
- closer monitoring of environmental incidents by marketing authorisation holders and by regulatory agencies;
- requirement in the marketing authorisation for active monitoring of environmental exposure by the marketing authorisation holder;
- the VMD to introduce a means of inspecting and auditing the records of marketing authorisation holders;
- greater publicity for the UK scheme in order to raise awareness.

In response, the Government has accepted the majority of the recommendations (23 of the 29). In the list above, this excludes establishing a separate pharmacovigilance section within the VMD, establishing causality for individual human adverse reactions (largely due to reasons of litigation), the proposal for active monitoring of environmental exposure as part of the marketing authorisation and, although it agrees with the recommendation, the concept of inspections and audits, as it believes that greater benefits can accrue from training and advising (Department for Environment, Food and Rural Affairs, 2004).

The UK's spontaneous reporting scheme provides valuable information to veterinarians and regulators on suspected adverse reactions to veterinary medicinal products. However, as its past published reports have been anonymous in terms of the products described, the value of the reports is perhaps less even, though comparable information is available from the open literature where individual drugs or even products are identified. The value of these reports and others published by other regulatory authorities worldwide could be enhanced by the provision of even limited information on the drugs they describe: for example, fluoroquinolone antimicrobial agent, potentiated sulphonamide or aminoglycoside antibiotic rather than 'antimicrobial'; benzimidazole anthelmintic or avermectin rather than 'wormer'; or anthelmintic drug and injectable or inhalation anaesthetic rather than 'anaesthetic'. This division of the reports into chemical classes rather than broad therapeutic classes would undoubtedly aid those using the products.

Nevertheless, the purposes of these spontaneous reporting schemes must not be overlooked. They are not a system of collecting data for the satisfaction of doing so or for publishing reports and papers. One of the most important aspects lies in their ability to detect signals, particularly after the launch of a new drug, the use of a drug in a new species or patient class (very young or very old), or with a new indication, the identification of temporal trends and new trends, and the estimation of changing benefit:risk ratios. Such concepts and practices are already firmly ensconced in the pharmacovigilance of human medicinal products (Smith-Rogers, 1987; Nelson, 1988; Haramburu *et al*., 1990, 1997; Alvarez-Requejo and Porta, 1995; Meyboom *et al*., 1997, 1999, 2000, 2002; Talbot and Nilsson, 1998; Meyboom and Egberts, 1999; Collet *et al*., 2000; Bongard *et al*., 2002; Figueras *et al*., 2002; Peachey, 2002; Breckenridge, 2003; Hauben and Zhou, 2003).

The UK's reporting scheme, together with those in operation elsewhere in the EU and throughout the world, provide a useful source of information for veterinarians and others on the adverse reaction profiles and adverse environmental effects of the veterinary medicinal products, and they complement the data available in the open literature and from other sources such as text books. Together, these provide a good overview of the adverse reaction profiles that might be expected with individual drugs or products in particular species, or even in some cases breeds of animal, during or following the treatment of animals with veterinary medicinal products. In the broader context they also provide useful information on the potential for violative residues of veterinary drugs occurring in food of animal origin, as well as valuable data on potential adverse environmental effects, although these do appear to be rare, and the vast majority of veterinary medicines in use in veterinary practice probably offer little scope to elicit adverse environmental effects when used properly.

Perhaps more important than any other aspect, the monitoring of spontaneous adverse effects and veterinary pharmacovigilance in general provide a sound basis for effective regulation. In the light of adverse effects, the total package of data available for a product can be reviewed by the regulatory authorities and, where appropriate, changes can be made to the terms of the marketing authorisation; in extreme cases, products can be subject to more restrictive use and even have their marketing authorisations suspended or revoked. Thus, pharmacovigilance activities contribute to knowledge of the safety profiles of marketed veterinary medicinal products, and make ongoing and positive contributions to product stewardship and the protection of those – animal, human and the environment – potentially exposed. The overall effect should be to increase confidence in the use of these products in the treatment of sick animals and in the prevention of animal diseases.

Pharmacoepidemiology, which can help to strengthen hypotheses regarding the causative role of veterinary medicines in adverse effects, and can lead to the generation of theories, is in its infancy in the veterinary sector. Nevertheless, well-conducted pharmacoepidemiology studies can make significant contributions to the

understanding of adverse reactions to veterinary medicinal products.

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7 Veterinary adverse drug event reporting in the United States, Australia and Canada

K.N. Woodward

The United States

The Food and Drug Administration (FDA) regulates medicinal products in the USA in an analogous manner compared to the way these are regulated in the EU (Frank and Schafer, 2001). Specifically, the FDA's Center for Veterinary Medicine (CVM) in Rockville, Maryland, regulates veterinary medicinal pharmaceutical products. However, in the USA, unlike the EU and most EU member states, different authorities regulate vaccines and ectoparasiticides. Vaccines are regulated by the Department of Agriculture (USDA) while ectoparasiticides are regarded as pesticides and are controlled by the Environmental Protection Agency (EPA) (Woodward, 1993, 2000).

The initial requirements for submission of adverse reaction data to the CVM were finalised in 1971 and have been updated since (see the CVM website for current requirements: http://www.fda.gov/cvm/). The official term for an adverse reaction in the USA is an adverse drug experience (ADE), as this term, unlike 'reaction', does not imply causality between the drug and the adverse event (Teske, 1983; Keller *et al*., 1998). Adverse drug experiences are submitted to the CVM on form FDA 1932A (Veterinary Adverse Drug Reaction, Lack of Effectiveness or Product Defect Report). Under US law, drug sponsors must report adverse drug experiences to the CVM within 15 days of receipt by the sponsor if the event is unexpected, or is an unexpected increase in incidence or severity of an expected event. As in the EU, the US system makes use of PSURs and these are submitted at 6-monthly intervals during the first year after authorisation (approval) and annually thereafter. Serious adverse experiences are subjected to expedited review (Keller *et al.*, 1998).

The CVM's website is convenient to use and has a Table of Contents which guides the user to the specific areas of the site:

- Home
- ADE Reports
- FAQs about ADEs
- Cumulative Reports
- How to Report an ADE
- ADE Report Description
- Glossary of Terms
- List of Abbreviations
- Form 1932A
- Pharmacovigilance Brochure
- Additional Information

The CVM characterises the adverse drug experiences using six criteria:

1. whether the event was consistent with known effects of the drug;
2. whether there is any other explanation for the event;
3. temporal relationships between administration of the drug and the event;
4. evidence of drug overdose;
5. the effects of dechallenge of the drug;
6. the effects of rechallenge with the drug.

A number of other factors are considered in the analysis, including the seriousness of the adverse drug experience, whether it was unexpected, the number of reports of similar events following use of the drug, the numbers of animals affected, geographic patterns and whether the effects are biologically plausible (Keller *et al*., 1998; Bataller and Keller, 1999).

The adverse drug experiences may be referred to the CVM's Monitored Adverse Reaction Committee (MARC) for evaluation. MARC may recommend label changes or product recalls, or it may call for further data. MARC may also recommend that the CVM send a 'Dear Doctor' letter to veterinarians, drawing attention to the adverse effects of a particular medicine or, as a last resort, it may recommend that the drug be withdrawn (Keller *et al*., 1998; Hampshire *et al*., 2004). Unlike the approach in the EU, causality is assessed by the regulatory authority, i.e. the CVM.

In many ways, the CVM's system is much simpler than that of the EU. It lacks the legislative complexities and some of the supporting guidance, but it does provide concise guidelines, including those developed through the VICH process (e.g. Anon, 2006a). However, it has to be recognised that the EU systems have been developed to improve communication and harmonisation, originally across 15 individual member states with differing philosophies, cultures, infrastructures, economic backgrounds and languages, and now across 27 countries or 30 taken together with those in the EEA. The EU legislation and guidelines are designed as much to address this issue as to address any of the regulatory or medical problems.

Reporting adverse drug experiences to the CVM is relatively straightforward. They can be submitted by mail on Form FDA 1932A or they can be submitted to the CVM by telephone. The form may be obtained from the CVM's website. Similarly, a brochure, which is a comprehensive guide to the scheme, can be found at this site (*Pharmacovigilance of Veterinary Drugs. Adverse Drug Events Reporting System*, April 2001), as can summaries of adverse events for past years. The FDA/CVM site has links to both the USDA and EPA sites so that adverse drug experiences can be submitted on vaccines and ectoparasiticides respectively, and to the Veterinary Practitioners' Reporting Network which is sponsored by the US Pharmacopeia, and is an independent pharmacovigilance reporting system.

The brochure stresses the importance of reporting adverse drug events to the drug sponsor and to the CVM. Specifically, it explains the unique position of veterinarians in recognising these, and how reporting contributes to the growing data on the safety of drugs and their use. It explains how side effects of drugs including those from off-label use, lack of efficacy and environmental effects all form part of pharmacovigilance activities and constitute adverse events which should be reported to the CVM. In fact, environmental assessments form an integral part of the pre-approval process for veterinary drugs, including those used in aquaculture, and thus recognition of post-approval adverse environmental effects is a critical part of veterinary pharmacovigilance in the USA (Haley *et al*., 1998). Lack of efficacy may under some circumstances be followed up further, as with the recent investigation into heartworm treatments (Hampshire, 2005).

The CVM's website provides guidance on the type of information that should be provided in ADE reports, including that which can be used to score for causality assessment (see Chapter 27). The CVM uses a causality algorithm for this purpose (Kramer *et al*., 1979). This includes:

- medical history of the animal;
- any concomitant drug use;
- all recent surgical procedures;

- the results of clinical examinations;
- clinical chemistry results;
- haematology;
- urinalysis;
- results of faecal examinations;
- radiographic results;
- blood pressure and other cardiac or related measurements;
- results of neurological assessments.

The 1932A form itself provides valuable prompts by requiring data on species, breed, age and gender as well as information to confirm the effects of dechallenge and rechallenge with the drug under suspicion.

Like the UK and other EU country systems, the CVM scheme is intended to alert the agency to adverse events arising from the use of veterinary pharmaceutical products so that a database can be built up and relevant regulatory action taken when needed. This might include, as elsewhere, some additional labelling and contraindications and, as mentioned above, in extreme circumstances, withdrawal of the product. Also like the EU systems, the CVM accepts reports on adverse reactions in humans following exposure to veterinary drugs and adverse experiences to human drugs administered to animals. Like the UK and EMEA approaches, the reporting system is integrated into the regulatory authority, thus allowing rapid regulatory action when required. Unlike the UK and EMEA pharmacovigilance systems where the annual numbers of reports are in the low hundreds, the CVM currently receives around 20,000 reports annually, with around 95% of these originating from animal health companies and the remainder from veterinarians and from animal owners or the US Pharmacopeia. It includes reports on human drugs administered to animals. Evaluation of these reports takes into account dosages administered, any concomitant drug use, the medical and physical condition of the animal at the time of treatment, environmental and management data, product defects and extra-label use of medicines.

As in the UK, dogs account for a large proportion of the adverse events reported. For example, in 1998, there were nearly 6000 adverse events reported to the CVM for dogs or approximately 63% of the total. Surprisingly, cats made up only 7.4% (692), coming in third place to cattle (9.0%; 692) (*FDA/CVM 1998 Adverse Drug Experience Report – A Descriptive Overview* – available from the CVM website). This may reflect the popularity of dogs, the wealth of their owners with respect to those of cats, and the economic value of cattle production. Similar findings were made for the previous year (Bataller, 1999, 2001).

The number of adverse drug experiences is rising on an annual basis, as shown below, reflecting the situation seen in the UK. In fact, in the period 1997–2001, the numbers increased from just over 4,000 to over 24,000 (Hampshire *et al*., 2004).

1992	1,011
1993	1,250
1994	1,746
1995	3,193
1996	3,112
1997	4,738
1998	9,385
1999	9,731

The reasons for this trend in the USA and elsewhere are unknown. However, it may represent a wider appreciation of the existence of spontaneous reporting schemes, the growth in animal ownership, a growth in wealth that allows more animal owners to seek veterinary help for sick animals, a change in attitudes or a combination of these factors. The schemes themselves may not answer such questions! As there is no breakdown of the frequency of reports versus the species and therapeutic types, it is difficult to make too many generalisations. However, of note is the fact that reactions to non-steroidal anti-inflammatory drugs tend to predominate in the CVM's statistics and that this group was a major contributor to the number of suspected adverse reactions in dogs in the UK. Hormones, CNS agents, anaesthetics and penicillins also featured strongly in the CVM's figures.

Label changes or other regulatory actions have been taken against a number of veterinary

medicines in the USA as a result of adverse drug experience monitoring. For example, the cattle milk yield enhancer bovine somatotropin was found to increase the frequency of udder oedema, injection site reactions and lameness, and label changes were imposed as a result. A 'Dear Doctor' letter was sent to veterinarians warning of the dangers of giving oral spectinomycin products by intravenous injection because of the risks from endotoxins, and a warning was added to the label for tylosin products providing a warning of the dangers of mortality from overdose in very young pigs (Bataller and Keller, 1999).

A compilation of the content of the cumulative list of adverse drug experience reports from 1987 to 2003 is available on the CVM's website. This provides information classified on the basis of active ingredient, species or clinical signs, but as with the UK and other pharmacovigilance reports, proprietary names are withheld. However, generic names are provided so that readers can determine if specific drugs are associated with particular adverse drug experiences (Anon, 2006b).

As with the UK scheme, under-reporting is acknowledged to be a problem and the CVM has taken steps to try to improve this by making reporting easier for veterinarians (Keller *et al*., 1998).

The cumulative list of adverse drug events received by the CVM is shown in *Table 7.1*. It should be emphasised that these figures do not represent incidence as the treated number of animals is unknown. So while for some drugs the number of reactions appears to be high, it must be borne in mind that the number of animals treated is also likely to be significant, meaning that the overall incidence in percentage terms may be low. Each entry is shown in descending order of number of adverse event and only the more frequent adverse events rather than the total are indicated.

With vaccines, the process of assessment by the Center for Veterinary Biologics, as with pharmaceuticals, includes attempts to determine causality. Interpretation of the data involves assessment of use and misuse and, as with pharmaceuticals, an analysis of the events surrounding the reaction. This interpretation and assessment may involve the use of complex mathematical models (Siev, 1999). Adverse events may be reported on the Adverse Event Report form available at (http://www.aphis.usda.gov/vs/cvb/html/vaccionvigilance.html). Adverse events to ecto-parasiticides and other medicinal products authorised through the EPA under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) should be reported to the EPA (http://www.epa.gov/fedrgstr/EPA-PEST/).

Australia

The Australian Pesticides and Veterinary Medicines Authority (APVMA, formerly the National Registration Authority, NRA) is responsible for the registration of veterinary medicines in Australia, and for dealing with adverse drug reactions, or adverse experiences (Dyke, 2003; Linnett and Dagg, 2005). It defines these as an

> '. . . unintended or unexpected effect on animals, human beings or the environment, including injury, sensitivity reactions or lack of efficacy associated with the clinical use of a veterinary chemical product'
> (Linnet and Dagg, 2005).

Adverse experiences were originally classified into four main categories, similar in nature, except for the last category, to the ABON system used in Europe. These were:

- product related;
- possibly product related;
- not product related;
- caused by not using the product according to the label directions.

However, the approach now used is virtually identical to that employed elsewhere using the ABON system. A causality algorithm is employed to determine the classification and this is the same as that used in the USA (Kramer *et al*., 1979). The data generated by the scheme are used to determine trends and, where necessary, to take corrective regulatory actions (Linnett and Dagg, 2005).

Table 7.1 Animal adverse reactions 1987–2007 taken from the FDA's CVM Cumulative List.

Drug	*Species*	*Route*	*Numbers*	*Major effects*
Acepromazine	Dog	Oral	44	Death, prolonged sedation, collapse
	Cat	Unknown	8	Death, reactive anaesthesia, epistaxis
	Cat	Parenteral	77	Apnoea, hypopnoea, death, deep sedation
	Dog		55	Prolonged anaesthesia, convulsions, death
	Pig		5	Penile prolapse
	Horse		18	Penile prolapse, collapse, death
	Rabbit		13	Injection site necrosis, alopecia
Albendazole	Dog	Oral	177	Bloody diarrhoea, melaena, anorexia, diarrhoea
	Goat		59	Death, diarrhoea, abortion
	Sheep		673	Death, anorexia, nasal discharge, collapse, lack of efficacy
	Cattle		1937	Lack of efficacy, diarrhoea, death, respiratory disorders, collapse
Amikacin	Dog	Parenteral	19	Pain, vomiting
Amitraz	Dog	Topical	162	Depression, death, vomiting, convulsions, ataxia
Amoxicillin	Cat	Oral	90	Diarrhoea, anorexia
	Dog		83	Vomiting, diarrhoea, anorexia, death
	Rabbit		1	Anorexia, death
	Cat	Parenteral	39	Injection site pain, anaphylaxis, death, anorexia
	Dog		89	Injection site pain, anaphylaxis, collapse, bradycardia
	Cattle		31	Death, cyanosis, dyspnoea, hypersalivation
Amoxicillin, clavulanate	Cat	Oral	355	Vomiting, diarrhoea, anorexia, depression, hypersalivation
	Dog		335	Vomiting, anorexia, depression, death, convulsions, ataxia
Ampicillin	Cat	Parenteral	41	Injection site pain
	Dog		54	Injection site pain, anaphylaxis
	Horse		13	Anorexia, injection site pain and inflammation, abdominal pain
	Cattle		840	Milk residues, dyspnoea, staggering
Arsenamide	Dog	Parenteral	115	Vomiting, death, depression/lethargy, icterus, anorexia, hepatic dysfunction
Atipamezole	Cat	Unknown	20	Hypersalivation, vocalisation, hyperactivity
	Dog		46	Depression, lethargy, death, polypnoea, fever
	Cat	Parenteral	60	Death, hypersalivation, anaphylaxis
	Dog		306	Death, convulsions, cardiac arrest, hyperactivity, apnoea, vomiting

Table 7.1 *Continued*

Drug	*Species*	*Route*	*Numbers*	*Major effects*
Bacitracin	Cat	Ophthalmic	19	Irritation, anaphylaxis, ulcerated cornea
	Dog		9	Irritation, conjunctivitis
Bacitracin, neomycin, polimixin B	Cat		157	Conjunctivitis, ulcerated cornea, discharge, epiphora, eye pain
	Dog		20	Eye irritation, blepharospasm
Betamethasone	Cat	Parenteral	2	Ataxia, death, diabetes mellitus
	Dog		20	Polyuria, diarrhoea, polydipsia
Betamethasone, clotrimazole, gentamicin	Cat	Topical	19	Deafness, ataxia, anorexia
	Dog		723	Temporary deafness, deafness, ear irritation, rash, urticaria
Betamethasone, gentamicin	Cat	Topical	10	Deafness, weight loss, anorexia
	Dog		35	Deafness, ear irritation
	Cat	Ophthalmic	9	Conjunctivitis, staggering, ulcerated cornea
	Dog		9	Eye pain, oedema, eye irritation
Butorphanol	Cat	Oral	17	Hyperactivity, death, aggression
	Dog		53	Sedation, anorexia, altered hepatic enzymes, convulsions
	Horse	Unknown	10	Lack of efficacy, death, apnoea
	Cat	Parenteral	128	Turbulent anaesthetic recovery, death, vocalisation, apnoea
	Dog		235	Diarrhoea, lack of efficacy, death, apnoea, cardiac arrest, ataxia
	Horse		41	Ataxia, hyperactivity, spasm, lack of efficacy
Carbadox	Pig	Oral	575	Lack of efficacy
Carprofen	Cat		489	Vomiting, depression/lethargy, anorexia, death
	Dog		14 854	Vomiting, anorexia, depression/lethargy, death, anaemia, icterus, convulsions, bloody diarrhoea, melaena, kidney failure, collapse, epistaxis, recumbency, dyspnoea, haematochezia, coagulation disorder
	Panda		2	Anaemia, death
	Rabbit		5	Urinary incontinence, anorexia, death
	Cat	Parenteral	60	Depression/lethargy, anorexia, death, vomiting
	Dog		598	Vomiting, anorexia, depression/lethargy death, diarrhoea

Cefadroxil	Cat	Oral	37	Vomiting, diarrhoea, skin congestion, pruritus
	Dog		17	Vomiting, depression/lethargy, death, anaphylaxis, anaemia
Cefpodoxime	Dog	Oral	192	Anorexia, vomiting, lethargy, anaemia
Ceftiofur	Pig	Parenteral	760	Diarrhoea, death, anaphylaxis, peritoneal effusion
	Horse		27	Death, diarrhoea, fever, abdominal pain, anaphylaxis
	Cattle		2 957	Lack of efficacy, injection site swelling, abscess or seroma, death, milk residues
		Intramammary	94	Milk residues, lack of efficacy, hypogalactia
Cephalexin	Dog	Oral	16	Vomiting, anorexia
Cephapirin benzathine	Cattle	Intramammary	327	Milk residues, death, mastitis, fever, anorexia, depression/lethargy
Cephapirin sodium	Horse	Parenteral	26	Death, diarrhoea, fever, abdominal pain, anaphylaxis
	Cattle		77	Milk residues, lack of efficacy, death
	Donkey		2	Death
	Cattle	Intramammary	1 267	Milk residues, lack of efficacy, death
Chloramphenicol	Cat	Ophthalmic	21	Eye irritation, conjunctivitis
	Dog		11	Conjunctivitis, anaphylaxis
Chlortetracycline	Pig	Oral	40	Death
	Cattle		62	Unpalatable, diarrhoea
Chorionic gonadotropin	Horse	Parenteral	12	Death, abdominal pain, anaphylaxis
Clenbuterol	Horse	Oral	8	Death, sweating, aggression
Clindamycin	Cat		49	Diarrhoea, anorexia, convulsions
	Dog		55	Depression/lethargy, death, vomiting, convulsions, ataxia
Clomipramine	Cat	Oral	21	Mydriasis, anorexia, depression/lethargy, tachycardia
	Dog		328	Depression/lethargy, anorexia, vomiting, aggression, ataxia, convulsions, vocalisation, mydriasis
Cloprostenol	Cattle	Unknown	35	Lack of efficacy
		Parenteral	343	Lack of efficacy, abnormalities of oestrous cycle
Clorsulon, ivermectin	Cattle	Parenteral	1 045	Lack of efficacy, injection site swelling and abscess
	Buffalo		3	Ataxia, convulsions, death

Table 7.1 *Continued*

Drug	*Species*	*Route*	*Numbers*	*Major effects*
Cloxacillin benzathine	Cattle	Intramammary	74	Milk residues, mastitis, death, udder swelling
Copper disodium edetate	Cattle	Various	100	Ataxia, blindness, collapse, depression/lethargy, death, cardiac and hepatic lesions
Copper glycinate	Cattle	Parenteral	114	Anorexia, anoestrus, reduced fertility, injection site abscess, pain and swelling, weight loss, depression/lethargy, death
Coumaphos	Cattle	Oral	20	Death
Cyclosporine	Cat	Oral	62	Anorexia, vomiting, depression/lethargy, diarrhoea, weight loss
	Dog		3101	Vomiting, diarrhoea, depression/lethargy, anorexia, pruritus, trembling, convulsions, polydipsia, polyuria
		Ophthalmic	73	Lack of efficacy, eye irritation, swollen eyelids, conjunctivitis
Cythioate	Cat	Oral	5	Gastroenteritis, weakness, death
	Dog		16	Convulsions, death, hypersalivation
Danofloxacin mesylate	Cattle	Parenteral	367	Lack of efficacy, collapse, anaphylaxis, recumbency, death, dyspnoea, ataxia
Deracoxib	Cat	Oral	36	Vomiting, depression/lethargy, fever
	Dog	Oral	3658	Vomiting, anorexia, depression/lethargy, diarrhoea, death, melaena, polydipsia, bloody diarrhoea, polyuria, convulsions, ataxia, peritonitis, weakness, fever
Deslorelin	Horse	Parenteral	351	Prolonged dioestrus, abnormal oestrous cycle, reduced fertility, cystic ovaries
Desoxycorticosterone	Dog	Parenteral	64	Vomiting, depression/lethargy, anaphylaxis, collapse
Detomidine	Horse	Parenteral	172	Lack of efficacy, urticaria, hypernoea, anaphylaxis
Dexamethasone	Dog	Parenteral	6	Bradycardia, collapse, death
	Horse		2	Laminitis, urticaria
Dexamethasone, neomycin, thiabendazole	Cat	Topical	20	Ataxia, balance disorder, anorexia, skin inflammation
	Dog		37	Skin congestion, vesicles/bullae, deafness, inflammation
Diazepam	Cat	Oral	4	Depression, death, icterus
Dichlorophen, toluene	Cat	Oral	266	Vomiting, ataxia, hypersalivation, depression/lethargy, death, anorexia
	Dog		117	Vomiting, ataxia, depression/lethargy, death, hypersalivation, trembling, diarrhoea, convulsions, anorexia

Dichlorvos	Dog	Oral	15	Lack of efficacy, anorexia
	Pig		222	Death, diarrhoea, depression/lethargy, skin congestion, skin irritation, abdominal pain, cyanosis
	Horse		6	Unpalatable, anorexia
Diclofenac	Horse	Topical	22	Swelling, inflammation, alopecia at application site
Diethylcarbamazine	Dog	Oral	211	Vomiting, depression/lethargy, diarrhoea, anorexia, convulsions
Diethylcarbamazine, oxibendazole	Dog	Oral	1086	Vomiting, altered plasma enzymes, anorexia, depression/lethargy, diarrhoea, death, weight loss
Difloxacin	Dog	Oral	72	Lack of efficacy, vomiting, anorexia, depression/lethargy
Dihydrostreptomycin, penicillin G procaine	Pig	Parenteral	605	Vomiting, depression/lethargy, diarrhoea, abortion, ataxia, convulsions, death
	Horse		5	Anaphylaxis, death
	Cattle		34	Death, thrashing/paddling, anaphylaxis
		Intramammary	76	Udder swelling, nasal discharge, hyperpnoea, head/face swelling, udder pain, urticaria
Dinoprost tromethamine	Cattle	Unknown	16	Lack of efficacy, injection site swelling, myositis, death, anorexia
	Horse	Parenteral	14	Abdominal pain, sweating, death, dyspnoea, collapse, ataxia
	Cattle		979	Lack of efficacy, injection site swelling, death, recumbency, myositis, swelling of limbs
Doramectin	Cat	Oral	20	Neurological disorders, depression/lethargy
	Dog		12	Ataxia, trembling, vomiting, anorexia, blindness
	Goat		1	Ataxia, blindness
	Cattle		1001	Anaphylaxis, dyspnoea, recumbency, head and facial swelling, blindness
		Topical	49655	Lack of efficacy, pruritus, alopecia, loss of condition, bloody diarrhoea, skin disorders, mucoid diarrhoea, abnormal hair, weight loss
		Unknown	10316	Lack of efficacy, pruritus, alopecia, loss of condition, anorexia
	Pig	Parenteral	21334	Lack of efficacy, skin lesions, abnormal hair, skin irritation, myositis, death, lameness, recumbency, ataxia
	Sheep		20	Death, depression/lethargy, trembling
	Cattle		6612	Lack of efficacy, pruritus, alopecia, abnormal hair, poor performance, death
Droperidol, fentanyl	Dog	Parenteral	38	Aggression, prolonged anaesthesia, head bobbing, death
Enalapril	Dog	Oral	32	Death, depression/lethargy, anorexia, bradycardia

Table 7.1 *Continued*

Drug	*Species*	*Route*	*Numbers*	*Major effects*
Enrofloxacin	Cat	Oral	300	Mydriasis, retinal abnormalities, blindness, abnormal reflex, partial blindness, papillary abnormalities, vomiting, anorexia, temporary blindness
	Dog		225	Vomiting, depression/lethargy, convulsions, anorexia, ataxia, death, lameness, birth defects, fever, blindness
	Turkey		8124	Lack of efficacy, death
	Chicken		12540	Lack of efficacy, death
	Cat	Unknown	21	Mydriasis, retinal abnormalities, convulsions, blindness, collapse, dementia
	Dog		12	Death, bloody vomiting, anaemia
	Cat	Parenteral	156	Mydriasis, blindness, retinal abnormalities, abnormal reflexes, convulsions, partial blindness
	Dog		48	Vomiting, convulsions, death
	Cattle		225	Lack of efficacy, death, injection site reactions
	Rabbit		20	Discomfort, injection site pain, irritation
Enrofloxacin, silver sulfadiazine	Cat	Topical	8	Shaking head and face, anaphylaxis, anorexia
	Dog		97	Ear congestions, ear irritation, application site erythema and inflammation
Eprinomectin	Cattle	Topical	540	Death, skin inflammation, alopecia, skin hyperpigmentation, skin ulcers
Erythromycin	Pig	Parenteral	150	Death
	Cattle	Intramammary	24	Udder pain, mastitis
Esiprantel	Cat	Oral	100	Lack of efficacy, depression/lethargy, anorexia
	Dog		261	Lack of efficacy, vomiting, diarrhoea, depression/lethargy, convulsions, death, anorexia
Etodolac	Dog	Oral	1816	Vomiting, bloody diarrhoea, anorexia, keratoconjunctivitis sicca, diarrhoea, depression/lethargy, altered plasma enzymes, melaena, anaemia, death, polydipsia, polyuria
Famphur	Cattle	Topical	3277	Lack of efficacy, hypersalivation, froth at mouth, recumbency, death, pruritus, anorexia, diarrhoea, depression/lethargy, abortion, straining, coughing, staggering
Febantel, praziquantel, pyrantel	Dog	Oral	70	Vomiting, diarrhoea, depression/lethargy, lack of efficacy, anorexia, death

Febantel, trichlorfon	Horse	Oral	65	Abdominal pain, diarrhoea, ataxia, distress, hyperpnoea, tachycardia, hypersalivation, death
Fenbendazole	Dog	Oral	230	Vomiting, depression/lethargy, anorexia, diarrhoea, death, dehydration, abdominal pain, hyperactivity
	Pig		460	Diarrhoea, lack of efficacy, reduced fertility, sperm abnormalities, death, severe diarrhoea
	Goat		212	Abortion, oedema, head bobbing, nystagmus, twitch, death
	Lion		1	Ataxia, confusion, convulsions, depression/lethargy, nervousness
	Horse		31	Diarrhoea, abdominal pain, anorexia, lack of efficacy, collapse, death
	Cattle		1356	Anorexia, lack of efficacy, coughing, diarrhoea, death, hypogalactia
Fenprostalene	Cattle	Parenteral	435	Lameness, injection site abscess or swelling, death, stiffness
Fenthion	Cattle	Oral	1500	Lack of efficacy
	Cat	Topical	11	Diarrhoea, trembling, vomiting
	Dog		77	Vomiting, depression/lethargy, skin disorder, trembling, death, alopecia
	Cattle		5237	Lack of efficacy, hypersalivation, death, diarrhoea, alopecia, ataxia, collapse, depression/lethargy, anorexia, recumbency, breathing abnormalities
Firocoxib	Cat	Oral	3	Anorexia, anaemia
	Dog		1344	Vomiting, anorexia, altered plasma enzymes, depression/lethargy, diarrhoea, drug interactions, death, anaemia, ataxia, lack of efficacy, melaena, convulsions, gastric perforation
Florfenicol	Dog	Parenteral	21	Death, injection site pain
	Deer		1	Anaphylaxis, death
	Cattle		595	Lack of efficacy, diarrhoea, anorexia, death, injection site swelling, recumbency, ataxia
Flunixin	Dog	Oral	5	Anorexia, depression/lethargy, vomiting
	Horse		14	Depression/lethargy, diarrhoea, projectile diarrhoea, hypomotility, tachycardia, death
	Dog	Parenteral	9	Haematochezia, death, depression/lethargy, vomiting
	Horse		161	Injection site swelling, collapse, convulsions, death, injection site abscess, anaphylaxis, injection site pain, fever, neck swelling, urticaria, ataxia, trembling
	Cattle		26	Death, injection site reactions, anaphylaxis, collapse

Table 7.1 *Continued*

Drug	*Species*	*Route*	*Numbers*	*Major effects*
Fluoxetine	Dog	Oral	8	Depression/lethargy, anorexia, apprehension
Follicle stimulating hormone	Cattle	Parenteral	671	Lack of efficacy
Furazolidone	Pig	Oral	633	Anorexia, constipation, lung lesions
Furosemide	Dog	Oral	19	Polyuria, depression/lethargy, hyperactivity, tachycardia
	Cat	Parenteral	3	Apnoea, convulsions, death, ventricular fibrillation
	Horse		3	Death, anaphylaxis, collapse, fibrillation
Gentamicin	Dog	Ophthalmic	15	Ocular congestion, impaired healing, corneal ulceration
		Parenteral	15	Abnormal plasma enzymes, deafness, anorexia
	Pig		3	Death, trembling, weakness
	Horse		22	Abdominal pain, shaking, anaphylaxis, distress, trembling
Gleptoferron	Pig	Parenteral	145	Death, depression/lethargy, weakness, diarrhoea, injection site abscess
Glycosaminoglycan polysulphate	Dog	Parenteral	76	Depression/lethargy, vomiting, anorexia, diarrhoea, fever
	Horse		73	Injection site swelling, lameness, joint swelling, joint effusion, injection site pain, urticaria
Griseofulvin	Cat	Oral	109	Depression/lethargy, depressed white blood cells, anorexia, diarrhoea, anaemia, fever, ear disorders
	Dog		12	Depression/lethargy, fever, icterus
Heptacillin	Cattle	Intramammary	122	Milk residues, udder swelling, lack of efficacy
Hyaluronate	Horse	Parenteral	281	Joint swelling, lameness, joint pain, joint effusion, arthritis, joint fever, cellulites, joint enlargement, limb swelling, injection site swelling
Imidacloprid, moxidectin	Cat	Topical	11	Hypersalivation, convulsions, depression/lethargy
	Dog		24	Vomiting, depression/lethargy, ataxia, anorexia
Imidocarb	Dog	Parenteral	36	Injection site swelling, death, vomiting, depression/lethargy, diarrhoea, hypersalivation
Insulin	Cat	Parenteral	19	Depression/lethargy, vomiting, convulsions, death
	Dog		116	Lack of efficacy, depression/lethargy, vomiting, anorexia

Iron dextran	Pig	Unknown	227	Death, paralysis, muscle lesions, thrashing/paddling, anaphylaxis, recumbency
	Dog	Parenteral	1 685	Death, vomiting, convulsions, diarrhoea, injection site swelling, kicking, thrashing/paddling, vocalisation, dyspnoea, recumbency, ataxia, anaphylaxis
	Cattle		100	Collapse, injection site abscess, death, fever, moribund, injection site emphysema
Isoflupredone	Cattle	Parenteral	180	Low potassium and phosphorus, ketosis, low magnesium, recumbency, weakness, torticollis
Isoflurane	Cat	Inhalation	401	Lack of efficacy, death, cardiac arrest, apnoea, blindness, cyanosis
	Dog		317	Lack of efficacy, death, apnoea, cardiac arrest, respiratory distress, bradycardia
	Rat		17	Lack of efficacy, death
Ivermectin	Cat	Oral	112	Vomiting, ataxia, depression/lethargy, weakness, diarrhoea, unconsciousness, mydriasis, hypersalivation, death, anorexia
	Dog		1 069	Depression/lethargy, ataxia, vomiting, mydriasis, death, trembling, anorexia, convulsions, diarrhoea, unconsciousness, hypersalivation, blindness, fever, recumbency, weakness, staggering, hyperactivity, confusion
	Horse		281	Lack of efficacy, abdominal pain, depression/lethargy, death, recumbency, ataxia, fever, diarrhoea
	Cattle		31	Anorexia, 'ill', rupture of oesophagus, choking, death
	Cat	Topical	24	Ataxia, anorexia, mydriasis
	Dog		5	Ataxia, convulsions
	Cattle		5 795	Lack of efficacy, hypersalivation, recumbency, depression/lethargy, hypopnoea, death, ataxia, bloat, anaphylaxis, diarrhoea, anorexia
	Sheep	Unknown	50	Ataxia, staggering, death
	Cat	Parenteral	52	Ataxia, unconsciousness, depression/lethargy, death, mydriasis, trembling
	Dog		76	Ataxia, death, depression/lethargy, hypersalivation, trembling, mydriasis, recumbency
	Pig		916	Depression/lethargy, death, recumbency, ataxia, thrashing/paddling, staggering, CNS disorders, vomiting, dyspnoea, anorexia, shaking
	Horse		33	Pain, oedema, swelling, cellulitis, ventral oedema, pneumonia, fever, death
	Sheep		71	Ataxia, ecchymoses, petechiae, blindness, collapse, confusion, difficulty rising, recumbency, thrashing/paddling, death
	Cattle		798	Lack of efficacy, abortion, injection site swelling and inflammation, death, diarrhoea, vaginal bleeding, recumbency, ataxia, dyspnoea

Table 7.1 *Continued*

Drug	*Species*	*Route*	*Numbers*	*Major effects*
Ivermectin, praziquantel	Dog	Oral	87	Ataxia, trembling, depression/lethargy, vomiting, mydriasis, convulsions, hypersalivation, blindness, stupor, death
	Horse		758	Swelling of mouth/lips, swelling of abdomen, abdominal pain, swelling at application site, hypersalivation, application site erythema, anorexia, diarrhoea, depression, irritation, mouth lesions, stomatitis, swelling of tongue
Ivermectin, pyrantel	Dog	Oral	9872	Lack of efficacy, vomiting, depression/lethargy, convulsions, anorexia, ataxia, death, trembling, pruritus, bloody diarrhoea, unpalatable, fever, hypersalivation
Ketamine	Cat	Missing	43	Lack of efficacy, death, pruritus, apnoea, prolonged recovery from anaesthesia
	Dog		31	Lack of efficacy, diarrhoea, vomiting, death
	Cat	Unknown	666	Lack of efficacy, death, congestion, prolonged recovery from anaesthesia, fever, convulsions, cardiac arrest
	Dog		230	Lack of efficacy, turbulent recovery, convulsions, fever, cardiac arrest
	Horse		83	Lack of efficacy
	Cat	Parenteral	4153	Lack of efficacy, prolonged recovery from anaesthesia, death, apnoea, convulsions, cardiac arrest, ataxia, fever
	Dog		667	Lack of efficacy, death, prolonged recovery from anaesthesia, fever, turbulent recovery, convulsions, cardiac arrest, trembling, apnoea
	Rat		82	Death, anaphylaxis, lack of efficacy
	Horse		459	Lack of efficacy, turbulent recovery, ataxia, turbulent induction, death
	Primate		47	Lack of efficacy, convulsions, death, gastritis
Ketoprofen	Cat	Parenteral	35	Diarrhoea, vomiting, death, fever
	Dog		95	Bleeding at incision site, coagulation disorder, vomiting, depression/lethargy, polydipsia, scrotal bleeding
Lasalocid	Dog	Oral	25	Collapse, death, unconsciousness
	Horse		5	Death
	Sheep		10	Death
	Cattle		2029	Lack of efficacy, cystic ovaries, reproduction disorders, recumbency, death

Levamisole	Sheep	Oral	194	Death, hypersalivation, dysmetria, ataxia, shaking, diarrhoea
	Cattle		74	Hypersalivation, depression/lethargy, recumbency, death, abortion, dehydration, oedema of head/face, rolling, miosis, trembling, ataxia
		Topical	2 531	Weight loss, staggering, rash, anorexia, nervousness, lack of efficacy, bloat, CNS disorders, pain, abnormal hair, skin ulceration, leukoderma
		Parenteral	1 084	Diarrhoea, lack of efficacy, depression/lethargy, death, anaphylaxis, abortion, hypersalivation, injection site swelling, convulsions
Lincomycin, spectinomycin	Pig	Parenteral	70	Circling, convulsions, trembling, vomiting
	Cattle		1 263	Death, dyspnoea, froth from nose, anaphylaxis, recumbency, hypersalivation, convulsions, trembling, dehydration, shock
Lufenuron	Cat	Oral	207	Vomiting, depression/lethargy, anorexia, diarrhoea, pruritus, alopecia, fever, ataxia, death, convulsions
	Dog		588	Vomiting, depression/lethargy, diarrhoea, pruritus, anorexia, urticaria, skin congestion, convulsions, alopecia, fever, ataxia, rash
	Ferret		4	Depression/lethargy, polydipsia, polyuria, collapse
	Cat	Parenteral	310	Injection site mass, depression/lethargy, anorexia, injection site abscess/swelling, vomiting, fever, injection site pain, inflammation, alopecia, ulceration
Marbofloxacin	Cat	Oral	125	Anorexia, depression/lethargy, convulsions, vomiting, blindness, ataxia, death
	Dog		246	Vomiting, depression/lethargy, anorexia, lameness, lack of efficacy, ataxia, convulsions, polydipsia, death, diarrhoea, anaemia
Mebendazole	Dog	Oral	58	Vomiting, anorexia, depression/lethargy, death, diarrhoea
	Horse		17	Lack of efficacy
Mebendazole/trichlorfon	Dog	Oral	58	Convulsions, hypersalivation, death
	Horse		270	Abdominal pain, diarrhoea, lack of efficacy, anorexia, hypersalivation, rolling, depression/lethargy
Meclofenamic acid	Dog	Oral	153	Vomiting, gastroenteritis, bloody diarrhoea, diarrhoea, anorexia, haematochezia, death, melaena
Melarsomine	Dog	Parenteral	1 713	Injection site swelling, death, depression/lethargy, vomiting, anorexia, fever, polypnoea, injection site pain, hypersalivation, ataxia, diarrhoea, pain, bloody diarrhoea, lack of efficacy, dyspnoea, hind limb paresis, anaphylaxis, cough
Melengestrol	Cat	Oral	34	Diabetes mellitus, mammary neoplasm, unpalatable, depression/lethargy
	Dog		6	Polydipsia, polyuria, depression/lethargy, diabetes mellitus

Table 7.1 *Continued*

Drug	*Species*	*Route*	*Numbers*	*Major effects*
Melengestrol, monensin, ractopamine, tylosin	Cattle	Oral	1280	Abnormal oestrus
Meloxicam	Cat	Oral	529	Elevated creatinine, anorexia, elevated BUN (blood urea nitrogen), vomiting, kidney failure, death, azotaemia, dehydration, weight loss, diarrhoea, anaemia
	Dog		1190	Vomiting, anorexia, depression/lethargy, diarrhoea, elevated alkaline phosphatase, death, elevated plasma enzymes, bloody diarrhoea, convulsions, melaena, anaemia, death, drug interactions, polypnoea, ataxia, pruritus
	Tiger		1	Haematochezia
	Cat	Parenteral	126	Anorexia, elevated BUN, depression/lethargy, vomiting, death, kidney failure, azotaemia, anaemia, dehydration
	Dog		111	Death, vomiting, anorexia, depression/lethargy, elevated BUN, bloody diarrhoea
Metetomidine	Dog	Oral	6	Death, oedema of head/face, anaphylaxis
	Cat	Missing	12	Death, drug interactions, hypersalivation
	Dog		181	Heart murmur, lack of efficacy, death, depression/lethargy
	Cat	Unknown	72	Lack of efficacy, death, vomiting, depression/lethargy
	Dog		291	Lack of efficacy, death, vomiting, bradycardia, depression/lethargy, convulsions, cardiac arrest, oedema of lungs/trachea, apnoea
	Cat	Parenteral	274	Death, vomiting, cardiac arrest, apnoea, depression/lethargy, cyanosis, dyspnoea, hypersalivation, oedema of lungs/trachea, prolonged sedation, anorexia, ataxia, convulsions, hypothermia, bradycardia
	Dog		1462	Lack of efficacy, death, vomiting, drug interactions, cardiac arrest, prolonged sedation, bradycardia, apnoea, convulsions, oedema of lungs/trachea, depression/lethargy, fever, twitch, collapse, hyperactivity, dyspnoea, aggression, blood in lungs/trachea, cyanosis, anorexia, pallor of mucous membranes, deep sedation, prolonged recovery, arrhythmia, ataxia, tachycardia, vocalisation, anaphylaxis, hypersalivation, hypothermia, relapse of sedation, confusion, cyanosis
	Rat		19	Death
	Monkey		7	Prolonged sedation, apnoea, death

(S)-methoprene	Dog	Oral	1 420	Depression/lethargy, vomiting, ataxia, anorexia, trembling, nervousness, diarrhoea, hyperactivity, apprehension, confusion, lack of efficacy, vocalisation, convulsions, shaking, alopecia, weakness
Methoxyflurane	Dog	Inhalation	53	Lack of efficacy, deep anaesthesia, cyanosis, death, prolonged recovery, confusion
Methylprednisolone	Cat	Parenteral	65	Diabetes mellitus, depression/lethargy, death, injection site mass, polydipsia, polyuria
	Horse		20	Joint swelling, lameness, joint effusion, anaphylaxis, collapse, death
Metronidazole	Dog	Oral	32	Convulsions, birth defects, ataxia
Miconazole	Cat	Topical	12	Skin congestion, pruritus, hypersalivation
Milbemycin	Cat	Oral	52	Depression/lethargy, ataxia, vomiting, anorexia, trembling
	Dog		4 759	Lack of efficacy, depression/lethargy, vomiting, diarrhoea, convulsions, anorexia, ataxia, death, dyspnoea, pruritus, hypersalivation, bloody diarrhoea, fever
Milbemycin, lufenuron	Dog	Oral	1 791	Lack of efficacy, vomiting, depression/lethargy, diarrhoea, pruritus, anorexia, convulsions, ataxia, trembling, fever, alopecia, death, bloody diarrhoea
Monensin	Dog	Oral	31	Death, ataxia, depression/lethargy, anorexia, neurological disorder, paresis of hind limbs
	Pig		3 388	Anorexia, ataxia, death, diarrhoea, myoglobin in urine, paresis
	Bird		1 050	Reduced egg production, reduced fertility, anorexia, death
	Deer		33	Death
	Goat		2 001	Depression/lethargy, anorexia, diarrhoea, death, recumbency, vocalisation, adipsia, abdominal distension, cardiac lesions, acidosis of rumen
	Horse		293	Death, recumbency, abdominal pain, tachycardia, anorexia, weakness, ataxia, diarrhoea, sweating
	Llama		1	Death
	Sheep		1 447	Anorexia, death, diarrhoea, weakness, lung lesions, joint pains, cardiac lesions, paralysis of hind limbs, ataxia, confusion, convulsions, stiffness, lameness
	Alpaca		50	Anorexia, dyspnoea, stiffness, trembling, death
	Cattle		124 466	Abnormal milk/udder, anorexia, diarrhoea, lack of efficacy, hypogalactia, unpalatable, watery diarrhoea, cardiac lesions, 'ill', death, mild diarrhoea, neurological disorders, chest oedema, lung lesions, depression/lethargy, melaena, reduced milk production, bloat, liver lesions, muscle lesions, dyspnoea, ataxia, cachexia, reduced fertility
	Chicken		38 300	Toxicity, death, paralysis, lack of efficacy, anorexia, collapse
	Water fowl		2 000	Anorexia, recumbency, death

Table 7.1 *Continued*

Drug	*Species*	*Route*	*Numbers*	*Major effects*
Monensin, ractopamine	Cattle	Oral	2 921	Anorexia, polyphagia, lack of efficacy, hyperactivity, abnormal hair colour, skin scabs, skin sores, death
Monensin, ractopamine, tylosin	Cattle	Oral	17 591	Lack of efficacy, death, anorexia, hyperactivity, abnormal GI system, unpalatable, behavioural disorder, polyphagia, meat abnormality, bloat, abnormal meat colour, digestion disorder
Monensin, tylosin	Cattle	Oral	48 467	Unpalatable, liver lesions, liver disorders
Morantel	Cattle	Oral	835	Hypogalactia, diarrhoea, anorexia, severe diarrhoea, rumenal acidosis, death
Moxidectin	Dog	Oral	365	Ataxia, unconsciousness, convulsions, death, trembling, depression/lethargy, recumbency, stupor, neurological disorders, hyperactivity, hypersalivation, vomiting, confusion
	Fish		7	Death
	Goat		70	Recumbency, anorexia, death, watery diarrhoea, diarrhoea, depression/lethargy, ataxia, abortion
	Horse		1 261	Ataxia, depression/lethargy, abdominal pain, recumbency, anorexia, lack of efficacy, death, pruritus, diarrhoea, abnormal hair, urticaria, abortion, fever
	Sheep		52	Depression/lethargy, abortion, death, collapse
	Cat	Topical	873	Lack of efficacy, bloat
	Cattle		52 163	Alopecia, pruritus, lack of efficacy, abnormal hair, anorexia, weight loss, hypogalactia, depression/lethargy, skin inflammation
	Fish	Unknown	150	Death
	Dog	Parenteral	5 473	Vomiting, anaphylaxis, depression/lethargy, urticaria, anorexia, diarrhoea, oedema of head/face, fever, pruritus, lack of efficacy, convulsions, death, pallor of mucous membranes, collapse, injection site swelling, shock, ataxia, weight loss, bloody diarrhoea, weakness, anaemia, skin congestion, polypnoea, recumbency
Moxidectin, praziquantel	Dog	Oral	21	Trembling, unconsciousness, hypersalivation, recumbency, ataxia, confusion, death
	Horse		74	Depression/lethargy, anorexia, lack of efficacy, recumbency, tachycardia, projectile diarrhoea
Narasin	Horse	Oral	45	Death, abdominal distention, abdominal pain, pericardial effusion, ataxia
	Chicken		1 100	Anorexia, paresis, death

N-butyl chloride	Cat	Oral	27	Vomiting, death, hyperactivity
	Dog		20	Vomiting, death, anorexia, dehydration
Neomycin, nystatin, thiostrepton, triamcinolone	Dog	Topical	93	Deafness, birth defects, premature births, temporary deafness, dystocia, partial deafness
Nicarbazin	Chicken	Oral	54 992	Death, reduction in egg production, abnormal eggs, abnormal hatching
Nitazoxanide	Horse	Oral	45	Death (euthanised), fever, recumbency, laminitis, lameness, difficulty rising, mild diarrhoea, anorexia
Nitenpyram	Cat	Oral	92	Hyperactivity, polypnoea, hyperpnoea, vomiting, depression/lethargy, fever, pruritus
	Dog		56	Vomiting, depression/lethargy, diarrhoea, pruritus, anorexia, convulsions, ataxia
Norgestomet	Cattle	Various	351	Lack of efficacy, early oestrus, abnormal oestrous behaviour
Norgestomet, oestradiol	Cattle	Parenteral	65	Oestrous behaviour, reproduction disorders, oestrous cycle abnormalities
Oestradiol	Cattle	Oral	50	Prolapse, death
		Parenteral	1 103	Oestrous behaviour, injury, hyperactivity, prolapse of vagina, rectum or uterus, death
Oestradiol, progesterone	Cattle	Parenteral	2 917	Abnormal oestrous behaviour, aggression, udder swelling, prepuce swelling
Oestradiol, testosterone	Cattle	Parenteral	432	Vaginal prolapse , udder swelling, reduced fertility, rectal prolapse, abnormal oestrous behaviour, death
Oestradiol, trenbolone	Cattle	Parenteral	706	Abnormal oestrous behaviour, dyspnoea, anaphylaxis, mouth froth, injection site abscess, aggression
Orbifloxacin	Cat	Oral	98	Lack of efficacy, anorexia, blindness, mydriasis, ataxia, death, depression/lethargy
	Dog		120	Vomiting, depression/lethargy, convulsions, anorexia, ataxia, death
Ormetoprim, sulphadimethoxine	Cat	Oral	21	Hypersalivation, oedema of pharynx
	Dog		602	Depression/lethargy, fever, convulsions, low platelet count, ataxia, anorexia, vomiting, neurological disorder, trembling, nervousness, apprehension, aggression
	Fish		1 000	Anorexia
Oxfendazole	Cattle	Oral	864	Lack of efficacy, anorexia, loss of condition, death

Table 7.1 *Continued*

Drug	*Species*	*Route*	*Numbers*	*Major effects*
Oxibendazole	Dog	Oral	41	Depression/lethargy, bloody diarrhoea, vomiting, death, abdominal pain
	Horse		667	Abdominal pain, diarrhoea, anorexia, depression/lethargy, hypersalivation, irritation of mouth and lips, fever, death, lack of efficacy, volvulus
Oxytetracycline	Dog	Oral	11	Vomiting, anorexia, diarrhoea
	Bird		500	Death
	Fish		28 000	Death, skin lesions, weakness
	Cattle		122	Milk residues, bloat, death, anorexia
	Chicken		51	Eye congestion, sneezing
	Pig	Parenteral	243	Injection site abscess, lack of efficacy, death
	Cattle		1 809	Injection site swelling, anaphylaxis, death, injection site pain, lameness, collapse, recumbency, front limb stiffness, froth at mouth, hypersalivation
Oxytetracycline, polimixin B	Cat	Ophthalmic	43	Swelling of eyes/eyelids, lack of efficacy, conjunctivitis, eye pain, vomiting
Oxytocin	Cattle	Unknown	202	Lack of efficacy
		Parenteral	340	
Penicillin G benzathine, penicillin G procaine	Pig	Parenteral	2 249	Vomiting, anaphylaxis, respiratory distress, depression/lethargy, death, diarrhoea, recumbency, trembling
	Horse		86	Death, injection site swelling, anaphylaxis, collapse
	Cattle		145	Anaphylaxis, death
Penicillin G procaine	Cattle	Unknown	59	Lack of efficacy, death, anaphylaxis
	Dog	Parenteral	69	Injection site abscess, swelling and seroma, reluctance to move
	Pig		1 558	Vomiting, shaking, anaphylaxis, respiratory distress, diarrhoea, dyspnoea, depression/lethargy, blindness, CNS disorders, thrashing/paddling, abortion, fever
	Horse		49	Death, collapse, anaphylaxis, ataxia
	Cattle		174	Anaphylaxis, death, milk residues, collapse, shaking, dyspnoea, hypersalivation
Pentobarbital, phenytoin	Cat	Parenteral	22	Lack of efficacy
	Dog		867	Lack of efficacy, vocalisation, periodic breathing, hyperactivity, delayed effect, injection site pain, convulsions, opisthotonos, spasm, twitch, biting/chewing
	Horse		81	Lack of efficacy, hyperactivity

Phenylbutazone	Dog	Oral	13	Anorexia, depression/lethargy, anaemia, diarrhoea
	Horse		22	Death, anorexia, depression/lethargy, abdominal pain, reluctance to move, colitis
		Parenteral	9	Death, abdominal pain, collapse, convulsions
Piperazine, phenothiazine, trichlorfon	Horse	Oral	82	Diarrhoea, abdominal pain, anorexia, hypersalivation, death, enteritis, depression/lethargy, abortion
Pirlimycin	111	Intramammary	111	Mastitis, death
Praziquantel	Cat	Oral	22	Lack of efficacy, depression/lethargy, anorexia
	Dog		50	Lack of efficacy, depression/lethargy, vomiting, diarrhoea, anorexia, death, ataxia
	Cat	Parenteral	103	Injection site alopecia, death, injection site inflammation, pain and sloughing, vomiting, injection site abscess
	Dog		104	Injection site swelling, pain and abscess, depression/lethargy, lack of efficacy, anorexia
Praziquantel, pyrantel	Cat	Oral	148	Ataxia, depression/lethargy, vomiting, hypersalivation, staggering, weakness, anorexia
Prednisolone	Dog	Parenteral	26	Anaphylaxis, collapse, vomiting, shock
Prednisolone, trimeprazine	Dog	Parenteral	36	Depression/lethargy, elevated serum enzymes, convulsions
Primidone	Dog	Oral	43	Lack of efficacy, death, elevated serum enzymes, depression/lethargy
Progesterone	Cattle	Suppository	402	Lack of efficacy, vulval discharge, tenesmus, vaginitis, reduced fertility, prolapsed rectum
Promazine	Horse	Oral	20	Lack of efficacy, hyperactivity
Propofol	Cat	Parenteral	19	Apnoea, cardiac arrest, temporary blindness, bradycardia, death
	Dog		109	Lack of efficacy, death, apnoea, cardiac arrest, bradycardia, convulsions, turbulent anaesthetic induction
Pyrantel	Cat	Oral	76	Diarrhoea, vomiting, ataxia, depression/lethargy, anorexia, death, hypersalivation
	Dog		625	Death, vomiting, depression/lethargy, diarrhoea, lack of efficacy, anorexia, convulsions, weakness, dehydration, bloody diarrhoea, hypersalivation, ataxia
	Pig		2 235	Lack of efficacy, loss of condition, death, diarrhoea
	Horse		559	Lack of efficacy, abdominal pain, diarrhoea, anorexia, depression/lethargy, unpalatability
	Dog	Parenteral	35	Injection site abscess, convulsions, diarrhoea, hypersalivation, vomiting, weakness

Table 7.1 *Continued*

Drug	*Species*	*Route*	*Numbers*	*Major effects*
Ractopamine	Dog	Oral	5	Depression/lethargy, tachycardia
	Pig		144 041	Hyperactivity, apprehension, recumbency, collapse, death, behavioural disorder, nervousness, reluctance to move, lack of fertility, difficulty rising, lack of efficacy, depression/lethargy, stiffness, vocalisation, spasm, pain, polydipsia, trembling, cyanosis, lameness, dyspnoea, aggression, hoof disorders, anorexia, drug interactions, twitch, limb stiffness, locomotion disorder, polypnoea, ataxia, reduced fertility
	Cattle		5 684	Anorexia, unpalatability, bloat, abnormal meat, polyphagia, death, hyperactivity, leg disorders, locomotion disorders
Ractopamine, tylosin	Pig	Oral	2 143	Vocalisation, biting/chewing, pain, tail disorder, lameness, hoof disorder, locomotion disorder, recumbency, stiffness, death
Roxarsone	Chicken	Oral	50	Ataxia, paralysis, weakness, death
Selamectin	Cat	Oral	44	Vomiting, hypersalivation, depression/lethargy, anorexia
	Dog		98	Lack of efficacy, vomiting, depression/lethargy, diarrhoea, anorexia, hypersalivation
	Cat	Topical	4 316	Alopecia at application site, depression/lethargy, anorexia, vomiting, lack of efficacy, diarrhoea, application site lesions, ataxia, death, alopecia, pruritus, fever, application site inflammation, trembling, application site pruritus and erythema, hypersalivation, convulsions, dehydration, dyspnoea, weight loss
	Dog		11 018	Lack of efficacy, vomiting, depression/lethargy, diarrhoea, pruritus, anorexia, convulsions, trembling, alopecia at application site, ataxia, hypersalivation, application site lesions, death, bloody diarrhoea, urticaria, skin congestion, skin inflammation, fever, application site inflammation, polypnoea, application site pruritus, hyperactivity, application site erythema
	Ferret		10	Anorexia, death, ataxia, weight loss
	Rabbit		120	Hyperactivity
Selegiline	Dog	Oral	2 114	Vomiting, lack of efficacy, diarrhoea, depression/lethargy, anorexia, nervousness, convulsions, polypnoea, polydipsia, ataxia, weakness, weight loss

Selenium, vitamin E	Sheep	Unknown	30	Abortion, anorexia, stiffness, death, dyspnoea
	Cattle		167	Anorexia, hypogalactia, depression/lethargy, stiffness, anaphylaxis, recumbency, ataxia, hypersalivation, injection site pain, death, abortion, dyspnoea
	Pig	Parenteral	42	Injection site lesions, oedema of lungs/trachea, lack of efficacy, injection site inflammation
	Horse		220	Anaphylaxis, abdominal pain, collapse, death, mucous membrane pallor, sweating, trembling, ataxia, depression/lethargy, injection site abnormality, dyspnoea, injection site swelling, recumbency, hyperpnoea
	Sheep		534	Depression, lethargy, nervousness, abortion, anorexia, recumbency, diarrhoea, hyperpnoea, hypersalivation, dyspnoea, bloat, abdominal pain
	Cattle		1233	Injection site abscess, depression/lethargy, anaphylaxis, injection site swelling, abortion, recumbency, premature birth, ataxia, death, neonatal death, lack of efficacy, trembling
Sevoflurane	Cat	Inhalation	30	Death, cardiac arrest, apnoea, anorexia
	Dog		89	Death, cardiac arrest, apnoea, bradycardia, cyanosis, hypopnoea
Sometribove	Cattle	Unknown	1142	Mastitis, elevated cell count in milk, injection site swelling
		Parenteral	54276	Elevated cell count in milk, reduced fertility, mastitis, hypogalactia, lack of efficacy, weight loss, injection site swelling, hoof disorders, multiple births, udder swelling, lameness, laminitis, abortion, injection site abscess and lesions, joint swelling, loss of condition
Spectinomycin	Cattle	Parenteral	124	Death, lung lesions, dyspnoea, frothing at mouth/lips
Stanozolol	Horse	Parenteral	19	Injection site swelling and abscess, anaphylaxis, injection site pain
Sulfadiazine, trimethoprim	Dog	Oral	59	Depression/lethargy, anorexia, fever, anaemia, death, vomiting
	Horse	Parenteral	34	Collapse, death, convulsions, anaphylaxis, sweating
Sulfadimethoxine	Cat	Oral	141	Lack of efficacy, depression/lethargy, ataxia, mydriasis, polyphagia
	Dog		341	Lack of efficacy, bloody diarrhoea, vomiting, depression/lethargy, diarrhoea, anorexia, convulsions, death, urticaria, hypersalivation, swelling of head/face, trembling, weakness, collapse
Sulfaquinoxaline	Chicken	Oral	2000	Depression/lethargy, respiratory distress, pallor, swelling at multiple sites, death
Tepoxalin	Dog	Oral	648	Vomiting, diarrhoea, anorexia, depression, bloody diarrhoea, melaena, anaemia, death, bloody vomiting, drug interactions, lack of efficacy, convulsions, ataxia, haematochezia, unpalatable, polydipsia, weakness, blood in urine

Table 7.1 *Continued*

Drug	*Species*	*Route*	*Numbers*	*Major effects*
Tetracycline	Cat	Oral	10	Fever, depression/lethargy
	Fish		25 000	Death
Tiamulin	Pig	Oral	822	Lack of efficacy, skin congestion, diarrhoea, nervousness, pain, ataxia, twitch, depression/lethargy, skin disorder, stiffness of hind limbs, death
Tiletamine, zolazepam	Cat	Unknown	110	Respiratory disorder, coughing, apnoea, death, lack of efficacy, mydriasis, oedema of lungs/trachea, congestion of lungs, blindness, apnoea, prolonged recovery, ataxia
	Dog		223	Diarrhoea, lack of efficacy, turbulent recovery from anaesthesia, fever, death, apnoea
	Cat	Parenteral	1 489	Death, lack of efficacy, prolonged recovery, oedema of lungs/trachea, ataxia, cough, inadequate sedation, apnoea, dyspnoea, hypothermia, congestion of eyes and mucous membranes, cardiac arrest, depression/lethargy
	Dog		989	Lack of efficacy, fever, turbulent recovery from anaesthesia, death, vocalisation, vomiting, prolonged recovery, apnoea, thrashing/paddling, convulsions, polypnoea, hyperpnoea, cardiac arrest, drug interactions, ataxia, cyanosis, hyperactivity
	Primate		6	Prolonged recovery, death
Tilmicosin	Dog	Oral	2	Death, pain
	Pig		7 757	Lack of efficacy, anorexia, unpalatable, poor performance, death, alopecia
	Sheep	Missing	21	Death, liver lesions
	Cattle		356	Lack of efficacy, death, joint swellings, injection site swelling, diarrhoea, recumbency
		Unknown	900	Injection site swelling, lameness, lack of efficacy, death, depression/lethargy, respiratory distress, acidosis, anorexia, ataxia, diarrhoea
	Pig	Parenteral	2	Death
	Deer		3	Dyspnoea, recumbency, death
	Goat		84	Death, vocalisation, collapse, ataxia, anaphylaxis, polypnoea, cyanosis, injection site necrosis, distress
	Alpaca		4	Death

	Cattle		4685	Injection site swelling, lack of efficacy, lameness, death, limb swelling, stiffness, injection site inflammation, recumbency, injection site oedema, anorexia, injection site stiffness, pain, reluctance to move, collapse, dyspnoea, injection site abscess, ataxia
	Rabbit		4	Death, shock
Tolazoline	Deer	Parenteral	8	Death, hyperpnoea, cardiac lesions, tachycardia, nervousness
	Horse		5	Death, collapse, respiratory distress
	Llama		7	Convulsions, death, anorexia, diarrhoea
	Cattle		3	Death
Triamcinolone	Dog	Topical	31	Alopecia, polydipsia, vomiting, depression/lethargy, polyuria, anorexia
		Unknown	35	Lack of efficacy, anaphylaxis, death, cardiac arrest, depression/lethargy
		Parenteral	88	Anaphylaxis, vomiting, shock, urticaria, lack of efficacy, collapse, polypnoea, death, polydipsia, polyuria, depression/lethargy, diarrhoea
	Horse		25	Arthritis, anorexia, depression/lethargy, fever
Tripelennamine	Cattle	Unknown	23	Anaphylaxis, dyspnoea, oedema of lungs/trachea, death, convulsions
	Dog	Parenteral	8	Convulsions, vomiting, death, difficulty rising
	Horse		20	Hyperactivity, convulsions, collapse, death, abortion, anaphylaxis, dyspnoea
Tulathromycin	Cattle	Unknown	106	Injection site abscess, skin lesions, anorexia
	Pig	Parenteral	36	Death, lack of efficacy, injection site pain and abscess
	Cattle		4223	Lack of efficacy, injection site alopecia, skin lesions, injection site oedema, abnormal hair colour, hyperpigmentation, injection site fibrosis, inflammation and ulceration, leukoderma, scaley skin, death, lung lesions, anaphylaxis, anorexia, hyperactivity, weakness, dyspnoea, dysmetria, injection site swelling and pain, collapse
Tylosin	Dog	Oral	29	Anorexia, vocalisation, diarrhoea
	Pig		9267	Lack of efficacy, enteritis, poor performance, bloody diarrhoea, abnormal hair, reduced fertility, diarrhoea, loss of condition, constipation, death
		Parenteral	510	Death, collapse, recumbency, thrashing/paddling, hypersalivation
	Cattle		68	Death, joint pain, joint swelling, recumbency, collapse, hind limb paralysis

Table 7.1 *Continued*

Drug	*Species*	*Route*	*Numbers*	*Major effects*
Virginiamycin	Horse	Oral	3	Death, diarrhoea, recumbency
	Cattle		49	Death
Vitamin A, Vitamin D	Cattle	Parenteral	54	Nasal discharge, vocalisation, pneumonia, death
Xylazine	Horse	Missing	20	Turbulent recovery from anaesthesia, lack of efficacy
	Cat	Unknown	13	Lack of efficacy, death, apnoea
	Dog		38	Lack of efficacy, apnoea, death, cardiac arrest
	Deer		8	Lack of efficacy
	Horse		60	Lack of efficacy, collapse, ataxia
	Cat	Parenteral	108	Lack of efficacy, deep sedation, prolonged recovery from anaesthesia, cyanosis, oedema of lungs/trachea, aspiration pneumonia, prolonged sedation, death, apnoea, cardiac arrest
	Dog		202	Lack of efficacy, death, convulsions, apnoea, vomiting, bradycardia, cardiac arrest
	Deer		58	Lack of efficacy, death
	Horse		317	Lack of efficacy, convulsions, collapse, trembling, sweating, death, anaphylaxis, hyperactivity, recumbency, urticaria, turbulent recovery from anaesthesia
	Llama		2	Death
	Tiger		2	Apnoea, death
	Cattle		40	Lack of efficacy, death, bloat
Yohimbine	Dog	Parenteral	26	Death, cardiac arrest, apnoea, cyanosis
Zeranol	Sheep	Parenteral	136	Prolapse of rectum and vagina, udder swelling, respiratory disorder, death
	Cattle		2733	Oestrous behaviour, prolapse of vagina, site abscess, prolapse of rectum, reduced fertility, udder swelling, aggression, prolapse of uterus, anorexia, 'ill', paresis, anoestrus,
Zinc gluconate	Dog	Parenteral	21	Testicular swelling, abnormal scrotum, depression/lethargy, scrotal pain, testicular lesions, testicular pain, skin necrosis, skin ulcers, scrotal swelling, vomiting

Figures for 1993 and 1994 were published and largely restricted to dogs, cats, horses and cattle (Maddison, 1992, 1994, 1996). These are shown in *Table 7.2*.

From 1995 on, the findings became available on the APVMA/NRA website, and a summary of these is provided in *Table 7.3*.

These data, and more recent information, are available in the annual reports of adverse experiences with veterinary medicines. These show a trend that over time, the number of reports submitted has steadily increased, probably as familiarity with these schemes has grown; this trend has also been seen in the UK and the USA.

Many of the reactions in cats and dogs were due to hypersensitivity reactions. As in the UK, a number of the reactions in horses arose from antimicrobial treatments and several animals died. Some of the incidents in goats involved off-label use of drugs, including ivermectin, while many of those in sheep involved ectoparasiticides. In one instance, over 35,000 sheep were treated with the endectocides ivermectin and over 600 died. It was found that incorrect administration was responsible and as a result severe damage to the throat occurred, which led to the deaths observed. Incidents in cattle often involved ectoparasiticides or antimicrobial drugs. In another instance, topical application of synthetic pyrethroids resulted in skin irritation. More recent reports suggest that many of the adverse reactions noted in the Australian scheme for cats and dogs are associated with vaccinations. Adverse reactions to propofol in dogs were also common (National Registration Authority, 1995, 1996, 1999, 2001; Australian Pesticides and Veterinary Medicines Authority, 1999, 2003–2006; Linnett, 2006).

The reactions in humans tended to involve contamination with ectoparasiticides, including synthetic pyrethroids giving rise to the expected dermal effects, or accidental self-injection with vaccines. There was one fatality following exposure to an ectoparasiticide in 2000. A case of photodermatitis occurred after exposure to olaquindox, an antimicrobial drug used in pigs (Fewings and Horton, 1995).

Table 7.2 Numbers of adverse reaction reports and animals treated – Australia, 1992–1994 (reports/animals involved).

Species	*1992*	*1993*	*1994*
Dog	23/24	30/43	32/44
Cat	23/30	11/14	18/31
Horse	4/4	8/10	17/48
Cattle	7/10	9/30	10/21
Ferret	—	1/1	—

Table 7.3 Numbers of adverse reaction reports and animals treated – Australia, 1995–2000. Where *1* is number of reports; *2* is number treated; *3* is number reacted.

Species	*1995*			*1996*			*1997 and 1998*			*1999*			*2000*		
	1	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*
Dog	20	41	25	42	87	69	67	101	83	16	32	32	30	43+	37+
Cat	19	30	28	18	26	18	18	19	18	7	7	7	18	44	31
Horse	10	15	11	31	35	35	39	112	104	3	3	3	18	66	31
Cattle	13	558	429+	35	830	356	35	2,036	586	16	1,388	211	57	10,239	7,800
Rabbit	—	—	—	5	51	7	2	2	2	5	5	5	2	2	2
Pig	—	—	—	—	—	—	—	—	—	2	1159	60	2	—	134+
Alpaca	1	60	1	—	—	—	—	—	—	—	—	—	—	—	—
Bird	1	6	3	—	—	—	—	—	—	1	1	1	1	100	11
Sheep	—	—	—	1	200	100	7	36,565+	870+	2	437	39	10	7,887	1,352
Goat	—	—	—	—	—	—	35	35	35	—	—	—	2	205	35
Human	1	1	1	1	2	2	2	2	1	20	1	20	20	—	19

In the UK, a major increase in suspected adverse reaction reporting in humans occurred in 1991. There were around 35 reports in 1990, but almost 200 in 1991. This increase followed a period of increased publicity by the VMD accompanied by increased media interest. The increases are not confined to the UK (and the USA). For example, the Australian scheme reported a 62% increase in adverse reactions in 2000 over the previous year. As with the US scheme, dogs and cattle were the species mainly affected, except for those occasions involving sheep. These increases are likely to reflect increased publicity for, and growing awareness of, the operation of the adverse reaction reporting schemes. Again, and like the UK scheme, adverse reactions in humans are also considered and 20 reports were filed in the 2000 period (Anonymous, 2000, 2001a, b).

Canada

In Canada, veterinary pharmaceuticals are regulated by the Veterinary Drugs Directorate within Health Canada. An adverse reaction to a drug is defined as any unintended or noxious side effect, injury toxicity or sensitivity reaction associated with clinical uses, studies, investigations and tests involving a drug and any unusual failure of a drug to produce its expected pharmacological activity. Veterinary vaccines are dealt with by the Veterinary Biologics section of the Canadian Food Inspection Agency. Adverse reactions are reported to the respective agency (http://www.hc-sc.gc.ca/dhp-mps/vet/index_e.html).

The Canadian system is intended to monitor adverse drug reactions in treated animals, potentially exposed humans and consumers, and manufacturers are required to report any such adverse reactions brought to their attention; veterinarians are also encouraged to report. The Veterinary Drugs Directorate has classified adverse reactions to veterinary drugs as follows:

- any unintended or noxious side effects, injury or sensitivity reaction associated with the clinical uses, studies, investigations and tests respecting a drug;
- any unusual failure of a drug to produce its expected pharmacological activity, that is lack of efficacy.

Adverse environmental effects are not explicitly covered, although they can be included because of the broad scope of the first bullet point above. The Directorate requires reporting for the following:

- all suspected adverse drug reactions that are unexpected, i.e. not consistent with product information or with the label;
- all serious suspected adverse drug reactions – those that contribute to a significant disability, that require hospitalisation or significant medical intervention, or reactions that are more severe or more frequent than indicated by the product information or the label;
- all suspected adverse drug reactions to recently marketed drugs – commercially available for less than 7 years, regardless of nature or severity;
- lack of efficacy;
- any suspected adverse drug reaction in humans arising from handling or accidental contact with a veterinary drug.

Full details are given on the Directorate's website on how and where to report, and a copy of a reporting form is provided. There is also a useful question and answer section covering the scope of pharmacovigilance, the role of manufacturers and veterinarians and the activities of the Directorate. Clearly, the latter concentrates on likely regulatory outcomes, which in turn depends on the nature of the adverse reactions reported. Any of the following actions may be taken:

- labelling changes;
- product recall;
- removal from market;
- no action if this is appropriate;
- requirement for post-marketing studies;
- re-assessment of benefits and risks;
- dissemination of information to veterinary and human health professionals, as appropriate;
- issue of public alerts.

In the case of the drug Micotil (tilmicosin) and adverse reactions seen in humans (see Chapter 20), the Directorate published full information regarding the nature of the adverse reactions, along with warnings and guidance to medical professionals.

From the limited information available, it would seem that adverse reactions to veterinary pharmaceuticals in Canada largely follow patterns seen elsewhere (Anonymous, 1992, 1995). However, there is surprisingly little veterinary pharmacovigilance data publicly available, unlike the situation in the UK, the USA and Australia. Health Canada also establishes maximum residue limits, but these are not explicitly linked to pharmacovigilance activities.

Veterinary biologics, largely vaccines, are regulated in Canada by the Veterinary Biologics Section (VBS) of the Canadian Food Inspection Agency (http://www.inspection.gc.ca/english/anima/vetbio/vbpbve.shtml). Like veterinary pharmaceuticals, veterinary biologics are assessed for safety, quality and efficacy before approval for marketing, and suspected adverse reactions must be reported to the agency. Adverse reactions are classified into four types:

- Type 1 events: systemic adverse reactions including anaphylaxis and hypersensitivity reactions requiring veterinary treatment, persistent fever (longer than 48 hours), muscle tremors, persistent lethargy, hypersalivation, eye and reproductive events and neurological disorders. In other words, what might be regarded as 'standard' adverse reactions.
- Type 2 events: death or an increase in mortality.
- Type 3 events: local persistent events such as oedema, alopecia, granuloma or fibrosis, or excessive pain at the injection site.
- Type 4 events: lack of efficacy.

Adverse events reported to the Agency are classified in a manner akin to that used in the ABON system: probable, possible, unlikely or unknown. The VBS provides a form for reporting adverse reactions.

Discussion

The veterinary pharmacovigilance systems operating in the USA, Australia and Canada have much in common with each other, and much in common with the approaches taken in other countries and notably those of the European Union (Woodward, 2005). This is perhaps not surprising in view of the nature of the task in hand and is encouraged by initiatives such as the work of the VICH discussed elsewhere in this volume (see Chapter 2). They strive to garner adverse reaction data to monitor the safety of veterinary medicinal products, and where necessary to change the terms of approval to reduce the potential for harmful side effects. The US and Australian systems also ensure that much of the data produced are available to a wider audience, including veterinarians and other health professionals.

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8 Veterinary pharmacovigilance: a South African perspective

V. Naidoo

Introduction

The monitoring of adverse drug reactions in veterinary medicine in South Africa is a fairly new field. Although the use of medicines in animals in the country was to some extent controlled from 1947, no formal system of adverse drug reaction monitoring was initiated until the late 1990s. The Veterinary Pharmacovigilance and Medicines Information Centre (VP & MIC) was initially established on an informal basis within the Department of Pharmacology and Toxicology of the Faculty of Veterinary Science, University of Pretoria, in 1998, as a purely academic venture by the then Head of Department (Gehring, 2001). With the system showing promise, a more formal system of recording was implemented in 2000, following the receipt of start-up capital from the May and Stanley Smith Charitable Trust (Naidoo and Gehring, 2002). This allowed the Centre to publish a proper reporting form (see Appendix), establish a computerised database and obtain the necessary resources to assist Southern African veterinarians with drug-related queries. At this point the Centre was run purely in-house within the department and had no interactions with the drug regulatory authorities. It was only in 2003, following consultation with the Medicines Control Council of South Africa (MCC), that the VP & MIC was officially recognised as the site for the monitoring of adverse drug reactions (ADRs) to veterinary medicines in South Africa.

South African legislation: a brief overview

South Africa is fairly unique in that the use of medicines in animals falls under a dual system of control. Over-the-counter products are controlled by Act 36 of 1947 and are under the control of the Minister of Agriculture, while veterinarian-restricted products are controlled by Act 101 of 1965 and are under the control of the Minister of Health (Anonymous, 1947, 1992). The histories of the two Acts are as follows.

The Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act (Act 36 of 1947) was promulgated in 1947 to assist the cattle farmers in the country. Following World War II, South Africa was very reliant on the livestock and agricultural industry to support the economy. As such it became very important for farmers to have direct access to medication that could stem production losses from tick- and helminth-associated diseases that were prevalent at the time due to the local subtropical climate. Although

the veterinarian could have made these medicines available to the farmer, very few veterinarians were available in the country at that stage to offer their assistance. Act 36/47 was instituted as a means to overcome this problem and gave the farmer direct access to certain medication(s) that could be used to treat animal conditions that were readily diagnosable without veterinary consultation.

These specific remedies were termed 'stock remedies' and are equivalent to over-the-counter products. The criteria for registration were set as: ease of diagnosis by a layperson; ease of use and administration; proven efficacy under South African conditions; and safety to the animal, user and environment. The specific remedies registered to date are the anthelminthics; ectoparasiticides; anti-protozoal agents; anti-rickettsial agents; mastitis and dry cow remedies; growth-promoting antimicrobials; vaccines; vitamin/mineral formulations/licks; and therapeutically used tetracyclines. Although the term is misleading, stock remedies is also applied to companion animal products, which fall into these classes.

The Medicines and Related Substances Act (Act 101 of 1965) was promulgated in 1965 and was intended to control the use, supply and sale of medicines for use in people. Although the Act had the potential to regulate veterinary medicines, this control was not extended to the veterinary profession until the early 1980s when it was applied to veterinary medicines:

> '. . . any substance or mixture of substances, other than a stock remedy or farm feed to be registered in terms of the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act, 1947 (Act 36 of 1947), used or purporting to be suitable for use or manufactured or sold for use in connection with vertebrates, for the treatment, diagnosis, prevention or cure of any disease, infection or other unhealthy condition, or for the maintenance or improvement of health, growth, production or working capacity, or for curing, correcting or modifying any somatic or organic function, or for correcting or modifying behaviour.'

Until this time, any person marketing veterinary medicines was able to do so without any restriction. At present, the rules and regulations of Act 101 of 1965 are enforced by the Medicines Control Council of South Africa (http://www.mccza.com).

In terms of the Medicines and Related Substances Act (Act 101 of 1965), veterinary medicines are grouped into various schedules (Schedule 0–8) based on their safety, use and habit-forming potential (*Table 8.1*). Schedule 0 medicines and stock remedies are over-the-counter products and are available directly to the public from any retail outlet, while Schedule 1 and 2 medicines may only be sold by a pharmacist, without veterinary prescription/permission. Veterinary permission is required for all products Schedule 3 or higher. In addition to the control of registered medicines, Act 101/65 also controls the use and sale of compounded formulations, the importation of non-registered drugs, the movement of drugs via South African ports and the use of illicit and banned substances.

In addition to the two Acts mentioned above, the use of veterinary medicines and stock remedies in South Africa are restricted by two other acts. The first defines the scope of practice of a veterinarian, while the second protects the consumer from residues of food origin. They are as follows:

- The Veterinary and Paraveterinary Professions Act (Act 19 of 1982).
- The Foodstuffs, Cosmetics and Disinfectants Act (Act 54 of 1972) (Anonymous, 1972).

The Veterinary and Paraveterinary Professions Act (Act 19 of 1982) regulates the veterinary profession (Anonymous, 1982). In addition to setting the rules and regulations that govern the veterinary profession locally, the Act allows veterinarians to use any medicine extra-label in any animal species, which unfortunately includes extra-label use in food-producing animals. The Act does, however, state that the veterinarian shall always protect the general public, which is locally interpreted to mean that no veterinarian is allowed to use a product extra-label in food-producing

Table 8.1 The schedules under which drugs are categorised in terms of Act 101/65, with some examples per schedule.

Schedule	*Group*	*Example of medicines*
0	Over-the-counter (OTC) medicines	All medicines not included in any of the other schedules
1	Pharmacy medicine	Topical bacitracin and polymyxin, atropine <0.1%, fenbendazole
2	Pharmacy prescription medicine	Most antihistamine formulations, atropine, ibuprofen, mebendazole
3	Frequently repeated prescription medicines	Digitals, most diuretics, insulin
4	Main group prescription medicines	Corticosteroids, antimicrobials, hormones
5	Dependence-producing prescription medicines	Sedatives, tranquillisers, anaesthetics, anabolic steroids
6	Dangerous dependence-producing prescription medicines	Etorphine, morphine, fentanyl
7	Undesirable dependence-producing substances not recognised for medical use, but which do have therapeutic benefit	Phencyclidine
8	Substances with extremely high potential for abuse or dependence, with very limited recognised medical uses	Amphetamine

animals unless a suitable dose and withdrawal period have been set for that product.

The Foodstuffs, Cosmetics and Disinfectants Act (Act 54 of 1972) regulates the maximum residue limits (MRLs) in products of animal origin (Anonymous, 1992). At present the Act follows the conventions of Codex Alimentarius and accepts the MRLs elaborated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (see Chapter 23).

Adverse drug reaction monitoring in South Africa

Both Act 36/47 and Act 101/65 make provision in their legislation for the monitoring and reporting of ADRs that result from the use of stock remedies and veterinary medicines respectively. More importantly in terms of Act 101/65, all members of the veterinary profession are legally obliged to report all ADRs they encounter in their daily practice of their profession.

With the VP & MIC gaining legal status in 2003, it has become the official monitoring centre for all veterinary medicines registered with Act 101/65. In addition, the head of the centre is seconded to the Veterinary Clinical Committee of the Medicines Control Council, which evaluates veterinary medicines for registration purposes, to ensure that important ADRs receive the appropriate regulatory attention (*Figure 8.1*). As yet, the Centre is not officially recognised by Act 37/47. According to the rules and regulations of this Act, all ADRs are reported directly to the registrar of the Act by the registration holder or any member of the public. Although the Act makes use of the Centre's monitoring form and database, the Centre has no official mandate to record ADRs on their behalf.

In the South African context, we include a wide variety of conditions as ADRs, such as untoward reaction from the correct utilisation of drugs, the use of human preparations, the extra-label use of drugs, use at incorrect dosages, failure to produce an effect at the recommended dosage, products

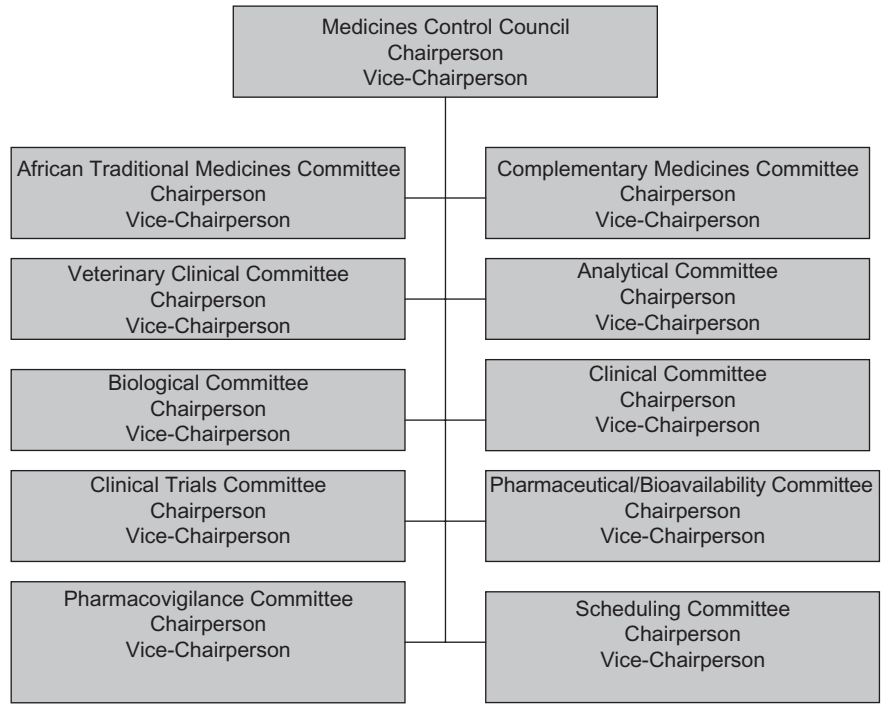

Fig. 8.1 Structure of Medicines Control Council of South Africa.

Table 8.2 Causality classification.

Classification	*Causality classification criteria*
Certain	There is a plausible time relation between the administration and the adverse event, which cannot be explained by the concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) is clinically plausible and the event is definitely pharmacological or phenomenological, using a satisfactory rechallenge procedure if necessary
Probable	There is a plausible time relationship between the administration of the drug and the adverse event, unlikely to be attributed to concurrent disease or other drugs or chemicals and which follows a clinically reasonable response on withdrawal. A positive rechallenge is not required to fulfil this definition
Possible	There is a plausible time relationship between the administration of the drug and the adverse event, but the event could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear
Unlikely	An adverse event with a temporal relationship to drug administration that would make a casual relationship improbable and for which other drugs, chemicals or underlying disease provide a plausible explanation

failing to meet the reported withdrawal period or reactions occurring in people following administration of veterinary drugs (either accidental or intentional).

As with other pharmacovigilance centres, full records are logged in an electronic database, evaluated for causality using the ABON system, and forwarded at regular intervals to the Veterinary Clinical Committee for evaluation (*Table 8.2*). The VP & MIC plays no role in complaint investigations, as all regulatory actions are the mandate of the MCC or the Registrar of Act 36.

Table 8.3 Persons reporting adverse drug reactions in South Africa.

Person	*Year*				
	98–01	*01–02*	*02–03*	*03–04*	*04–06*
Pharmaceutical company	32	29	1	2	1
Veterinarians	18	28	35	17	18
Veterinary nurses	0	12	1	1	1
Veterinary specialists	5	7	1	0	0
Other	2	1	2	0	0

Table 8.4 Numbers of reports received by species, from 1998 to 2006.

Species	*Year*				
	98–01	*01–02*	*02–03*	*03–04*	*04–06*
Canine	19	31	25	55	10
Feline	15	21	6	30	32
Bovine	7	15	3	10	4
Equine	2	6	2	0	1
Ovine/caprine	6	2	2	0	1
Poultry	4	2	1	0	0
Porcine	3	0	1	0	0
Other*	1	0	0	5	2

*Refers to wildlife treated with specific products intended for use in food-producing animals: when animals are treated in a group, it is recorded as a single adverse reaction.

Trends in adverse reactions in South Africa

Although the process of adverse drug reaction monitoring has been in existence for nearly 10 years, reporting is very apathetic by not only veterinarians but also the pharmaceutical industry (Naidoo and Gehring, 2002; Naidoo, 2003; Naidoo and Sykes, 2005, 2006). From 1998, only 214 reports were received, with an average of 27 reports a year (*Table 8.3*). The reason for the poor reporting is unknown. One would assume that the promotion of safer drugs would be a goal that the veterinary profession would strive for. Nonetheless, the veterinary profession still accounted for 70% of the total reports. One of the reasons put forward by veterinarians for the poor reporting is that the majority of South African veterinarians are in single-man practices and therefore find it difficult to fill out tedious forms. Although this might be plausible, it is considered poor veterinary practice not to keep and report these valuable records.

More importantly, the poor response from registration holders is confusing, as many of the international pharmaceutical companies have well-established internal reporting systems in Europe and the United States of America (*Table 8.3*). Although our recording system is for Act 101/65 registered products, the reporting of ADRs directly to the Registrar of Act 36/47 is also non-existent at the present moment (personal communication, Act 36/47).

The majority of the reports received in South Africa are for products registered for use in small animals (*Table 8.4*). The reason for this may result from either owners treating their own pets with over-the-counter products (stock remedies) (*Table 8.5*) or the closer relationship of owners with their pets, i.e. reports may result from better

Table 8.5 Adverse drug reactions according to the Act regulating their use in South Africa.

Registration	*Year*				
	98–01	*01–02*	*02–03*	*03–04*	*04–06*
Stock Remedies (Act 36/47)	34	58	36	20	13
Veterinary Medicines Act 101/65	21	41	11	9	10
Extra-label*	4	15	11	2	1

*Includes human medicinal products used in animals and registered products used for other indications.

Table 8.6 Classification of adverse drug reactions by persons administering the product when the ADR resulted.

Person	*Year*				
	98–01	*01–02*	*02–03*	*03–04*	*04–06*
Veterinarian	31	47	16	14	9
Veterinary nurses	0	2	1	2	0
Owners	24	23	3	5	10
Other*	2	5	20	0	2

*Includes medical doctors, dentists and pharmacists.

observations. The larger percentage of reports being for stock remedies may be because these products are registered for easily diagnosable conditions such as tick and flea remedies and are therefore freely available to the public. Unfortunately these products are extremely toxic, which, when used without veterinary supervision, could be the reason for the observed toxicity. With such free access, a large volume of these products are sold annually. The higher number of reports could therefore be a reflection of the widespread use as opposed to the actual inherent product safety (*Table 8.6*).

Specific adverse drug reactions

Although the total number of adverse reactions reported appears to be non-significant, a series of interesting cases have been reported to the Centre during the last 5 years.

Vitamin A toxicity in cattle

In two separate reports, severe adverse reactions were reported in cattle treated with a vitamin A-containing formulation. In the first reaction, 30 out of 674 heifers treated on the same day presented with signs of swelling around the eyes, foaming at the nose (with or without blood specks), lying down and heavy breathing. One animal died within 20 minutes, a second required resuscitation with adrenaline, while six aborted. At this stage the reaction was attributed to a hypersensitivity reaction to either the cremophor excipient or vitamin A.

The reaction was recorded a second time, approximately a year later on a feedlot during the processing of 80 calves. In total, two animals died, with 60% reacting with signs of salivation, increased breathing and drowsiness. To determine the causality, six formulations, oil and emulsion, were tested by the veterinarian on the same farm. Although the oil formulation took

longer, the same clinical signs were evident between the two formulations. Interestingly, similar but milder signs were also seen in the dams of the affected calves on further investigation. Once again the study concluded that hypersensitivity was occurring to the vitamin A within the formulation.

Diminazene

At present, babesiosis remains the most important clinical infection of dogs in the country and is characterised by severe intravascular haemolysis, anaemia and weakness (Jacobson, 2006). Even though the babesial parasite is known to respond to the lincosamides group of antimicrobials in Europe, in South Africa it responds to only the extremely toxic dimidines or acrylic dyes (Lindsay and Blagburn, 2001; Namikawa and Sunaga, 2006). From this group, the most commonly used drug is diminazene, an aromatic diamidine. Although this product is registered for use (it is a stock remedy) as a single one-off treatment for babesiosis in dogs, horses and cattle, it is known to be highly toxic, specifically to the dog, and causes signs of poisoning which are evident clinically as behavioural changes such as depression and stupor, imbalance, spasticity, nystagmus and coma. Some animals show clinical signs shortly after administration, while others tend to develop clinical signs after a few days, during which time the animal appears to recover from the babesiosis.

Unfortunately there is no redress once an animal shows clinical signs of toxicity. Interestingly, the drug appears to be toxic when used at doses above 4.2 mg/kg bodyweight as a single dose or when repeated within a period of 4–6 weeks. This is in contrast to reported doses in other countries where the drug is used at a dose of 7 mg/kg divided over 2 days (Lindsay and Blagburn, 2001). Unconfirmed reports also tend to suggest that the drug is more toxic in clinically healthy animals that do not have babesiosis, making it necessary to make a proper diagnosis. The latter is problematic as it requires the evaluation of a thin blood smear for the identification of the parasite – something many owners cannot do, despite their using the drug freely as a result of its registration as a stock remedy.

Alpha 2 agonists

A reaction to detomidine was reported in a lion immobilised with a combination of detomidine and ketamine. Following darting, the lion was followed until it had gone down into lateral recumbency. When the animal was touched by the veterinarian, it appeared to be fully awake in a few seconds, proceeded to run over the veterinarian, and then fall under deep sedation a few metres away. Reactions similar to those noted with the lion have also been recorded for cats and dogs sedated with either xylazine or medetomidine. In most cases it has been reported that animals that appeared to be completely sedated suddenly woke on administration of the local/general anaesthetic and jumped off the induction table, to only once again become sedated. Although not completely explainable, it would appear that high levels of adrenaline temporarily overcome the agent and thereby result in the rapid recovery and re-sedation (Moens, 2000).

Sulphonamides

On a feedlot during processing, calves injected with one of two potentiated sulphonamides intramuscularly into the lateral neck muscle administered concurrently with routine vaccines started demonstrating signs of severe toxicity. Within 5–10 minutes five animals went down and approximately 100 of the calves had a wide range of clinical signs, including incoordination, ataxia, twitching of the tail and head shaking; these signs varied from being mild to moderate. In total two animals died peracutely, with another two succumbing approximately 4–6 hours later, while 24 calves eventually had to be euthanised due to poor prognosis. Six animals were paralysed in the front limbs and six demonstrated

clinical signs of pain of the neck muscles at the site of injection. Some of the animals recovered following treatment with intravenous cortisone. On histopathology, the peripheral nerves of the affected muscles revealed various degrees of Wallerian degeneration characterised by micro-vacuolation of myelin sheaths, often associated with segmental swelling of axis cylinders. While the reaction may have been caused or exacerbated by the concurrent vaccines, no reoccurrence was reported at the farm on cessation of the use of the potentiated sulphonamides.

At present there is one report of potentiated sulphonamides being a cause of peripheral neuritis in cattle and chickens, in addition to their association with local intolerance, nephrotoxicity, nervous disorders, blood disorders and reaction hypersensitivity. With potentiated sulphonamides being a known cause of neurological reactions in humans, which may range from aseptic meningitis, ataxia, benign intracranial hypertension, convulsions, dizziness, drowsiness, fatigue, headache, insomnia, mental depression, peripheral or optic neuropathies to psychoses, this reaction appears to be purely related to the use of potentiated sulphonamide drugs.

Morphine in dogs

Two cases of extreme excitation were reported in dogs that presented at a specialist hospital in the country. According to the reports, both dogs showed signs of hyperexcitability on intravenous morphine administration and attacked staff at the hospital. Both animals had to be restrained with a catching pole, for sedation with diazepam, which was only partially effective. The second animal required full induction with propofol prior to it calming. On recovery the dog once again started showing signs of hyperexcitation, which only subsided immediately on administration of naloxone. It was confirmed that different batches of drugs were used in the two animals.

Ascending polyradiculoneuritis in dogs

Ascending polyradiculoneuritis or Coon-hound syndrome has infrequently been reported around the world. The disease, characterised by ascending weakness in the hind-quarters, appears to be immune-mediated. A report was received for a 4-month-old puppy describing this exact presentation 1 day after being vaccinated with a rabies and a multivalent vaccine combination (distemper–parainfluenza–parvovirus–adenovirus type 2). The animal recovered following 3 weeks of anti-inflammatory/immune suppression therapy. Although the veterinarian at the time believed the reaction to have resulted from the rabies vaccination, we believe that this is most likely a distemper vaccine-related spinal cord infection (Raw *et al*., 1992; Gehring and Eggars, 2001).

Fipronil in a cat

A case of severe recurrent adverse reaction to fipronil spot-on formulation was reported in a cat. Clinical signs of hyperaesthesia characterised by repeated kicking and scratching of all parts of the body, sudden twitching and accelerated running as if trying to escape appeared within a couple of hours of administration and progressively became more severe. The same symptoms were reported to have occurred 6–7 months previously following the use of the same product. The animal completely recovered 2–3 hours later following treatment with prednisolone and mepyramine maleate.

Teratogenicity in a puppy

Following the use of doramectin (extra-label) and praziquantel for verminosis in a bitch at mating, a litter of seven puppies were all born with congenital abnormalities 3 weeks later. The one puppy that survived had signs of cleft palate, atrial septal defect and an umbilical hernia.

Future perspective

The Centre's role in veterinary drug utilisation will hopefully develop further. Currently it is envisioned that the Centre will start monitoring compliance with the withdrawal periods set for production animals by the Department of Health. The role of the Centre in the monitoring of antimicrobial resistance is also being discussed.

Conclusion

The reporting of adverse drug reactions in South African is a fairly new field. Nonetheless the response from both the veterinary profession and the pharmaceutical industry remains poor. Until such time that the pharmaceutical industry and veterinary profession begin taking full responsibility for adverse drug reaction monitoring and reporting within our boundaries, drug use in South African cannot be considered to be prudent.

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Appendix: Advice about reporting suspected adverse reactions

This form should be completed whenever a suspected adverse reaction is observed during the use of a veterinary medicinal product in:

- animals (including birds and fish);
- incidents involving humans.

Please complete the form in BLOCK LETTERS and send it to the Section of Pharmacology and Toxicology, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Ondertepoort, 0110, or fax to (012) 529 8304. Reports may be electronically submitted to vinny.naidoo@up.ac.za.

For further information please write to the above address or telephone (012) 529 8239.

What to report

- Suspected adverse reactions to registered veterinary medicines, stock remedies and vaccines.
- Suspected adverse reactions to medicines used extra-labelly in animals.
- Suspected adverse reactions to herbal, homeopathic or other alternative remedies.
- Suspected lack of efficacy of a product.
- Suspected lack of efficacy of a vaccine.
- Misuse of products.

Report even if:

- You are not certain that the product has caused the event.
- You don't have all the details.

We are particularly interested in:

- Adverse reactions to recently marketed products.
- Serious adverse reactions and interactions with all products.
- Adverse reactions that are not clearly reflected in the package insert.

Confidentiality

- Identities of the reporters, client and patient will remain strictly confidential.
- The report does not constitute an admission that the veterinarian or the product caused or contributed to the event.

Veterinary Medicines – Report of Suspected Adverse Reactions

Section One: Reporter Details

Name and Address of Reporter:

_____
_____
_____

Code: _____ Tel: (_____) _____

Name and Address of Veterinarian involved or, in the case of a human suspected adverse reaction, the doctor involved:

_____
_____
_____

Code: _____ Qualifications:_____

Section Two: Animal Details

No. of animals treated: _____ No. of animals reacting: _____ No. of deaths: _____

Species	Breed	Sex (M/F)	Age	Weight	Pregnant (Y/N)	Neutered (Y/N)

Section Three: Medicine Details

Please list all veterinary medicines, stock remedies and vaccines administered. Indicate the product suspected by writing next to the trade name.

Trade Name	Batch No.	Actual amount administered	Route	Date started	Date stopped	Reason for use

Product Administered
By Veterinarian ☐ Owner ☐ Paraveterinary professional ☐ Other ☐

Has the product registration holder been informed? Yes ☐ No ☐

Veterinary Medicines – Report of Suspected Adverse Reactions (*continued*)

Section Four: Adverse Event Details

Date of onset: _____ Duration of adverse event: _____
Description of event or problem (include relevant diagnostic test? Post mortem results)

If you need to continue on a separate sheet of paper please attach and tick this box ☐

Are there any results to follow? Yes ☐ No ☐

Section Five: Adverse Event Outcome

☐ Died ☐ Recovered Event reappeared on rechallenge ☐
☐ Euthanised ☐ Ongoing Yes ☐
☐ Congenital anomaly ☐ Other: . . . . . No ☐
☐ Intervention required to prevent permanent impairment

Treatment given, if any _____

Were there any sequelae? Yes ☐ No ☐

If yes, please describe sequelae: _____

Section Six: Adverse Reactions in Humans

Name/Initials	Sex	Age	Date of reaction	Nature of reaction

9 Pharmacovigilance inspections in the European Union

K.N. Woodward and C.W. Evans

Introduction

Prior to 2001, pharmacovigilance inspections in the EU for human medicines had been somewhat of a rarity and, indeed, general compliance among the EU regulatory authorities was at best patchy (Koster *et al*., 2000; Bleumink *et al*., 2001). The same was almost certainly true in the veterinary sector, and the limited information available suggests that the situation here was even more 'relaxed'.

In 2004, and as discussed in Chapter 2, both the veterinary and human pharmaceuticals legislation was significantly updated in the EU. Directive 2001/82/EC as amended by Directive 2004/28/EC strengthened the requirements for the provision of inspections. Article 80.1 of this Directive states that:

> 'The competent authorities of the member state concerned shall ensure, by means of repeated inspections and, if necessary, unannounced inspections . . . that the legal requirements relating to veterinary medicinal products are complied with.'

Clearly, this is a very broad and sweeping requirement which encompasses other aspects of the veterinary legislation and Article 80.1(d) is more specific, requiring the inspection of

> '. . . premises, records and documents of marketing authorisation holders or any firms performing the activities described in Title VII (of the Directive) and in particular Articles 74 and 75 thereof, on behalf of a marketing authorisation holder'.

Title VII is the section of the Directive that deals with pharmacovigilance, while Article 74 sets out the role of the Qualified Person for pharmacovigilance and Article 75 the role of the marketing authorisation holder in reporting activities, including electronic reporting. Thus, Article 80 introduces, inter alia, a specific requirement for EU regulatory authorities to conduct veterinary pharmacovigilance inspections.

Articles 72 and 73 of the amended Directive require the EU member states to put in place reporting systems and to encourage adverse reaction reporting. Thus, together, the Directive places the onus for pharmacovigilance, and for pharmacovigilance inspections, on the competent authorities of the member states. On the other hand, Regulation (EC) No. 726/2004, under Article 57(1)(c), makes it clear that while the European

Medicines Agency (EMEA) may be responsible for several aspects of veterinary (and human) pharmacovigilance, it is also clearly responsible for the monitoring of pharmacovigilance activities, including activities conducted by the EU competent authorities (i.e. the EU veterinary regulatory authorities). Moreover, Article 44(1) sets out the requirements for competent authorities to ensure that marketing authorisation holders comply with the requirements laid out in Directive 2001/82/EC, including pharmacovigilance requirements (Title VII). Finally, Article 84 of the Regulation permits EU member states to effect and impose penalties on marketing authorisation holders for infringement of requirements, including pharmacovigilance requirements that 'shall be effective, proportionate and dissuasive'.

Quite clearly, the EU veterinary pharmaceuticals legislation not only makes provision for pharmacovigilance activities but also introduces requirements for monitoring, implementation and enforcement, including the introduction of pharmacovigilance inspections to be conducted by the competent authorities of EU member states.

There has certainly been an increase in pharmacovigilance inspection activities with respect to human pharmaceuticals in recent years, and particularly since the review published in 2004 mentioned earlier in this Introduction (Koster and van den Oetelaar, 2005). Until recently, this was not true for the veterinary sector. However, the evidence, including personal experience, suggests that this is now changing, and at the time of writing in late 2008, a number of animal health companies based in the EU have been subjected to pharmacovigilance inspections, with the British and German authorities being particularly active in this regard. The answer to the question 'European legislation: has it led to implementation of pharmacovigilance inspections?', the title of the paper by Bleumink and colleagues in 2001 referred to previously, was 'maybe' or even 'not directly'. In the light of the revised human and veterinary pharmaceutical legislation and the guidance provided by the European Commission (European Commission, 2007) which will eventually become a part of Volume 9B, the answer now is a clear 'yes'. Indeed, it has not merely led to the introduction of inspections, it has demanded them.

The purpose of pharmacovigilance inspections

As discussed, pharmacovigilance inspections and audits are a new phenomenon for veterinary medicines. However, the introduction of pharmacovigilance inspections has been accompanied by a change in emphasis on the importance of the renewal procedure for marketing authorisations. The responsibility for monitoring the safety and efficacy of veterinary medicinal products has been placed firmly on the shoulders of the marketing authorisation holders. Consequently they need to have effective means for the post-marketing surveillance of their marketing authorisations.

The inspection process is a way for competent authorities to ensure that marketing authorisation holders both are aware of their responsibilities and obligations and observe these appropriately.

Preparing for pharmacovigilance inspections

Having accepted that pharmacovigilance inspections, at least in the EU, are a reality, one clearly needs to know what to expect. This is particularly true if the 'one' in question is the Qualified Person for Pharmacovigilance, but it is also true for others working in pharmacovigilance elsewhere in the same company. Of course, dealing with pharmacovigilance inspections is easy – you only need to comply with the letter and spirit of the legislation. Nothing else is required – is it? Well, how sure are you that you comply? You may collect spontaneous report data on adverse reactions, but is the information stored appropriately? Are serious, adverse reactions reported in an

expedited manner as required, are all adverse events recorded, is the electronic reporting system compliant, does the Qualified Person reside in the EU, and so on? How can what is actually being done in practice be reconciled with what ought to be done under the requirements of EU legislation?

Perhaps the first response to such questions involves ensuring that EU pharmacovigilance guidelines, and through these EU pharmaceutical legislation, are being complied with. There is plenty of guidance, and guidance in a form that can be incorporated into standard operating procedures (SOPs), to assist in the compliance programme. This is particularly true of Volume 9B of the Rules Governing Medicinal Products in the European Union, *Guidelines on Pharmacovigilance for Medicinal Products for Veterinary Use*. While available only in draft form (EMEA/CVMP/PhVWP/430286/2007 – draft 13) at the time of writing, draft 14 (November 2008) was recently released for consultation (see Chapter 2), and there is extensive guidance provided on compliance with pharmacovigilance requirements. This document provides extensive advice to marketing authorisation holders and competent authorities on the conduct of inspections, the targets of such inspections and what is generally expected. Specifically, the guideline focuses on a number of key areas relevant to implementation of pharmacovigilance aspects of the veterinary legislation, in addition to providing useful background information and describing the legislative basis for the requirements. One of the first requirements of the pharmacovigilance legislation is the need for the marketing authorisation holders to have a pharmacovigilance system. This system, and how it operates, forms the basis of pharmacovigilance inspections by the competent authorities.

Detailing the pharmacovigilance system

There is now a requirement for marketing authorisation holders to provide details of this system in their marketing authorisation application. This should include the reporting system itself, the computer and databases used, the role of overseas subsidiaries, the duties of the Qualified Person and all other functions related to the routine operation of pharmacovigilance and adverse reaction reporting (Borner *et al*., 2006). The guideline on monitoring for compliance, as mentioned above, initially focuses on the detailed description of the pharmacovigilance system in place, or proposed, at the time of the marketing authorisation application, and specific attention is given to the following:

- Proof of services of a Qualified Person for Pharmacovigilance as required by Article 12(3) of the amended Directive, and of the necessary means to notify adverse reactions. The applicant should provide:
  - a statement signed by the marketing authorisation holder and the Qualified Person, to say that they have the services of the Qualified Person and suitable means for notification of adverse reactions;
  - the name of the Qualified Person, located in the European Economic area (EEA), with address and contact details and a summary of their curriculum vitae;
  - a summary of the Qualified Person's job description;
  - a description of the back-up procedure to apply in the absence of the Qualified Person.
- Detailed description of the pharmacovigilance system. The applicant should provide the following details:
  - There should be a description of the company and its organisation, location and sites of databases. The sites of data entry for pharmacovigilance information must be specified along with high-level organisational charts and brief descriptions of activities carried out by the organisations and sub-organisations involved.
  - Flow diagrams should demonstrate the way in which pharmacovigilance data are moved around within the organisation and how reports are processed from the

initial source/reporter to the point of receipt by the competent authorities.

- Evidence that clear written procedures or SOPs exist to include:
  - the activities of the Qualified Person and the back-up procedure to apply in his or her absence;
  - collection, processing (data entry, coding), assessment (classification, veterinary review) and reporting of adverse reactions;
  - handling of third country reports;
  - follow-up of missing or further information;
  - detection of duplicate reports;
  - expedited reporting;
  - electronic reporting;
  - preparation of periodic safety update reports (PSURs), including a full safety evaluation, and their submission;
  - global pharmacovigilance activities, including signal detection issues, benefit:risk review, reporting to competent authorities and communication of changes to the benefit:risk assessment to health professionals and others;
  - responding to requests from competent authorities;
  - handling of safety variations;
  - handling of commitments made to authorities with respect to pharmacovigilance requirements;
  - management and use of databases;
  - internal audit of the pharmacovigilance system;
  - pharmacovigilance training;
  - archiving of paper and computer records.

If there are any organisations contractually involved in pharmacovigilance activities (e.g. a third-party supplier of the medicinal product, an external Qualified Person or a computer contractor involved in dealing with databases), then these too should be outlined. All co-licencing agreements with other animal health and related companies should be described. Staff involved in pharmacovigilance activities must be adequately trained, but in addition their training should be recorded and documented so that this can be provided to the authorities. There should also be documentation of all the procedures used, the quality management systems introduced and currently in place and any further supporting documentation that might be relevant.

Compliance monitoring by EU competent authorities

The guideline goes on to outline what the competent authorities should do to monitor the marketing authorisation holder's compliance with their pharmacovigilance obligations. The competent authorities need to check the marketing authorisation holder's compliance, from the initial assessment of the pharmacovigilance system in the marketing authorisation application dossier to the noting of their pharmacovigilance practice with regard to expedited reports and PSURs. The guideline outlines the activities to be monitored, which include:

- checking that the Qualified Person's contact details, including out-of-hours contact, are correct;
- monitoring the expedited suspected adverse reaction reports for content and timeliness;
- checking PSURs for under-reporting, for example, to see that all serious incidents were originally reported within 15 days.

PSURs are a very important mechanism for marketing authorisation holders to report on the safety profile of their products. There is a section of the guideline dedicated to the submission of PSURs, and indeed to their non-submission. Areas of non-compliance include poor-quality PSURs, failure to comply with the requirements of Volume 9B, unauthorised changes to the summary of product characteristics and ignoring previous requests from competent authorities.

The guideline also extends to providing advice on responding (or not responding) to pharmacovigilance commitments imposed by the Committee for Medicinal Products for Veterinary Use (CVMP) during consideration of products authorised through the centralised procedure. Here, the following will be regarded as non-compliance:

- failure to supply the requested data;
- submission of data after the agreed deadline (without prior approval);
- failure to implement a specific obligation;
- failure to implement a specific follow-up measure;
- poor quality of report requested as a follow-up measure;
- poor quality of a report requested as a specific obligation;
- failure to implement an urgent provisional measure.

The conduct and reporting of post-marketing surveillance studies and failure to comply with EU requirements are also dealt with at this point.

Before moving on to details of the pharmacovigilance inspection itself, it is perhaps helpful to look at the other preparations a marketing authorisation holder should consider before an inspection takes place. How do you ensure compliance in your pharmacovigilance activities? A clue appears in Section 3.3 of the guideline (detailed description of the pharmacovigilance system) under (c) *Procedures in place which are documented in writing*, with a simple bullet point and no further explanation – *Internal audit of the pharmacovigilance system*. Done properly and effectively, an internal audit is the single most important tool in ensuring regulatory compliance – and preventing embarrassing and damaging findings as a result of an inspection that a marketing authorisation holder can put into place. Combined with the observance of good pharmacovigilance practices and data quality management, internal pharmacovigilance audits should ensure regulatory compliance.

Internal pharmacovigilance audits

Pharmacovigilance systems, associated activities and procedures, and the staff involved in using all of these, together form a complex web of interacting subjects. This raises the question of exactly what should be audited in order to determine the functioning of the whole. What precisely is, or should be, the scope of an internal pharmacovigilance audit? There is very little written on this issue. More precisely, there is very little written in the open literature, but each company must have this documentation in place. Fortunately, the British Association for Research Quality Assurance (BARQA) has addressed this in one of its guidance publications (BARQA, 2006a). This section relies heavily on the contents of this document.

In reality, it is difficult to define the full scope of a pharmacovigilance audit. Each audit will depend on the structure of the company in question and how it arranges its pharmacovigilance activities – and its audits. BARQA makes recommendations on the possible scope of a pharmacovigilance audit but recognises that these cover the more critical aspects and that other issues may be included at the discretion of the auditors. In other words, the list is not exhaustive and auditors should consider what other activities, processes and documentation may need to be included. The key points, amended where necessary for the animal health industry and for veterinary pharmacovigilance in particular, are shown in *Table 9.1* (see also Luker, 2007).

Prior to an internal pharmacovigilance audit (or any other audit for that matter), those to be audited must plan ahead and be prepared for the process. Auditors are likely to ask in advance for critical documentation, including relevant SOPs, organisational charts, internal guidelines, PSURs, computer validation data and, indeed, almost any other piece of documentation dealing with one or other aspect of the pharmacovigilance system or systems and regulatory procedures and guidelines. An audit may range from being product specific or process specific or it may

Table 9.1 Critical issues, processes and documentation for inclusion within the scope of an internal veterinary pharmacovigilance audit. (Adapted from BARQA, 2006a.)

Area	*Comments*
Collection and evaluation of pharmacovigilance data from veterinary clinical trials	Reporting as per internal SOPs, evaluation, dissemination to other investigators and, where appropriate, reporting to regulators, depending on local regulations (McPhee and Reimers, 2006)
Reconciliation of spontaneous report data received from each operating territory, regulatory authorities, technical services, sales representatives, third party companies and related sources	Receipt verification between operations in different countries or regions, and between different groups involved. Avoidance and handling of duplicate reports if they occur
Collection, dissemination and evaluation of spontaneous adverse reaction reports in affected animals and in exposed human subjects	Notification to others with a justifiable interest, e.g. technical services, company veterinarians, marketing, regulatory affairs, case processing and analysis, retention of documentation, veterinary, medical and toxicological evaluation, quality check of source data, case validation, coding (causality) and quality assurance, where appropriate
Compliance with submission timelines to regulatory authorities of expedited reports, PSURs and any data specifically requested by regulatory authorities	Ensuring that expedited reports, PSURs, requests for data and responses to other correspondence are submitted in a timely fashion and in accordance with regulatory requirements. Operation of electronic reporting systems
Collection and evaluation of data from post-marketing studies, including pharmacoepidemiological studies or further clinical trials	Any data or the results of studies demanded by a regulatory authority either because of past events or as a condition of issuing an authorisation or change to the conditions of an authorisation must be submitted in accordance with the imposed or agreed schedule or after agreement has been reached for a reasonable extension or an explanation provided as to why the information cannot be provided
Procedures for searching published literature to determine availability of safety data to support reports, PSURs and other relevant submissions	Full description should be given including databases used, frequency of searches, search strategies and related information
Maintenance of summaries of product characteristics and associated labelling in all territories where a product is marketed	Procedures, SOPs in place, timelines and timescales involved, liaison with regulatory department, manufacturing, label production, variations and other regulatory procedures for label change approval and authorisations. Provision of updated safety information to veterinary health professionals
Responding to queries from regulatory authorities, requests concerning product safety issues and related correspondence	Procedures, SOPs
The role and activities of the Qualified Person for Pharmacovigilance	Procedures, SOPs, job description, clear manifesto of responsibilities, training and duties
Pharmacovigilance management and operational structure, with delegation of responsibilities for all of those involved in the process	Organisational charts, clear description of responsibilities, line management, reporting arrangements

Table 9.1 *Continued*

Area	*Comments*
Signal detection and benefit : risk analysis	Procedures for periodic safety review of products, documentation of outcomes, dissemination of results, notification of regulators, signal detection procedures including automated procedures, SOP for benefit : risk evaluation
Risk management plans	Documentation of risk management plan for each product affected, SOPs for relevant roles, clear requirements of risk management plans, periodic updating
Production and review of PSUR line listings and other PSUR information such as benefit : risk evaluation	Accuracy, identification of anomalies including potential duplicates, case omissions, causality, numbers affected
Pharmacovigilance activities and responsibilities with third party supplier, co-promotion partners and licensees	Contracts, SOPs, warranties, reporting arrangements
Filing and archiving of pharmacovigilance information	Arrangements, SOPs, fire protection, compliance (e.g. GLP archive)
Validation, operation and functioning of computer systems, databases and computer hardware involved in pharmacovigilance activities. Maintaining the description of the pharmacovigilance system as it evolves/changes	All validation documentation, SOPs, procedures, reports, and results of validation testing and frequency of repeat or routine testing, automated functions, data migration, breakdowns of hardware, system security
Ensuring adequate training of those involved in pharmacovigilance systems, including training required when systems or processes change	SOPs for training, training records, internal vs external training, methodologies to identify training requirements
Procedures for dealing with crisis issues	Crisis management plan, SOPs, personnel and procedures involved, composition of crisis management group or committee
Disaster planning and business continuity procedures	Contents of plan as a) relevant to pharmacovigilance specifically, and b) the company generally, amendments to the plan – past and future, pharmacovigilance reactions in the event of a disaster e.g. a fire, explosion, on site air crash

cover the entire operation at a country or ultimately a regional or global level. Whatever, an agenda should be obtained from the auditors and the appropriate personnel should be available for the duration of the procedure. In the EU, the Qualified Person for Pharmacovigilance or the deputy should be available except in the most exceptional of circumstances, which usually means (serious) sickness or death! Vacations are not a suitable or acceptable excuse for absence, although most auditors will agree a mutually acceptable time with those to be audited.

The end result of this exercise is the audit report itself which, in addition to noting all that is well, must, if it is to have any real value, emphasise all that is out of compliance, especially that which is not functioning or is malfunctioning. The follow-up to the audit and the emergence of the report is as important as the audit itself. Remedial actions, shortcomings and outright failures will

be noted in the report, almost certainly with deadlines for their resolution, and it is clearly vital, if regulatory compliance is to be maintained, that while realistic deadlines should always be considered, serious failings will need urgent attention. In the event of such findings, a follow-up audit is highly likely to ensure that remedies have been introduced, and that the remedies are working. Many companies may consider disciplinary measures in the event of serious shortcomings, and notably in those affecting regulatory compliance, particularly in the event of repeat findings at subsequent audits. This is perhaps not surprising when issues of lack of trust by regulatory authorities, coupled with enforcement activities, are taken into account.

One of the most important aspects of maintaining pharmacovigilance data in such a way as to protect its integrity as well as its physical well-being is maintaining data quality management (Lindquist, 2004). This should be seen as a cyclical process involving data entry, storage and maintenance, retrieval and analysis. The pharmacovigilance data should be regarded as assets with a positive value rather than as a nuisance with only negative ramifications. If the former view is taken, it will not only contribute to the maintenance of data integrity and the positive use of the information, but also reinforce a positive attitude towards pharmacovigilance and its associated tasks and procedures, and ensure that both pharmacovigilance audits and inspections have successful outcomes.

Conducting pharmacovigilance inspections

As mentioned previously, pharmacovigilance inspections are a way for competent authorities to check that marketing authorisation holders are aware of their responsibilities and obligations, and that they are taking these seriously. The guideline is quick to point out that usually the inspecting authority will be the competent authority, usually the veterinary regulatory authority, of the member state in which the facility operates. However, under some circumstances, the CVMP rapporteur or co-rapporteur may form part of the inspection team. Alternatively, a representative of the reference member state for products to be considered through or already authorised under the mutual recognition or decentralised procedures may be included. A number of types of inspections are foreseen by the document:

- Routine inspections – these may be repeated at regular intervals and probably will form part of an inspection schedule (e.g. every 3 or 4 years). Hence, they are likely to be announced and thus expected by the company in question. Alternatively, they may be at the request of a body such as the CVMP (for example when considering for the first time a marketing authorisation application from a company that has not been previously inspected).
- Targeted inspections – these may occur, as described above, when a marketing authorisation holder has not been previously inspected, when the company has been involved in a merger or acquisition or if the company has changed its pharmacovigilance system or systems. In addition, the guideline makes it clear that a number of triggers may instigate an inspection. These include:
  - delays or failure in complying with obligations and commitments;
  - delays in expedited or periodic reporting;
  - poor quality or incomplete PSURs;
  - inconsistencies between source data and those that appear in reports;
  - changes in the benefit:risk balance;
  - previous experiences during inspections;
  - information received from other regulatory authorities;
  - poor follow-up on requests for information;
  - product withdrawal with inadequate advance warning to European regulatory authorities.

Inspections may be product specific or system specific and could involve inspection of contractors and others involved in the company's

operations. Furthermore, inspections may be carried out in facilities in third countries, not merely in the country or countries involved in EU or EEA operations. The majority of inspections are likely to be announced, that is, arranged to suit the convenience of both parties, with adequate warning being given of when the inspection is likely to occur. However, the authorities have the powers to conduct unannounced inspections, although these will usually be restricted to situations where there are serious failings in compliance such as delays in submission of spontaneous reports, major inaccuracies in PSURs or other safety concerns which arise and which give rise to suspicions that a company's pharmacovigilance system leaves something to be desired.

The actual procedures for veterinary pharmacovigilance inspections may vary from one competent authority to another as they have not yet been agreed within Europe. However, most inspectorates will work to their own standard operating procedures. When the procedures have been discussed and agreed by the pharmacovigilance inspectors and the CVMP's Pharmacovigilance Working Party, they will be adopted and published.

The following guidance on pharmacovigilance inspections is based on some of the experiences of marketing authorisation holders and competent authorities to date.

The inspections usually have a duration of between 1 and 3 days, although this can be longer depending on findings, and are usually conducted with one to two members of the competent authority in question. They can focus on any aspect or the majority of aspects of a company's pharmacovigilance operations, and the limited experience up to this point suggests that the latter is likely to be the pattern for future activities.

Inspections generally involve interviews with key personnel, reviews of documentation, demonstration and examination of databases and the pharmacovigilance system in general, demonstration of search tools, signal generation systems and report production, and tours of relevant locations including the pharmacovigilance offices, archiving and computer facilities. Inspectors may provide an inspection plan or request completion of a pre-inspection questionnaire. These plans, questionnaires and the inspection itself may cover a variety of areas including (but not limited to) the following:

- an overview of the company staff structure and organisation, with clear delineation of responsibilities and management. An organogram is usually required. Also the marketing authorisation holder's activities including R&D, regulatory and administrative, and those of its subsidiaries;
- confirmation of the marketing authorisation holder's contact details, including 24-hour availability for reporting of adverse events;
- in the EU, confirmation of the availability and contact details for the Qualified Person for Pharmacovigilance (QPPV) and if relevant the local QPPV, and the back-up procedures in his or her absence. Job descriptions and curriculum vitae for personnel involved in pharmacovigilance may be requested, but are already included within the marketing authorisation application;
- details of all veterinary medicinal products registered and marketed in the territory subject to the inspection;
- details of the pharmacovigilance system with information on data collection, data processing, use of the database, retrieval of data, and follow-up procedures for individual adverse event reports. Also a record of (timely) submission of expedited adverse event reports and PSURs;
- information on the role of the sales force, technical advisors, marketing in pharmacovigilance and provision of advice to clients on safety issues;
- preparation of PSURs and submission procedures. In the EU, this can be extended to details for products registered nationally, those authorised through the mutual recognition or decentralised procedures, and those authorised through the centralised procedure. Information may be required on interactions with the various regulatory agencies involved;

- literature search procedures employed and databases involved; also the search strategies employed;
- signal generation and trend monitoring;
- training schemes in place including training in aspects of pharmacovigilance, regulatory requirements, database operation and routine use and availability of training records;
- validation details for the computer system and associated software, plus details of global systems if relevant, and responsibilities for routine maintenance, updating and repair;
- archiving of data, reports, PSURs and processes used for their retrieval;
- review of local and global SOPs used in pharmacovigilance activities and procedures in place for their generation, review and revision;
- pharmacovigilance arrangements in place for co-marketed products, licencing agreements and third-party products;
- interaction between product quality and pharmacovigilance including product recall procedures, where appropriate;
- systems in place for notification of safety issues to competent authorities and veterinary health care professionals, etc., when appropriate;
- crisis management procedures including disaster planning and business continuity procedures where these impact on pharmacovigilance activities.

Under some circumstances, the following may also be reviewed:

- reports from internal audits and from previous inspections, including reports of inspections in other territories conducted by other regulatory authorities;
- quality control procedures;
- information flow in those parts of the organisation responsible for various aspects of pharmacovigilance and other statistics associated with regulatory timelines;
- demonstration of the involvement of a safety committee with a description of its membership, functions, responsibilities and operation;
- systems schematics, workflow, server, external links and overall database operation;
- quality management systems and their operation and management.

In view of the scope of pharmacovigilance inspections, careful preparation is critical. Key documentation should be gathered in advance and personnel briefed clearly on what is expected and what they might be expected to be asked by the inspectors. Training records, job descriptions, reporting diagrams and organograms, SOPs, working practices and a description of the pharmacovigilance systems should be collated so that these are readily available. Where necessary, they should be updated and otherwise amended although, strictly, this should be unnecessary as this type of routine maintenance should be conducted on a rolling and continuous basis. Nevertheless, if required, it should be done *before* the inspection and certainly in response to any pre-inspection questionnaire. An overview presentation describing the company, its operation and its pharmacovigilance activities should be prepared as a useful introductory measure to provide to the inspectors.

The key points in any inspection are honesty and integrity – answering inspectors' questions as fully as possible, supplying information when asked and providing any demonstration requested where possible. Inspectors will almost certainly request a demonstration of the operation of the database and this may involve data input, retrieval or manipulation. Remaining polite and helpful at all times is also critical; evasiveness is not a successful strategy. Where necessary, refer to source material – it is not a good idea to rely on memory when asked for crucial information, names, dates or other data. If asked to provide copies of documents then these should be made available, marked *confidential*. A record should be kept of all information provided and of all questions asked. Indeed, it is good practice to produce a report of the entire inspection to be used for future inspections or for inspection of other ter-

ritories within the company. More information on the conduct of inspections has been provided in another useful BARQA publication (BARQA, 2006b).

The inspectors will probably provide or agree to an exit meeting where their main findings will be made (see below). At this point, challenges can be made if this seems appropriate, but otherwise the outcome of the inspection, in the form of the report from the regulatory authority, must be awaited.

Post-inspection reports and possible regulatory action

Inspections are followed by the production of inspection reports which are intended to highlight the findings, good and bad, and to identify follow-up actions necessary for remedial measures to ensure regulatory compliance. Inspection findings may be shared with other EU or EEA regulatory authorities. Findings may be classified as critical, major or other (see *Table 9.2*). However, the definitions in *Table 9.2* have not yet been discussed nor agreed for veterinary pharmacovigilance, and other more appropriate ones may ultimately be used. Whatever definitions are used, the findings should be addressed in the timelines established in the report and a response made back to the inspectorate. The report itself should be forwarded to all involved in the pharmacovigilance process including senior management, quality assurance, R&D, regulatory affairs and the legal department. If there are significant findings classified as critical or non-compliant, a follow-up inspection, announced or otherwise, should be expected and anticipated. In some countries and under some circumstances, these findings may be made public and so the importance of pharmacovigilance inspections, and their ramifications for those inspected, should never be underestimated. The best strategy for any pharmacovigilance operation must always be to remain compliant and vigilant, and do not wait for the announcement of an inspection to put the house in order. That could be too little and far too late.

Penalties for failure to comply with pharmacovigilance requirements, including adverse findings following an inspection, are listed in the guideline. They may depend on the regulatory authority in question. However, and depending on the seriousness of the offence, they are graded and generally comprise:

- education – advice is provided on how to rectify faults in order to be in compliance;
- further inspection to ensure that remedial action has been taken;
- warnings – formal warnings may be issued;

Table 9.2 Possible findings from a pharmacovigilance inspection. (Note: these definitions are provisional, as the guideline only talks of non-compliance. They may differ from authority to authority; what is considered major by one may be considered critical by another, for example.)

Finding	*Definition*	*Example*
Critical	Deficiency in a pharmacovigilance system that adversely affects the health of patients or exposed humans or that represents a serious violation of applicable legislation	No appointed Qualified Person, inadequate database, failure to submit PSURs
Major	Deficiency in pharmacovigilance that could adversely affect the health of patients or exposed humans or that represents a violation of applicable legislation	Late submission of expedited spontaneous reports and PSURs, inaccurate description of the pharmacovigilance system, failure to update reporting structures within company
Other	A violation of a pharmacovigilance system that would not be expected to adversely affect the health of patients or exposed humans	Poorly stored records, inadequate archiving facilities

- naming – a list of non-compliant companies may be published;
- urgent safety restrictions – for products that are found to have serious safety issues;
- suspension of the marketing authorisation/licence/approval – where a restriction is thought to be inadequate but where the results of further research may assuage concerns;
- revocation of the marketing authorisation/licence/approval – where the safety concerns are such that a safety restriction is inadequate to address the risk and where further research is unlikely to provide any mitigating factors.

Discussion

The purpose of education is *not* solely to pass examinations. It has a wider remit. The purpose of regulatory compliance is *not* solely to pass regulatory inspections. It too has a wider remit. Thus regulatory inspections and, specifically for the purposes of this chapter, pharmacovigilance inspections should not be viewed as yet another audit, but this time by outsiders, nor should they be regarded as being a reason to put everything right until the next inspection (or internal audit) comes around with a 2- or 3-year period of relaxation before the next one intrudes. Pharmacovigilance inspections, coupled with internal pharmacovigilance audits, are there to ensure that everything really *is* as it should be and that full compliance is a permanent matter of daily routine and not a biennial peak on the otherwise flat-lining output of pharmacovigilance action, or worse, inaction. Put another way, no-one working in a pharmacovigilance environment should feel at all threatened if reception or site security calls to say that there are two inspectors from the local regulatory agency in reception asking to conduct a pharmacovigilance inspection. Many people working in the pharmacovigilance field would probably experience anything from blind panic to suicidal tendencies in this event, possibly for good reasons.

A pharmacovigilance system, for all the reasons discussed in this and other chapters, should be fit for purpose and compliant. If it is operated properly and audited accordingly then an unannounced inspection, while causing apprehension, should not induce morbidity let alone mortality, and an organisation should always be prepared for a routine inspection. For the main part, unannounced inspections will usually only be visited on those who have transgressed, either by hosting a previously unsatisfactory inspection or by giving cause for concern to the regulatory agency in some other way (e.g. poorly written and inaccurate PSURs, failure to submit spontaneous reports and PSURs in a timely manner, failure to respond to regulatory correspondence related to pharmacovigilance, biased reporting, and any of the other myriad ways to engender a lack of trust in a highly regulated environment, as mentioned previously).

In the EU, veterinary pharmacovigilance inspections are a new phenomenon. Indeed, human pharmaceutical inspections are only a recent development, with a history going back only as far as 2004 (Wright, 2006). Veterinary pharmacovigilance inspections commenced in 2006 and 2007, initially in the UK and Germany.

Conclusions

Pharmacovigilance inspections are a reality in many parts of the world. Diligence and a culture of regulatory compliance will help to ensure that inspections need not be feared. They will also provide reassurance for personnel, including senior management, that the operation meets regulatory requirements and that it provides adequate information to the company and to regulators. Internal audits provide an essential service by ensuring that shortcomings and errors are identified early on so that these can be rectified and the system can be regarded as fit for purpose. In addition to providing confidence in the pharmacovigilance system, the system itself will provide robust data on adverse reactions,

which can extend the marketing authorisation holder's information about its own products and lead to improvements in their safety and efficacy. The data can also be useful in contending with regulatory challenges or questions. It should be remembered that pharmacovigilance data are frequently useful in responding to safety-related questions from regulatory authorities either as part of routine product defence or arising from new applications or product extensions.

Pharmacovigilance data should be treated with the respect they deserve and the degree of respect depends on the successful and compliant operation of the system, and its respect for Good Pharmacovigilance Practices as espoused by several authoritative sources, including the International Federation for Animal Health (2004) and others covering human drug pharmacovigilance (Meyboom, 2000; Nelson *et al*., 2002; Brown, 2005; PDUFA III Pharmacovigilance Working Group, 2005). The aim of pharmacovigilance inspections should be for marketing authorisation holders and competent authorities to work together to make pharmacovigilance more effective.

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10 Essential elements of veterinary pharmacovigilance and the role and duties of the Qualified Person

B. Cornez

Introduction

Veterinary pharmacovigilance in the EU is an important but complex amalgam of tasks and responsibilities which to a certain extent even exceed the requirements and scope of human pharmacovigilance requirements. It has evolved over recent years from a niche area in the regulatory environment into one of the core areas. However, even within the regulatory community it is often one of the least understood disciplines of the regulatory framework, and is restricted to a limited group of experts. It is often considered as just an additional legal obligation to be fulfilled and it is thus forgotten that pharmacovigilance finds its roots in the will and desire to provide high-quality products and services, ensuring the safety and the efficacy of veterinary medicinal products (VMPs) throughout the entire supply and use chain.

After the recent reform of the EU's veterinary pharmaceutical legislation, which included a major reduction in the frequency of renewal procedures, pharmacovigilance has become the major control mechanism available to regulatory authorities to monitor products after their market introduction. Last but not least, it fits well with the demands from today's society for maximum safety for any human activity. In this sense it may provide companies with a powerful tool to enhance the trust of the community in veterinary medicines.

In spite of this increased importance it often remains difficult to integrate the pharmacovigilance activities in the structure of regulatory authorities and industry. This is partially caused by the fact that the core activities of pharmacovigilance are situated beyond the classical milestone on which most regulatory actions are focused: the initial granting of the marketing authorisation for the product.

While its importance is now more generally recognised and the process is organised centrally, the fact that it has evolved from initiatives by some of the more motivated EU member states, sometimes even driven by a single individual within a regulatory authority, and depending on several factors such as product type and geographical location, the practical working of pharmacovigilance systems in companies and in regulatory authorities alike can differ fundamentally. One common factor, however, remains the same in all circumstances: the requirement for a company to have access to the services of a

Qualified Person for Pharmacovigilance (QPPV). The full responsibilities for pharmacovigilance within the company fall on this individual.

In this chapter we will describe in a broad sense, but with a degree of pragmatism, all of the aspects of veterinary pharmacovigilance at a company level, including what these tasks are and how they can and should be achieved by the QPPV. Apart from this, attention will be given to some of the more practical aspects or even spin-off tasks which the QPPV will have to focus on when performing his or her duties.

General principles of veterinary pharmacovigilance in the EU

In the EU, any authorised veterinary medicinal product is subject to pharmacovigilance.

Pharmacovigilance activities come within the scope of the criteria of quality, safety and efficacy. In practice, pharmacovigilance examines the possible and undesirable effects a product can have on its exposed environment in the broadest possible sense, once it has obtained its marketing authorisation from the competent authorities and once it is introduced into the market place. In this sense it is not limited to effects on efficacy and safety in the target animal, but applies also to the possible effects it can have on non-target animals, the environment and exposed humans. Furthermore it can be used as a control on the adequacy of the approved withdrawal periods for produce from animals treated with the concerned product in circumstances where residue requirements are violated.

It should be noted that this broad approach is typical in the EU. The USA and other regions apply other, often more restricted definitions of pharmacovigilance, which is not always conducive to facilitating the establishment of global veterinary pharmacovigilance systems within multi-national companies.

The fact that any approved veterinary medicinal product is involved implies that any company or person responsible for placing such a product on the market in the EU – the marketing authorisation holder – needs to have access to the services of a QPPV, as described earlier, residing within the EU. In practice, since the description of the pharmacovigilance system including the name of the QPPV is an integral part of the marketing authorisation application, even companies that currently have no marketing authorisations, but only the intention to obtain them in the future, must have access to a QPPV and a pharmacovigilance system in place.

However, having access to the services of a QPPV does not imply that this person has to be employed by the marketing authorisation holder. It is perfectly acceptable that these obligations are contracted out to a third party. While this can be a very pragmatic solution for small-scale operations, this does not imply that all obligations can be fulfilled with a simple contract. A close follow-up of the actual performance of the contractual partner will be necessary, especially in view of the important possible impact of non-compliance with pharmacovigilance obligations on the conditions under which the concerned marketing authorisation has been granted.

Legal guidance on pharmacovigilance

Several legal texts, instruments and guidance documents are available in the EU. In fact it is the domain within the regulatory environment that has seen the most new texts develop over recent years. This is not surprising in view of the importance of pharmacovigilance and the information that can be derived from it, combined with the fact that it emerged late as a specific discipline within the veterinary regulatory field. A good knowledge of all of these documents by the QPPV is essential, so while it is not our intention to elaborate in depth on them, a brief summary cannot be avoided in the frame of this chapter.

Most legal texts and guidance documents at EU level can be found on two websites. These are the EMEA's site under the guidance section on veterinary medicines (http://www.emea.europa.

eu) and the European Commission's site where the veterinary legislation and further guidance documents are assembled (http://ec.europa.eu/enterprise/pharmaceuticals/index_en.htm). Additional data can be found on the EU's Head of Medicines Agencies website (HMA; http://www.hma.eu.) and at the VICH site (http://vichsec.org). This topic is also discussed in more detail in Chapter 2. Furthermore the majority of the national agencies have detailed information available on local requirements and related systems and these provide a wealth of material and documents that can also sometimes be used for educational purposes. The interested reader should refer to the specific website for the relevant EU national authority, e.g. the UK's Veterinary Medicines Directorate at http://www.vmd.gov.uk/.

In general, there are four major groups of texts available for use in the EU. These are the compulsory pharmaceutical legislation, the technical guidelines, the (draft) guidelines generated by the VICH process, and the *Notice to Applicants*.

Pharmaceutical legislation

The most important and relevant text is the amended version of Directive 2001/82/EC (as amended) which forms the framework for the European legislation on veterinary medicinal products. The concept of pharmacovigilance and its obligations is embedded throughout the text. Chapter VII of the Directive is even fully devoted to pharmacovigilance and how it should be performed in the EU.

Technical guidelines

Apart from the Directive which is the backbone of veterinary pharmacovigilance in the EU, the practical implementation is defined by the different relevant technical guidelines. There has been a notably high output in this area. The guidelines range from purely scientific to practical guidance, procedural rules on implementation of the system and the corresponding possible inspections, to sometimes highly technical documents, especially with respect to the set-up and implementation of the electronic reporting system as demanded by the Directive.

The VICH process

In parallel, pharmacovigilance has become one of the subjects of the harmonisation process on regulatory issues between essentially the USA, the EU and Japan, as part of the VICH project (International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Products). In view of the sometimes different interpretations of what pharmacovigilance should embrace, this process is progressing very slowly, but, for the same reasons, this is exceptionally important. Nevertheless the (draft) texts sometimes provide useful hints and indications on how pharmacovigilance may look in the future, and, furthermore, how they will eventually be adopted into national and supranational guidance (e.g. that of the EU).

Volume 9B

From a daily use point of view, probably the most useful and understandable text is Volume 9 of the *Rules Governing Medicinal Products in the European Union* (see Chapter 2). As noted in Chapter 2, this is currently being revised as Volumes 9A (pharmacovigilance of human pharmaceutical products) and Volume 9B (pharmacovigilance of veterinary products. While it is, or rather will be, a rather bulky document, it provides a reasonable transcription and interpretation of all legal aspects and guidance notes in a comprehensive and readable document. Unfortunately the new purely veterinary version, 9B, is not yet available, which means the historical version combining the veterinary and the human aspects is still valid, together with a limited document entitled *Guideline on Monitoring of Compliance with Pharmacovigilance Regulatory*

Obligations and Pharmacovigilance Inspections for Veterinary Medicinal Products. This is a to-do list for regulatory authorities to verify the compliance of an MA holder with the relevant legislation forms – a good albeit brief checklist for anyone wanting to verify 'How am I doing?'

The Qualified Person for Pharmacovigilance

Definition and profile

As indicated above, each MA holder of a veterinary product in the EU will need to have access to the services of a QPPV and he/she will be the backbone of the pharmacovigilance system. At the outset, it is fair to say that this terminology ('Qualified Person') is to be regretted. To be clear, the Qualified Person for Pharmacovigilance (QPPV) is not the same as the Qualified Person (QP) responsible for manufacturing related quality issues and controls, and hence there is scope for significant confusion. Fortunately the explanatory documents make clear this distinction and the abbreviation QPPV is used commonly.

The QPPV has been present from the beginning in the legal concept and texts on pharmacovigilance. However, the duties have been gradually increased over time and in general it can be stated that in most cases the QPPV is the centre of the company pharmacovigilance system. Unlike the QP for quality and manufacturing issues, the relevant requirements on education, skills and background of the QPPV are poorly described in the basic texts. In fact, it is limited to the following basic requirement:

> 'The marketing authorization holder shall have permanently and continuously at his disposal an appropriately qualified person responsible for pharmacovigilance.
>
> That qualified person shall reside in the Community.'
> (Article 74 of Directive 2001/82/EC as amended)

The consequence is that different member states may (and do) give their own interpretation on the abilities or qualifications of that person. While most countries, like the UK, have a very pragmatic approach – 'any person who is capable of competently performing the specified duties would meet the requirement' – some member states have a more formal approval system in place, with specific requirements with respect to educational background and experience, sometimes even linked to formal recognition (e.g. Belgium). This often goes beyond the requirements for a QPPV for human pharmacovigilance in the EU. In practice, however, it appears that the pragmatic approach prevails. Note also that it is expected that in an MA application, 'a summary Curriculum Vitae of the QPPV with key information relevant to their role (main qualifications, training and experience)' will be provided.

It must also be highlighted that in the recent version of Volume 9B of the *Rules Governing Medicinal Products in the European Union* mentioned above, it is stated that 'the competent authorities will maintain a list of QPPV's within the EEA'. This could indicate that a more formal acceptance or qualification procedure may eventually be developed.

It must also be noted that the requirement 'That qualified person shall reside in the Community' has been added in the last amendment of the Directive, and due attention must be given to this.

It is important to note that in the draft Volume 9B mentioned above, it is foreseen that at the moment of submission of an MA application a signed statement must be provided confirming that the proposed MA holder has access to the services of a QPPV.

Tasks of the QPPV

The general tasks of the QPPV are legally defined and are described in the Directive (and repeated here *in extenso* in view of the importance of the text):

'(a) the establishment and maintenance of a system which ensures that information about all suspected adverse reactions which are reported to the personnel of the company, including its representatives, is collected and collated in order to be accessible at least at one point within the Community;
(b) the preparation for the competent authorities of the reports referred to in Article 75, in such form as may be laid down by those authorities, in accordance with the guidance referred to in Article 77(1);
(c) ensuring that any request from the competent authorities for the provision of additional information necessary for the evaluation of the benefits and risks afforded by a veterinary medicinal product is answered fully and promptly, including the provision of information about the volume of sales or prescriptions of the veterinary medicinal product concerned;
(d) the provision to the competent authorities of any other information relevant to the evaluation of the benefits and risks afforded by a veterinary medicinal product, including appropriate information on post-marketing surveillance studies.'

It is also interesting in that the legal texts also list 'other tasks for the Marketing Authorisation holder' in addition to those set out for the QPPV. These include:

- follow-up and handling of (possible) duplicate reports;
- communication of information relating pharmacovigilance concerns to the general public;
- collection of specific pharmacovigilance data from targeted groups of animals.

Although these do not fall directly under the QPPV's responsibilities, it is clear that in practice they almost certainly will be handled completely or in large part by the QPPV or come under his or her direct responsibility.

Most of the tasks indicated above will be discussed in more detail later.

Position of the QPPV within the company

In most cases the QPPV will be an employee of the company, but it is possible to subcontract or outsource these pharmacovigilance obligations. This implies that the QPVV may be employed by a third party or, as seen recently, be a self-employed consultant. In most companies the pharmacovigilance services are an integral part of the Regulatory Department, which makes sense since pharmacovigilance can be considered as the last phase of the development and licencing process for a veterinary medicinal product. It is also appropriate in view of the potential impact of the system and its findings on the product licence or marketing authorisation. However, in view of these responsibilities, the QPPV's internal contacts or operational field within the company can be rather elaborate. Furthermore, the 'empowerment' he or she needs could be rather important. In order to allow for the correct execution of the QPPV's functions, his or her rights jurisdiction could be close to those normally attributed to an internal auditor. While this may sound exaggerated, one should bear in mind that the core of the QPPV's duties is to make sure that the company, the MA holder, is fulfilling its legal duties. Non-compliance may have serious consequences for the marketing authorisations, for the products concerned and, ultimately, for the MA holder itself.

The actual fulfilment of these duties is clearly not limited to and indeed is the beyond the scope of a specific person. It involves the active support of all personnel at any level in the company, including senior management. The clearest example of this need for support is the time constraint imposed by the need for expedited reporting: serious cases in animals or human cases occurring in the EU should be reported 'promptly, and in no case later than 15 calendar days from receipt'. Receipt should be understood as the time point when *any* person employed by the MA

holder becomes aware of a possible suspected adverse reaction. Fifteen calendar days is not a great deal of time, especially in larger multinational organisations where the pharmacovigilance duties are handled centrally and possibly on another continent. Failure to comply with this requirement may result in serious consequences, including financial penalties in addition to any adverse effects on the marketing authorisations for the products. This need for having access to, and the requirement for, support at all levels in the company implies that, besides a sound scientific background, organisational as well as communicative skills are almost a prerequisite for any QPPV. As we will discuss further, these skills will also be crucial for the part of the QPPV's functions that involve communications outside the organisation.

For those QPPVs operating within a company that handles both veterinary and human products it may be useful to have a good connection with their human QPPV counterpart. As discussed later, this may be useful in the reporting of adverse events of veterinary products in humans and vice versa. Furthermore, the legislative initiatives from the various regulatory authorities operating in the human medicinal product field are generally ahead of those in the veterinary field, which means valuable experience can be obtained from this source. For the sake of completeness, it should be mentioned that nowhere is it mentioned in the legal texts or in guidance documents that the QPPV for the human and the veterinary products must be a different person.

Relationships with the competent authorities

The actual relationships and contacts with the competent authorities with respect to pharmacovigilance are very important and exist under different conditions. Several different situations will occur: for example, during the submission of expedited reports and periodic safety update reports (PSURs); in the event of an inspection of the pharmacovigilance system; during an MA application and subsequent procedure; and as part of information sessions.

Under normal circumstances the contacts between the QPPV and the competent authority occur much more frequently than for other sections of the regulatory disciplines for the very simple reason that cases are a continuous phenomenon, unlike dossier submissions and registration procedures which tend to concentrate around peak moments. Reporting is often a routine, sometimes a daily or weekly procedure, and it is likely that the electronic reporting system will both formalise this and encourage it. However, feedback and follow-up are frequently important aspects of the QPPV's work, requiring direct contacts with regulatory authorities. This continuous contact, along with the need to cooperate closely during critical situations, where it may be necessary to act rapidly in order to obtain further data as well as to solve a possible clinical problem in the field, makes it important that a good relationship based on trust and openness is established between the QPPV and the regulators. As discussed under different headings, probably the best way to gain this trust is to apply a consistent policy of efficient and open reporting, complying with temporal reporting requirements where relevant. In the case of doubt as to whether an event is reportable, the best choice is to report.

A further factor is that the high interest in veterinary pharmacovigilance generated by regulatory authorities and others and the continuous output of new requirements and guidelines mean that everyone involved in veterinary pharmacovigilance is on a continuous (and sometimes steep) learning curve. This is true for both company personnel and the regulatory authorities. In these circumstances, and in any cases of doubt, it is better to consult and debate and, more importantly, achieve some degree of consensus.

As a result of the dynamic status of veterinary pharmacovigilance in the EU (and elsewhere) there are several initiatives and forums to allow industry and the regulatory authorities to engage in lively discussions on this topic. It is clearly

advisable for those involved in pharmacovigilance to participate as much as possible in these discussions and particularly to keep the shape of pharmacovigilance in the future within a workable and proportionate balance.

The good relationship and organisational skills needed for the QPPV's internal activities in the company are equally essential for a good relationship with the competent regulatory authorities.

How to design a pharmacovigilance system and make it operational

Establishing a pharmacovigilance system has already been discussed – a legal requirement for an MA holder and normally a core task of the QPPV. Obviously just having a system is not sufficient. A key task is to have a system and then to make it function in an optimal way. This means that the system should be capable of receiving and channelling all pharmacovigilance cases that may occur in the field, and be designed in an almost tailor-made fashion in view of the MA holder's specific requirements. It must be capable of meeting the reporting obligations imposed by the competent authorities. This will require a correct mix of equipment, software, procedures and human skills.

Getting support from within the organisation

The core of the duties of the company is to have and maintain a system that:

> 'ensures that information about all adverse reactions which are reported to the personnel of the MA holder, including its representatives, is collected and collated, so that it may be accessed in at least one point within the Community'.

As discussed earlier, this implies that there must exist an organic network throughout the whole organisation which is operated in such a way that cases reaching the company at any possible point will effectively reach the QPPV, who will then have access to the necessary resources to process them administratively and scientifically. The set-up and maintenance of such a system is probably the most important task of a QPPV. Databases can be purchased or custom built. Collaboration and support have to be gained.

In order to build and maintain this information chain, the QPPV will need to have the right to impose obligations on other employees and departments, frequently with people who may be on a higher echelon. The major problem the QPPV may encounter in obtaining the necessary support from within the structure is the common opinion that pharmacovigilance is not within the scope of the activities of the respective departments. For some services, especially those providing technical and representative support, it is relatively easy to obtain the necessary support. The key message here will be that pharmacovigilance is actually a tool to provide assistance to your customers when they are encountering problems with a product. Ultimately, it will ensure that there are better products on the market. However, most departments (for example, the logistics and finance departments) will perceive little benefit from pharmacovigilance and will not assess their personnel's performance in relation to how well they acted on it. While the number of cases received in these 'non-target' departments normally is limited, it is necessary to have their support, for example in obtaining sales figures to permit calculation of incidence. Any missed case may cause doubts as to the overall performance of the pharmacovigilance system of the MA holder and this may amount to a missed opportunity to help a customer.

The best and probably the only way to get the necessary help from these departments is by garnering the support from higher management. So the first target of the QPPV should be to reach as high as he/she can within the organisation. It may not always be easy to reach this level and a good approach is to try to present the information package for the relevant pharmacovigilance

issue not so much as an education, but rather as a request for approval of information before it is distributed throughout the company. A correct mix of clear advantages, such as customer assistance and possible product improvement as well as possible (adverse) impact on marketing authorisations and marketed products as a result of failure to comply, should usually be sufficient to obtain the required endorsement.

Procedures

Apart from a good network, the system will have to rely on clear procedures outlining its structure and functionality. This will not only be of assistance in the actual organisation and functioning of the pharmacovigilance system within the company, but also be a great help in the event of inspections or in preparation for the description of the pharmacovigilance system required in regulatory submissions for marketing authorisations. Therefore when drafting the pharmacovigilance procedures it is a good idea to make sure that all elements, as listed in Volume 9B and in other guidance documents, addressed in the detailed description in the MA application are actually addressed. A pragmatic approach may be to work with a master procedure, which addresses all these data without too much detail, but which refers to secondary procedures covering all the specific issues in more detail. The best approach is to keep these procedures as lean as possible by creating a specific procedure for each topic, rather than to group them. Then this master procedure could be even used as the actual description of the pharmacovigilance system presented in the MA application, which then reduces the risk of possible inconsistencies between various documents.

When writing the procedures it may be wise not to put all the necessary details in the body of the text itself. Details such as names and contact details, other than those of the QPPV, can be a problem since these are quite often subject to changes and updates. Listing these in an annex to the procedure, which is described as not being a formal part of the procedure and which can be updated whenever necessary, may eventually save much subsequent administrative work. It may be tempting to call these procedures Standard Operating Procedures (SOPs), but it should be taken into account that SOPs are normally part of the Quality system of a company and therefore subject to all related (internal) audits and external inspections of this system. Since there is no legal obligation on the exact nomenclature, it may be wise to name them differently (for example, business procedures or working instructions) and keep them as a standalone set.

Normally the procedures describe in-house tasks, so with the necessary empowerment this should not be excessively difficult. A specific issue, however, involves possible co-operations with third parties such as distributors and agents. Clearly these form an important, though often weak, part of the information chain. Experience suggests that having a specific procedure addressing this aspect and the relevant obligations of these outside partners, though as general as possible, is an effective tool and should form a standard part of any co-operation agreement.

Databases

It is the common understanding that the MA holder needs an electronic database to file the pharmacovigilance reports and data that are received. This is not entirely correct. The current legislation requires a mandatory electronic reporting system, but not the obligation to have an electronic data storage system. It can be anticipated that especially for small operations the option to keep a paper-based system could be the solution of choice.

Whatever the choice is for an electronic data filing system, the question remains: whether to build your own system or to buy a commercially available system. Making this choice will be influenced by a number of important considerations. First of all the number of commercially available systems for veterinary pharmacovigi-

lance is rather limited. In practice only two systems are commonly encountered. The acquisition and maintenance costs of these may be high, especially if the purpose is to have a system that can act globally and is accessible at multiple entry points. On the other hand, these systems are more than just a simple database and they can contribute significantly to structure and reduce the workload by preparing reports in an adequate and acceptable format, through the integration of terminology databases such as VeDDRA (Veterinary Dictionary for Drug Regulatory Activities), by automating submission of reports via the Gateway system to EudraVigilance (which will indeed reduce the workload significantly once the system is fully operational), by allowing in-depth analysis of trends via build-in statistical elements, and so on.

The decision to build your own system is normally an option if the size of the organisation and the number of reports remain limited and electronic reporting will be done via EVWEB or SERF (see the section 'Submission of expedited reports to the authorities' below) or if, on the contrary, the internal information technology resources of the company are adequate and able enough to build a system capable of meeting all of the disparate requirements necessary for a fully operational system. A professional commercial database may be the best option.

Receiving and storing data

A major problem in running a validated pharmacovigilance system is the receipt of data and especially the need to ensure that all data received are correctly recorded and stored or archived. The major problem is that the format of the original reporting from a source outside the company cannot be standardised. It will be frequently chosen by the reporter. In spite of this it is important that all the data received can be correctly registered. Normally written messages such as letters, faxes and emails should pose no problem. The main problem lies with the most common form of first reporting: by telephone.

When the case is reported directly to the QPPV or to one of his or her collaborators, there should be no problem. An easy way to be sure that the data are recorded correctly is to develop, in analogy with Good Clinical Practice principles, a standardised 'note to file' document, either on paper or electronically, allowing an immediate transcription of the oral data. The real problem lies when the first report is received outside of the pharmacovigilance department. A recurrent problem is the incomplete recording of contact data or essential data on the animals and case.

Therefore details and instructions on how to act upon such initial reports should be part of the education programme provided to non-pharmacovigilance personnel. However, it should also be taken into account when setting up the pharmacovigilance system. From experience it can be said that there are some valuable actions that can be taken in this respect. For example, it is a good idea to include in documentation given to the employees a copy of the EU reporting form which they can follow when noting down a reported case. Whenever possible it is an even better idea to make sure that the form is accessible electronically on a common web server, allowing the contacted employee to complete the form online. The same server could even have a folder acting as a 'Pharmacovigilance Post Box' where the electronic form can then be saved. Alternatively, a specific recording form can be developed for use in the company. This could consist of the EU reporting form but stripped of the secondary data fields, and supplied with some annotations and instructions on what data are essential. Whichever system is applied, clarity, accessibility and ease of use will be essential.

The actual storage of case-related data should be addressed at two levels: electronic and physical. The storage of the electronic data in the database should pose no problems. Obviously the relevant procedures should be in place to protect these electronic data, including making the necessary back-ups at regular intervals. As for the physical storage, the numbering system of the cases in the database should be used to store all

physical data consistently. In principle, storage should be done in a place with limited access since the documents can contain sensitive data and information for the company, some of which may also be subject to legislation on data privacy. If possible it is a good idea to scan each document and store it on a central server, so that the paper file is fully reproducible and accessible from an electronic server.

Case data entry

At a certain moment during the handling of a case the data received have to be entered into the electronic database, if available. A few rules of thumb should be considered. First, the sooner the data are entered after receipt the better in ensuring exactness and completeness. Second, the fewer people allowed to enter the data into the system the better in view of consistency and robustness. Traceability and consistency between the original paper documents and the electronic data are crucial.

For large-scale operations the question arises as to whether data entry should be done only at a central point within the EU or if decentralised data entry by the respective affiliates and local offices where the original case report was received is preferable. Obviously this will depend largely on the structure of the organisation itself. However, the rule 'less is better' will also apply here. (For completeness, it should be mentioned that in theory the actual data entry and handling can also be performed outside the EU. The legal texts only stipulate that the data must be accessible at a point in the EU, but they do not prescribe where the actual data entry and handling must take place.) As will be discussed in detail under the heading 'Pharmacovigilance system', in certain circumstances specific precautions should be put in place at the moment of entry of the data in order to avoid errors, especially if the company's product portfolio contains products prone to confusion due to their name, composition or use.

Privacy of the stored data

When introducing data on an adverse event into whatever database is employed, it must be appreciated that these data are subject to European legislation (Directive 95/46/EC) on the protection of individuals with regard to the processing of personal data and the free movement of such data. Reporters and/or owners should be informed that the data they are providing, including their personal details, will be entered into a database, and in principle they have the right to consult these data. They should also be informed that these details will be forwarded to the competent regulatory authorities. Some companies have general procedures on this and it may also be useful to involve the legal services of the company on how data privacy issues should be addressed. This is particularly useful when the pharmacovigilance system is initially set up.

The impact of this privacy aspect should not be underestimated. Owners may object to having their personal data registered and transferred, for whatever personal reasons. If this happens, it is of little use objecting. Instead, it may be better to propose that the reporter contacts the competent authorities directly via the available national reporting systems. That way, a brief notification can be given to the authorities on the status of the complaint, with the sensitive data rendered anonymous. By doing this, the MA holder will still have complied with his or her reporting duties.

Electronic submission of cases

The submission of pharmacovigilance data to EU regulatory authorities in an electronic form is obligatory according to the current legal texts, and the actual choice of a submission system is an important part of the pharmacovigilance system. The details and implications of the different systems available are discussed later in this chapter (see 'Choosing an electronic reporting system').

Validation of the databases and electronic systems

Specific attention should be given to the validation of the databases. In fact, this should be part of the overall validation process of the pharmacovigilance system, but in view of its particularities it needs to be given some additional attention. It cannot be assumed that the QPPV is an expert on electronic data management, and this leads to the conclusion that almost certainly assistance will be required from a specialised team or person. For commercially available databases these services can normally be obtained from the supplier. However, there are frequently differences in the level of services offered. This aspect is therefore an important factor in the final decision on which system to acquire. For in-house systems, the burden will of course fall on the company.

The degree of validation required will depend on the functionalities built into the electronic system. If the database is limited to a filing system, the validation can be limited to verification of whether data entered can be retrieved consistently and whether there is any confusion in data packages linked to the relative cases. For those systems capable of generating reports it will be necessary to verify the consistency of a number of generated reports for each available format (for example expedited reports and PSURs). Attention also needs to be given to circumstances where external datasets from systems such as VeDDRA are attached to the database to ensure that these are integrated correctly. It is an interesting question whether functionalities, such as statistical analysis, which are not directly within the legal scope of the system, should also be validated.

It may be tempting to omit aspects such as this, but in most cases these functionalities will be used anyway. Consequently, it is advisable to include them in the overall validation of the electronic system. The most complex exercise is likely to be the validation of the data transfer if a submission is made via the Gateway system to EudraVigilance, especially if this includes all partners linked to the system. This is a very complex task since at a certain point, the data package leaves the control of the submitter, making it difficult to validate the correct arrival of the data in the receiving system. Problems have been encountered at several levels, including the correspondence between data fields, the sending and receiving of acknowledgments and other functionalities. Currently, this part of the process remains cumbersome because of the current status of the central receiving database.

Validation of the pharmacovigilance system

Often the validation of a pharmacovigilance system is limited to the electronic components, as discussed above. However, it is a good idea to validate the entire handling of case procedures from the moment of entry of information on a particular case to the moment of the closure of the process, and to base this on the functionality of the procedures. A ‘dry-run’ approach is an effective way to do this. The practical set-up for a dry-run will of course be defined by the actual structure of the company and the design of the system. In principle, one should begin with an outside source reporting via a possible case entry point using a specifically designed mock-up case. The scenario can be constructed in such a way that follow-up and technical support from other departments is unavoidable. This exercise can be repeated on a regular basis, testing each time different case entry routes and different types of support and follow up. This approach often brings to light weak points. Although the practical advantages of this approach might be questioned, if these exercises are well documented they provide strong supportive evidence on the quality of the system and supportive evidence that can be employed in the event of an inspection of the pharmacovigilance system by the competent authorities.

In addition to the actual reporting chain and dataflow, other aspects of the system should be validated. For companies using commercially available specific databases, a large part will be linked with or even covered by the validation of the database. Operations not using such large systems will be obliged to assess what parts of their operations may be vulnerable to errors. A few classical issues are:

- recognition of duplicate reports; and
- confusion between different products.

Duplicate reporting can in principle occur when the veterinarian and the owner each report a case separately. However, it is normally relatively easy to distinguish between these. The most reliable approach is to make a critical review of all cases reported in a certain time span (monthly) and to cross-check and verify all cases that have several parameters in common. For expedited reports, this should be a standard check before despatching the actual report.

The main problems lie with cases that are not subject to expedited reporting which are received from regulatory authorities. Often these are reported on an annual or periodic basis and they may contain duplicates if owners or veterinarians have filed the same complaint with both the company and the authorities, without informing either party that they have done so. The only way to identify such duplicates is to systematically screen the reports for potential matches with the database. A simple check of all the data fields will not be sufficient. These duplicate reports may sometimes have different information on certain fields (owner's name, husband/wife/farm, number of animals involved, etc.), complicating the identification process. A critical review of the line listing of each PSUR can reveal such duplicated cases and this should be a standard part of the preparation process.

Confusion between different products is a particular problem for portfolios with older products which may contain a disparate list of products with different indications or compositions and active substances but with comparable and confusing names. In these circumstances the risk of errors arising while recording the correct product details are clear. These can happen at any stage before the case is introduced into a standardised system, which in practice means from the owner of the animals up to the entry point into the company. The major problem with these kinds of errors is that it is virtually impossible to resolve them retrospectively once they are in the database. The only way to prevent them is proactively. A thorough analysis of the portfolio should be made upfront to identify the possible scenarios that may lead to confusion. The best way to do this is in cooperation with the technical and sales staff who are better placed regarding what may be happening in the field. Based on that exercise, a list of critical combinations ('product × use') can be identified. On receipt of a potentially affected case, a specific double-check through the reporting chain can be made to confirm the authenticity of the reported data.

In theory, when PSURs are generated manually rather than automatically by the database, the same process can be useful: initially identify which products are prone to confusion and then implement a double check on the consistency of the data. This should in fact be a standard procedure. With PSURs it is also necessary to ensure that the correct administrative data for the products, including date of authorisation, common birth date and other relevant data are entered correctly into the system.

Description of the pharmacovigilance system

As foreseen in the new pharmaceutical legislation, the description of the pharmacovigilance system must be included in the MA application (specifically as Annex 5.20 of the application form). As already stated above, probably the best approach is to develop a master procedure for the system which contains all the elements as listed. The procedure can then be used as the description. An alternative and somewhat better idea is to embed it in or attach it to another document which explains in a more narrative way how the

pharmacovigilance system is structured and operates. The elements that need to be addressed are detailed in the relevant sections of Volume 9B.

As already discussed, it is essential to keep the description as general as possible, while still providing the essential detail. The reason for this approach is that any change to the description will be considered as a change in the documentation provided in support of the MA application. This in turn means the change will be the subject of a variation procedure, and since at this moment this change is not yet foreseen as a so-called type I variation, it will automatically be classified as the more complex (and frequently in terms of fees more expensive) type II variation. Attempts are being made to rectify this, for example by replacing the inclusion of the description of the system in the MA application by a separate procedure. Here, the description of the pharmacovigilance system is submitted separately (for example, as a type of master file) at national and/or EU level. This could then be referred on submission of an MA application. Currently, if a change occurs in a pharmacovigilance system, not only does that have to be included in all future MA applications, but also variations (see above) need to be submitted for all affected existing MAs. The major advantage of the master file approach would be that a change would be limited to a single procedure covering all the products referred to in the master file. Unfortunately, the current EU pharmaceutical legislation does not permit this pragmatic approach!

A peculiar requirement in the legislation is that in addition to a description of the system, the applicant should also provide 'where appropriate, (a description) of the risk-management system which the applicant will introduce'. Not much can be said about it at this moment since the subject is too novel and no real experience has been gathered, at least in the veterinary pharmacovigilance field. It is only possible to make extensions from observations in the human pharmacovigilance field. While there exists little experience and reports on it are minimal, it is highly interesting to consult the extensive Volume 9A of the *Rules Governing Medicinal Products in the European Union*, and specifically to Chapter 3 'Requirements for risk management systems'. This makes it evident that risk-management policies in pharmacovigilance and the tools developed for it will become a major factor in the future. This will undoubtedly be extended to veterinary pharmacovigilance as suggested in the draft of Volume 9B.

In addition to the description of the pharmacovigilance system, the submission of an MA application will also require a statement that the proposed MA holder does indeed have access to the services of a QPPV. This must be signed by the QPPV but also by a senior person in the company.

Education

As indicated above, education programmes are an essential part of a good pharmacovigilance system. The programmes in place will be a part of any inspection by a regulatory authority and will also have to be detailed in the description of the pharmacovigilance system. This makes sense since the need to have an effective chain of operations implies that an important 'education' effort is inevitable. This effort should not be limited to the company. It will involve to a certain extent the user level, since having a good system in place is of little use if the necessary data are not reaching the company and its pharmacovigilance system.

Education within the company

As discussed, one of the main problems in setting up a good pharmacovigilance system in a larger organisation is the 'not-my-business' approach that for understandable reasons may exist within several parts of a company. So a key objective will be to shape this view to an approach where pharmacovigilance is considered as a valuable tool to garner information about the company's products (information in the broadest possible environment can be obtained), to improve the

use of those products and to proactively protect the customer – in addition to being a legal obligation. Furthermore it is a valuable tool in providing professional support in the field.

A major pitfall involved in communications surrounding pharmacovigilance is the enormous amount of guidance and information that is available. It will be almost inevitable to develop in-house guidance material that describes in a concise way the essentials of pharmacovigilance and what actions should be taken in the event of a case being encountered. However, focus should be maintained on the concept that pharmacovigilance is a useful tool. A small reference booklet that is distributed amongst company personnel has been shown to be effective. This is reinforced if people are asked to sign for receipt of it. Through this, the importance of pharmacovigilance is stressed to the receiver and this will also form an important part of the documentation on the functionality of the system, and be useful to the authorities. It is perhaps even a better idea to link the distribution of such a booklet to a short informative session where the essentials are presented to a wider audience. This is particularly true if there are representatives of marketing and sales, and persons with a scientific background in that audience; 'case studies' are very well received. These could be hypothetical, but it is better to distil a few cases from the company's own databases. Key questions could be:

- Is this a case and why?
- How is it reportable?
- How would you classify it within the ABON causality assessment system?
- Regular follow-up sessions to refresh and update knowledge on the topic and some feedback on 'How are we doing?' are indispensable.

Education outside the company

Promotion of good reporting of pharmacovigilance cases outside the company should be strongly encouraged. This can be achieved indirectly by making sure that the technical and sales staff have the right tools and education to pass the message on to their customers, or directly via short presentations attached to commercial sessions given to veterinary practitioners and other clients.

When discussing pharmacovigilance issues outside of the company, special attention should be given to ensure that the right message is passed on. The primary message should always remain 'veterinary medicines are useful and good'. Pharmacovigilance should always be presented as a tool to make products and treatments even better, something that is in anyone's interest. However, any message that suggests that veterinary products are dangerous commodities that require stringent controls to avoid disasters must be avoided. In some countries the competent authorities have developed attractive and informative leaflets and brochures on pharmacovigilance and how this is organised in the respective concerned EU member states. Often they can be easily downloaded from the websites of the regulatory authorities. Including these publications in with the company's own documentation when communicating on pharmacovigilance to end users may help to convince veterinarians and the general public on its importance, and assist in removing some of the uncertainties that may continue to exist.

Inspection of the pharmacovigilance system by the competent authorities

The possibility of an inspection of the pharmacovigilance system by the regulatory authorities is a new element foreseen by the updated pharmaceutical legislation (see Chapter 9). It should be highlighted that inspections can only be made by the competent authorities of the member state where the MA holder is located. However, authorities of other EU countries may request an inspection by the authorities of the member state where the MA holder is actually located.

Until now only a few EU member states have proceeded with effective inspections. These are mainly those member states that historically already have a good pharmacovigilance culture

and structure in place. There are indications that several other member states will start to inspect in the near future. It is also clear that the authorities themselves are going through a learning curve and it appears that the inspections will tend to be focusing on guidance and learning. A specific complication in some member states is that the inspections are performed by persons experienced in inspections in the human pharmacovigilance field. This can sometimes make it difficult to have the practical situation in the veterinary world taken into account. Normally the inspections are rather intensive and may involve several inspectors or multiple day procedures. In most cases MA holders were given prior notification of forthcoming inspections and these announcements are often accompanied by a list of what information will have to be provided during the course of the inspection.

Clearly an inspection should not be an issue if a few major points are taken into account. The basic instructions as detailed in the law and the guidance document should be complied with and adhered to. Accessibility to and transparency of the data and pharmacovigilance system are paramount. It must be possible for an outside person to understand, in the limited time available, how the system is functioning. Again, good procedures and even brief narrative summaries that can be distributed to the employees (as discussed above) are very helpful. Performing a self-inspection at regular intervals is very helpful and, indeed, could be written into the system procedures. This is particularly useful in those cases where the pharmacovigilance system is decentralised to local affiliates. Such self-inspections could be conducted by the QPPV or by a local consultant, specialised in veterinary pharmacovigilance and perhaps better acquainted with the local expectations of the competent authorities.

Languages to be used in pharmacovigilance

The EU currently recognises 21 official languages. This fact alone illustrates the complex operating environment for veterinary regulatory issues including pharmacovigilance. Guidelines foresee that reporting for national licences should be done in the national language, unless the local authorities are willing to receive reports in another EU language. Obviously a report is an important aspect of the communication with authorities, so consideration should be given, especially for those organisations that have local affiliates, whether the correspondence and reporting should be in the national language or not. Local companies can more easily handle their reporting in the local language. Note that versions of the EU reporting forms are available for any country in the local languages, both in PDF and Word format, on the EMEA website.

However, it can be argued that the final position for multinational operations should always be that the EU accepted language for pharmacovigilance will be English. This is definitely the case for electronic reporting in the central system through EudraVigilance. While reporting in the national languages may improve contacts with the local competent authorities, it should be borne in mind that this may be cumbersome and even impractical for storing and processing the reports in centrally organised systems. One should also take account of the fact that the general commercially available databases and lists of terms such as VeDDRA exist in English only. So the risk of introducing errors by having data in different languages in the system is real. Translation remains possible, but the classical exclamation '*traduttore traditore*'[1] is also valid in veterinary pharmacovigilance. In conclusion, the general recommendation for pharmacovigilance is to stay with English whenever possible!

Handling of veterinary pharmacovigilance cases and data

The current section will discuss the practical handling of data and cases including expedited reporting. PSUR preparation and submission will be addressed under a separate heading.

[1] 'Translator, you're a traitor.'

Specificity of veterinary pharmacovigilance cases

A general consideration needs to be made of the specificity of veterinary pharmacovigilance cases. As discussed, veterinary pharmacovigilance, and especially its legislative organisation in the EU, is largely based upon human medicinal product pharmacovigilance. That is, the two sets of legislation are parallel. This may initially seem logical, but there is an important difference to bear in mind. In human pharmacovigilance, the reported adverse reaction may be initiated by the patient; the patient may complain about one or more signs and symptoms that indicate an adverse reaction (pain, loss of feeling, blurred vision, nausea, etc.). Clearly, this does not occur in the veterinary field. Reported cases here are initiated by a person responsible for the animal. While this may appear to be but a detail, it is in reality an important factor and a filter. It will greatly influence which cases *are* reported, independently from the objective nature of the event. As discussed later, it will also have an important influence on how pharmacovigilance activities are influenced by a company's product portfolio and the geographical location of its markets.

Follow-up to adverse reaction reports

Once a pharmacovigilance report has been received, it is crucial, apart from meeting the deadlines on possible expedited reporting, to follow the report case correctly at the user's end. This follow-up phase has two aspects. First, it is to ensure that all necessary data are gathered to document the event. This will allow a correct causality assessment, but it also provides valuable information to improve the management and positioning of the product. A rapid reaction is crucial. Most data can be gathered when the case is still 'fresh'. Early reporting to the authorities is important. If there are questions from the authorities, then this is the best approach to allow a high-quality follow-up. This means that as soon as, or sometimes even before, the minimum data set that constitutes a case is obtained, initial reporting should be made if the case qualifies for expedited reporting.

Apart from this fact-finding work, one should never forget that many if not most cases are reported not by owners or veterinarians for compliance reasons. In the first place, they are a product complaint or at least a request for help. So while this is not formally within the legal duties of the QPPV, it is clear that any responsible company will make sure that a correct follow-up is given to the reporter and the patient when a complaint is received. For obvious reasons this close follow-up, from a support as well as from a fact-finding viewpoint, is often well done for cases that are classified as unknown or serious. The main risk lies with the more common and well-known side effects. From a company point of view they may appear as less interesting, but one should never forget that behind each report lies a problem that is perceived as real by the reporter.

Furthermore, it should not be forgotten that a change in trend or an increase in expected cases is defined as a possible trigger for further investigative actions. This implies that a good follow-up of such 'expected' cases may already provide the answer as to why there is a change in a trend before the question is raised by the regulatory authority. It should also be remembered that providing correct support is probably the best way to promote pharmacovigilance and to ensure good future relationships and reporting from the concerned partners.

Opening a case is not that difficult. Data are flowing in and this will determine the actions taken within the company. It is often more complex to close a case, but this is an essential point in its handling. At a certain moment the decision should be taken that no further data can be retrieved and the causality assessment (ABON classification) has to be made. One should be aware that there can be different closing dates for the same case within a company. Obviously the case can be completed from a scientific point of view while the discussion for any responsibility

and compensation is still ongoing. The latter is, however, not within the scope of pharmacovigilance and so emphasis should be on the scientific aspects. It is not possible to construct a standard checklist, but in general it is probably advisable to consider a case closed when four questions plus one major question can be answered positively:

- Do I have all necessary and relevant administrative data?
- Do I have all possible laboratory data?
- Do I have all the descriptive information I wanted?
- Are my technical colleagues satisfied with the data set?

After these four questions a final assessment can be made and a (final) report should be submitted to the competent authorities for cases subject to expedited reporting, stating that this is the final report and that the MA holder considers the case as complete. This leaves the 'plus one' question:

- Does the competent authority agree with the closure of the case?

As will be discussed under the heading 'Causality assessment of the case or ABON coding', it always remains possible that a case may have to be re-opened in the light of new data or new scientific insights.

Incorrect or incomplete reporting

Obtaining a good data set is essential for good pharmacovigilance. Special situations arise with those cases where incorrect or incomplete reporting occurs.

Incorrect reporting

Some reports, for whatever reasons, may contain deliberately falsified or partially falsified information. Fortunately these cases are rare, but they do occur. The most common are those where financial benefit is sought or where the intention is to harm veterinarians, employees or the company. The wisest approach is to accept the data as for any other case. In fact, this is a legal obligation since a company does not have the right to refuse or decline adverse event reports. As soon as possible the data should, however, be transferred to the competent authorities and discussed with them in the light of the suspicions of the MA holder. The case should then be handled in close cooperation with the authorities. The experience is that a direct request from the competent authorities to those making the report is often sufficient to put the case in the right perspective. It should be remembered that financial gain should never be a trigger for reporting a pharmacovigilance case and that companies have an important duty in enforcing this approach. This message should be clearly given in the educational pharmacovigilance programmes for commercial personnel since they may be tempted to opt for a compensation approach when confronted with a dissatisfied customer.

Incomplete reporting

Owners may be reluctant to report adverse events, or they may not allow their veterinarians to report all the data necessary to constitute a case. This is often caused by the fear of some kind of recrimination by the company, which is obviously without any grounds but is nevertheless real to some individuals. In principle one could claim that since the necessary data are not available, the report then fails to meet the requirements to qualify as a case and the process could be halted there. This is obviously not a very constructive approach and useful information may be lost this way. In most cases, insisting and persisting with the reporters may not be very useful and productive. The best approach is either to recommend the reporter to make direct contact with the competent authorities via the appropriate national reporting schemes, or to inform the authorities on their behalf while proposing that they take the lead in the further handling of the case.

Specific issues in handling different types of cases

Cases in the target animals

Cases in the target animals after use of the product in accordance with the condition of the MA and the approved labelling are relatively straightforward and common and should normally not pose special problems to the MA holder. They will therefore not be further discussed in this chapter.

Human reactions after exposure to veterinary medicines

Human cases necessitate a very specific approach for several reasons. By definition they are not the result of a normal use of the product but always the consequence of a contact, accidentally or deliberately, for which the product was not intended. Note, however, that there must be an exposure to the product. Exposure to an animal treated with a product normally does not qualify, except, for example, with pour-on formulations and ectoparasiticides, where transfer is possible. All of this implies that a wide range of scenarios are possible, and this does not facilitate their handling. As such they do not fit within the normal classification used for animal cases, but they involve an area where often only limited or even no knowledge is available.

For the larger companies where both a human and a veterinary department exist, scientific and medical support can be gained from colleagues working in the human sector. This is particularly relevant if the concerned molecule is available both for human and for veterinary use. Even in this case support can be restricted since the formulations in veterinary medicines are often very different to those used in human products. Another complication, especially for third country reporting, is the fact that the USA and the EU have a different definition of human cases. As for the classification, it is commonly accepted by regulatory authorities that no ABON approach or classification is given by the MA holder unless the relation or non-relation is very obvious or endorsed by sufficient data (e.g. laboratory or hospital test results).

Apart from accidental exposures, some other scenarios exist that may require a special approach. A specific additional class are those cases where criminal acts may be involved. The abuse of veterinary products with narcoleptic properties is relatively common. However, the deliberate abuse on third persons for reasons ranging from practical jokes to attempted murder has been recorded. In such cases the first report is often received via police and it can be difficult for a company to obtain the requested data, even to constitute a case, via that route. Experience suggests the most appropriate approach then is not to try to get direct information from the field, but to inform the competent authorities immediately and, if possible, to agree with them that the lead in further investigations of the case rests with them.

It should also be noted that since 2006 there is a specific database, actually an addendum to the VeDDRA database, listing specifically the clinical terms to be used for reporting human adverse drug event cases in relation to the use of veterinary medicines.

Suspected lack of expected efficacy

This class of reports falls within the scope of pharmacovigilance, but it is not always easy to frame them within the pharmacovigilance system. Historically this type of case was called ‘suspected lack of efficacy’, which is misleading. In analogy with the American pharmacovigilance system, the term ‘expected’ has been added to give ‘suspected lack of expected efficacy’ or SLEE. This extension is important. It means that only cases where the product has been used in accordance with the label claims and where no or insufficient efficacy as described in the approved product literature has been obtained should be considered as pharmacovigilance reports. So any complaint after a clear off-label use in the approved or another species, or for a disease not mentioned in the product literature, will not meet

this standard and should not be considered as a genuine reportable case. The information itself of course may be very useful from a technical point of view.

Another important remark is that laboratory findings, such as elevated MIC values for antimicrobial drugs, should not be considered as reportable pharmacovigilance cases except where an association can be made with apparent clinical failure in the treated animals. Currently SLEE cases are normally not coded on causality in the ABON system. This may change, however, since the French authorities have developed a standardised approach to the coding of SLEE cases. While this is not yet embedded in an EU guideline, it is possible that in the future such coding may become a standard requirement.

In principle SLEE cases are not considered as meeting the definitions of 'serious' and therefore not are reportable via expedited reporting. However, it becomes complicated when the apparent failure of the treatment results in effects linked to the disease which can be considered as 'serious'(for example, if animals die because of the failure of an antibiotic or antiparasitic treatment; presumed prophylactic failure of a vaccination is another common example). Experience suggests that in cases of doubt a prudent attitude is to inform the authorities in exactly the same way as one would for a serious event.

Off-label use

The off-label use of a product, defined as any use not in compliance with the specifications of the approved product literature or the Summary of Product Characteristics (SPC), as such, is not a pharmacovigilance issue. Indeed, it is permitted in some circumstances under EU legislation (the cascade) where a treatment is unavailable for a particular condition or for a particular animal species. Intrinsically unwanted effects observed due to off-label use are not within the legal scope of pharmacovigilance since the legal definition of adverse events limits it to

> '. . . a reaction which is harmful and unintended and which occurs at doses normally used in animals for the prophylaxis, diagnosis or treatment of disease or the modification of physiological function'.

However, effects observed as a result of off-label use can be very informative. For example, they can provide useful information on the hazards and risks of using certain substances in certain species or on tolerance levels in target species. Therefore these data should be recorded when reported and, if deemed appropriate, the information should be passed on to the competent authorities. Consideration should also be given as to whether such reports need to be treated as expedited, or if they can be included in periodic update reports.

Use of human medicinal products in animals

For completeness sake this should also be mentioned since this topic is addressed in the legal texts and, indeed, the use of human medicinal products in animals is permissible in certain circumstances of non-availability of an appropriate veterinary medicinal product. However, it is not really within the scope of pharmacovigilance.

Under the so-called cascade system mentioned above, human products can under well-defined conditions be used on animals. Occasionally this may lead to adverse effects in the animals. The *Notice to Applicants* foresees that these should also be reported by 'the veterinary representative of the Marketing Authorisation Holder of the human medicinal product concerned'. Obviously this could only be the case for companies effectively having a veterinary and a human department. What should happen in cases where the concerned company only possesses a human medicines department is not very clear. However, in practice it is not very common that such cases are reported by the owner or veterinarian to an MA holder or to a regulatory authority.

Transfer of contagious agents

One of the latest additions in the scope of pharmacovigilance is the request to report 'any suspected transmission via a veterinary medicinal product of any infectious agent'. According to the legal texts this is limited to cases happening outside the EU. Furthermore it is well understood that this transfer is considered to be linked directly with the quality of the product, which implies the transfer of infectious agent present within the product at the moment of opening the packaging. The transfer of an agent due to poor hygiene measures or poor veterinary techniques is not within the scope of this requirement.

Infringement of maximum residue limit values in animal produce

The exceeding of an approved maximum residue limit (MRL) for a substance after the correct use and correct observation of the withdrawal of an approved product is placed firmly within the scope of pharmacovigilance. Such cases are often reported by authorities but can also be reported by an owner after the rejection of his or her animals or products. The necessary follow-up is defined by several parameters and it is probably not possible to develop a standard approach for this. Several issues have to be taken into consideration. For example:

- Is the level of infringement of the MRL value within reasonable limits or, in other words, can it be caused by a variation in excretion level or is this due to dosing or withdrawal period errors?
- Is it a single report or are there several comparable cases?
- The confidence interval to calculate withdrawal periods implies that some cases will be observed.
- Is it linked to an individual or group treatment? As discussed below, group treatments are vulnerable to dosing errors due to either mixing errors in the feed or drinking water, or a misjudgement on the average bodyweight of the animals dosed with an injection or using a drenching gun.

Causality assessment of the case or ABON coding

Quite remarkably, Volume 9B states that MA holders 'may' comment on whether they consider there is a causal association between the reaction and (the use of) their product. In practice it is expected that the MA holder does this de facto. This classification should be done via the so-called ABON system. The meaning of each class is well explained in several legal texts and elsewhere in this work and it will not be repeated here. However, it should be indicated that in some documents (e.g. EMEA/CVMP/552/03, *Guideline on harmonizing the approach to causality assessment for adverse reactions to veterinary medicinal products*) it is foreseen that the 'O' code can be further split up into two sub-codes, namely O1 and O2, where O1 stands for 'inconclusive' (cases where other factors prevent a conclusion being drawn, but a product association cannot be discounted) while O2 stands for 'unclassified' (cases where insufficient or unreliable information does not allow any conclusion to be drawn). In practice this subdivision has indeed proven to be useful and is a correct reflection of the real-life situation, so it is highly advisable to use it wherever possible and appropriate.

The causality assessment itself is important since it has a direct impact on the incidence calculation, which in turn may have an effect on the approved product literature and the conditions under which the product is allowed on the market. Volume 9B details how the assessment should be done and what parameters should be taken into account. However, it remains difficult to standardise a classification of biological events and in practice there often remains a discrepancy between the MA holder's assessment and that of the authorities.

In an attempt to harmonise the approach to causality assessment, an EU guideline was prepared and published (EMEA, 2003). Through a

standard set of questions, a set of tentative classifications for each specific parameter is provided. Based on the different sub-classifications, a final classification using one of the ABON categories is obtained. This is indeed a valuable and constructive addition to the legal tools, although in reality a difference in classification can be observed between that achieved by the regulatory authority and that arrived at by the MA holder. The guideline recognises that the current approach is only an attempt to harmonise causality assessment and states that it should indeed be re-evaluated in 2007–2008; at the time of writing in late 2008, this re-evaluation has yet to occur. In practice one should bear in mind that any algorithmic classification will remain an aid. A final validation by an expert clinician or toxicologist is essential and decisive.

The question remains how to act on or to assess the non-clinical cases. As discussed above it is acceptable in most cases to not classify human cases using the ABON system, apart of course from cases where the actual relation or non-relation between product and the effect is obvious. With infringements of MRLs in animal produce or with environmental cases it can be argued that it is inappropriate to use the ABON system. A good justification for this view is that these cases normally are not considered in the incidence calculation, which is itself largely based on the ABON coding.

SLEE reports remain a special case. Until recently the standard approach was not to classify them using the ABON system. However, as discussed above, the French authorities have recently developed a classification system which may eventually become embedded in an EU guideline.

Submission of expedited reports to the authorities

The legal texts well define, even to the extent of providing some tabular guidance, which reports have to be submitted within 15 calendar days after receipt and which should be included in the PSUR submission. Because of this available guidance it is not necessary to repeat this information in the framework of this discussion. However, some guidance and advice can be given on the actual submission procedure.

The electronic reporting of adverse events has become compulsory following implementation of the new pharmaceutical legislation in November 2005. This should be done via specifically developed central systems and the authorities have spared no efforts to make the electronic systems operational. However, the actual use of these systems remains limited, as stated in the EMEA *Public Bulletin 2006 on Veterinary Pharmacovigilance*:

> 'The veterinary pharmaceutical industry's major players have further used 2006 to set-up and test their internal systems of reporting to the EU central database. The number of marketing authorisation holders actively using either the gateway or the web reporting tool for actual reporting in 2006 was disappointing. It is however expected that many more companies will move to electronic reporting in early 2007.'

This is a true representation of the situation as it existed at the end of 2006, but to date (in 2008) it appears that the industry is still struggling with the implementation and use of the central electronic reporting systems. There are many reasons that can explain this and it is not our intention to enter into technical discussions on the backgrounds and possible solutions. In a nutshell, it can be said that the main problem lies in the lack of a reliable and robust communication between the different systems of the respective stakeholders, in combination with some technical shortfalls within the central systems themselves. This is not surprising. Actually we are looking at the integration of a central EU system with several disparate systems of the national competent authorities. Some of these were in existence before the creation of the central system. This complex of systems has to communicate via three different levels of data entry – Gateway, EVWEB and the 'Simplified Electronic Reporting Form (SERF) –

with the industry's own systems and all these systems having to be integrated with several terminology databases (VedRRA, VeDDRA on human terminology, database on species and breeds).

All of this has to operate in a validated way ensuring that the necessary feedback is received and the necessary procedures are in place to prevent double reporting. In addition, the data must be stored, analysed and processed while integrity of the data must be maintained.

All parties are working hard and are motivated to make this system robust and reliable, but it remains a huge and complex project. This situation is unpleasant but a reality, and one that the QPPV has to deal with on a daily basis while fulfilling all his or her legal obligations.

Choosing an electronic reporting system

Even though the overall system is not yet fully operational it is important that a choice is made as to which entry to the electronic reporting system will be used by companies, as this needs to be registered with the central system for reporting. This registration is part of the pharmacovigilance system and it should be included in the description of the pharmacovigilance system. It will almost certainly be a part of a possible inspection on pharmacovigilance compliance.

The registration form and all the necessary information can be found on the EudraVigilance website (http://EudraVigilance.emea.europa.eu/veterinary/index.asp).

While electronic reporting is entangled in a complex network where procedures and science are interlaced with highly specialised IT terminology elaborated in several guidance documents, the actual EudraVigilance website is very user friendly and sufficient guidance is foreseen online to facilitate a smooth registration procedure.

In principle there are three different systems or reporting routes available (as already indicated above):

1. the Gateway system;
2. the EVweb system;
3. the SERF or Simplified Electronic Reporting Form (html based).

From a compliance point of view, all systems are equivalent. The system chosen is in principle the option of the MA holder, with the remark that the first two are considered as the standard systems. The latter, the simplified format, should be restricted to (very) small operations and used with the prior agreement of the local competent authority.

The Gateway system is designed as a fully automated reporting entry point where the database of the MA holder and the EudraVigilance database communicate directly, sending back and forth data on cases and the relevant confirmations of receipt and acknowledgments, or as it is described by the authorities:

> 'The purpose of the EudraVigilance Gateway is to operate a single, common, European Economic Area (EEA)-wide Gateway for receiving regulatory submissions in a fully automated and secure way including all aspects of privacy, authentication, integrity and non-repudiation of all transactions in pharmacovigilance.'

This reads like an ideal solution, but it implies of course that the MA holder will need to have a database capable of corresponding with the authority's database. In view of the practical complications already discussed, and the complex experience with this today, this is almost certainly limited to (some) commercially available databases. When making the decision on acquiring such a database this capability can be an important decisive factor.

The EVweb system also allows a direct input into the EudraVigilance database, but this has to be done manually and requires assistance by an operator from the MA holder. So this system, which is more labour intensive, is probably the best choice for smaller operations or those operations with limited reportable cases.

The SERF is, as indicated, an html-based reporting system which includes a form that can be opened via the EudraVigilance or national authority sites and on completion is sent via email to the concerned competent authorities. Typically the SERF has less functionality than both the other systems and it also contains fewer data fields to be entered. Although this is not the right forum for such a discussion, one could question why some cases may be reported with less detail than others.

As stated in the Introduction to this chapter, the actual daily use of the electronic reporting system is still often flawed, in spite of the goodwill of all concerned parties. So while electronic reporting is obligatory, it appears evident that until the system is fully operational, no MA holder can be blamed for not using the system. Fortunately, this risk of apparent non-compliance is addressed in the Directive which indeed foresees that the system may not be usable:

> 'Save in exceptional circumstances, these (adverse) reactions shall be communicated electronically.'

It is obvious that a non-operational system should be regarded as an 'exceptional circumstance' and if a judgment must be made whether adherence to electronic reporting or certainty that safety data are made available within the allotted time span is most important, one has to realise that the latter will prevail and reporting via alternative means should be used. All competent national authorities today still accept reporting via other means such as paper and email.

Periodic Safety Update Reports (PSURs)

Periodic Safety Update Reports or PSURs are, in addition to expedited reporting, the second standard route of reporting. The principle is fairly simple: at regular intervals the MA holder submits an overview of all the cases he or she has become aware of over a well-defined period of time. This information is combined with other relevant data such as the numbers of doses sold in the field, allowing the determination of whether the risk: benefit ratio of the concerned product remains unaltered and the current SPC is still valid. Alternatively, this may highlight whether there is a trend that needs to be investigated in more depth, possibly leading to a change in the MA conditions, including warnings on the label or other regulatory action. While the principle is fairly simple, the practical implementation and applied submission schedule are certainly not; they are subject to variable interpretations by different competent authorities. So in practice this is one of the most time-consuming activities of the pharmacovigilance department of a company. Consequentially it also appears to be a large part of the work of regulatory authorities. As will be discussed, it is rewarding to observe that authorities have taken action and an initiative is underway to reduce and optimise the workload both for MA holders and for authorities.

Another complicating factor is that at present the transition period between the provisions of the old and new (current) legislation is not yet completed. All previously approved products are still going through their last renewal phase as foreseen in the current legislation. This renewal involves a PSUR submission which does not fully fit into the standard schedule as defined in the current legislation. However, this is only a temporary phenomenon and one that hopefully will be addressed by the current initiative to optimise the whole PSUR procedure.

Apart from the submission required at renewal, the current schedule for submission of a PSUR is:

- every 6 months until the placing of the product on the market;
- every 6 months for 2 years after the placing of the product on the market;
- then yearly for the next 2 years;
- then once every 3 years.

This is a minimum provision. Authorities have the right to ask for a PSUR at any moment they deem appropriate.

While this schedule is not very practical, a transition measure to bridge the requirements from the old legislation to the updated texts has been agreed and put in place, and can be summarised as follows:

- For products having an MA with an expiry date no later than 30 April 2009 the next PSUR should be submitted with the renewal application no later than 30 October 2008 (taking into account the requirement in the revised legislation that a renewal application should be submitted 6 months before expiry of the current MA). Thereafter PSURs should be submitted every 3 years, unless other requirements have been laid down as a condition of the MA.
- For products having an MA with an expiry date after 30 April 2009 the next PSUR should be submitted no later than 30 October 2008. The precise date of submission should be agreed with the national competent authorities. Thereafter PSURs should be submitted every 3 years, unless other requirements have been laid down as a condition of the MA.
- Furthermore a PSUR is required at the 5-yearly renewal application.

Submission dates are defined by the birth dates and data lock points (DLP) of the product. The whole procedure is rather complicated but is explained in detail in Volume 9B. In order to reduce the workload MA holders are allowed to produce their PSURs based on the first approval date in the EU. The DLP is the moment when the data set on cases received on a product is 'frozen' in preparation for the PSUR.

In spite of all these efforts the coordination and preparation of the PSURs often remains a cumbersome and ineffective exercise for the MA holder. Furthermore, and without going into examples, based on the submission schedule described, situations can occur for established products where two different PSURs will need to be submitted within a 1-year time span to the same competent authority.

Clearly the situation is equally inefficient for the regulatory authorities. In order to address this, the European Surveillance Strategy (ESS) group, an initiative taken by the EU Veterinary Heads of Medicines Agencies (HMA-V), which include member state representatives, the CVMP, its Pharmacovigilance Working Party and the EMEA, was recently established to explore the actions needed for co-ordination of veterinary pharmacovigilance throughout the European regulatory network. ESS has started up a project to address this situation. The group has promulgated a proposal to harmonise the preparation and submission of the PSURs to the different member states. In principle, PSURs for a specific substance, regardless of specific products, should be submitted by all the respective MA holders to the competent authorities. The actual evaluation should then be coordinated by a member state (the Pharmacovigilance Reference Member State) that will assume the responsibility for the assessment of the given substance, or at least take the lead in this assessment. This change should be possible within the current legal framework without requiring a formal legal initiative because it has been foreseen in the current amended Directive that the actual submission schedule of the PSURs can be altered via a so-called comitology procedure (put simply, introducing the requirements of EU legislation through a committee procedure).

While this would be a substantial simplification of the current schedule and would serve to dramatically reduce the workload for both sides – industry and the competent authorities – it is clear that several hurdles must be overcome before this final goal can be realised. Until then the current schedule remains in place. Moreover, agreement must be reached on how pharmaceutical *and* biological (mainly vaccines) products can both participate and care must be taken to ensure that the specificity of the different formulations is respected.

A joint working group made up of representatives of the ESS group and industry has recently been established (PSSG) in order to ensure that this project progresses. A pilot phase study has begun involving a limited number of substances and EU member states. It is also encouraging to

note that other initiatives have also been taken, largely because of developments in human pharmacovigilance; for example, the proposal that the PSUR system, and the evaluation of PSUR data, should be part of a greater risk-management policy. In this respect, it is highly questionable whether the requirements and schedules applied to newer products are really appropriate for older products whose safety profiles are well known and understood.

It may appear peculiar, but for an authorised but non-marketed product in an EU country, a PSUR has to be submitted. This is justified by the fact that the product may be marketed outside the EU or in other member states. This means that pharmacovigilance reports may become available with data drawn from these markets before the product is introduced into the EU market and this may shed light on some safety aspects of the product. The international scientific literature may provide interesting data with respect to the safety:efficacy ratio of the yet-to-be-marketed product.

Data to be included in a PSUR

The data that have to be presented in a PSUR are well described in Volume 9B of the *Notice to Applicants* and other guidelines, so there is little need to repeat the requirements here. However, for some topics some practical considerations and suggestions should be given.

Update of regulatory or MA holder actions taken for safety reasons

Significant regulatory actions taken anywhere in the world have to be listed in the PSUR. In most cases such actions are only taken if there is an intrinsic problem with the product that calls for immediate action, and consequentially if any such actions have been taken, it is very likely that they will affect any licence or authorisation anywhere else in the world before a PSUR is prepared according to the submission schedule. In many circumstances this will only be a listing of actions taken previously in the member state where the PSUR is to be submitted.

Sales volumes of the products

Although this appears to be a straightforward requirement, in practice it is often confusing and the source of disputes between MA holders and the competent authorities. The legal texts are clear: for national licences, the doses sold in the concerned country should be reported; for EU marketing authorisations, the doses sold in each member state should be reported. However, some EU countries may require additional data. The sales volumes may need to be listed in specific magnitudes (vaccines in doses, tablets in numbers, powders in kilograms, etc.). The *Notice to Applicants* provides sufficient guidance on this. For multi-species products an estimate should be made on how the sales are distributed over the different approved species. The only way to obtain these data is to address the question to the sales and marketing departments. Once obtained, it is tempting to maintain the applied distribution for all countries and for all submission time points. However, the question should be repeated regularly and specifically for each market since market conditions are different in different markets and change over time. Keeping this information up-to-date may avoid the unnecessary reporting of changes in incidence rates.

These data should then be used for incidence calculation. The calculation itself is rather straightforward and the guidelines give sufficient guidance, except for the number of treated animals to be taken into account. For some reason, the core texts fail to foresee the need for standard body weights of the different animal species that could be applied to calculate the number of doses administered. These data can be retrieved (e.g. in a footnote of CVMP guideline EMEA/CVMP/183/96-Rev.1-Consultation), but for convenience they are given here:

- Horse, 550 kg
- Dog, 20 kg
- Cat, 5 kg

Table 10.1 Determination of animals treated based on sales data.

Criteria	*Pigs (55%)*	*Broilers (30%)*	*Layers (8%)*	*Turkeys (6.5%)*	*Ducks (0.5%)*
Meat sold over the period (kg) (total 782,340)	430,287	234,702	62,587	50,852	3,912
Dosage (mg/kg bw) applied	20	25	25	25	30
Average bodyweight (kg)	50	1	2	10	2
Number of days treated	7	7	7	7	7
Total dosage (g/animal treated)	7.00	0.18	0.35	1.75	0.42
Total animals treated over the period	6,146,957	134,115,429	17,882,057	2,905,834	931,357

- Cow, 550 kg
- Beef calf, 150 kg
- Newborn calf, 50 kg
- Sow or boar, 160 kg
- Finishing pig, 60 kg
- Weaner, 25 kg
- Sheep, 60 kg
- Lamb, 10 kg.

For other species the following values are suggested:

- Broiler, 1 kg
- Layer, 2 kg
- Duck, 2 g
- Turkey, 10 kg
- Pigeon, 30 pigeons/litre of drinking water.

It has recently been suggested that these weights should be replaced with those used in EU environmental risk assessments, largely in the interests of harmonisation. However, as these are yet to be fully defined, this suggestion is rather premature and at the moment it merely contributes to further confusion.

A question that remains to be answered is what happens to products with a dose range? A correct estimation of the average applied dosage is important since it may also have a direct impact on the trigger value and on the results of incidence calculations. Again it would be wise to rely on the information that can be obtained from the market, and from the sales and marketing teams.

In order to facilitate the consistent assessment of all this information, it can be convenient to include it in a simple computer model. An example is given in *Table 10.1*. Such a table can be easily attached or incorporated into the PSUR to increase transparency.

A continuous discussion exists concerning which cases of the different ABON classes should be taken into account in incidence calculations. Where there is little discussion regarding the A's and B's, the O's remain a point of debate. Since, as stated above, the EU legislation foresees that the MA holder 'may' comment, it is suggested not to include the adverse drug reactions classified as O or N in incidence calculations.

Cases published in the international literature

Published adverse reaction reports should be included in the PSUR and this can be a complicated request. It is of course impossible to follow every possible publication worldwide that may ever contain such a publication or report. The normally accepted approach is to perform a search via some major electronic databases. It should then be clearly stated in the PSUR which information sources and parameters were used during the search and of course the eventual outcome of what was found. In this respect, keep in mind that data on events obtained from the internet itself through search engines, posted on

uncontrolled websites, should not be considered as genuine and reportable pharmacovigilance cases. They should be excluded from the PSUR.

Narrative review of the individual case histories

Volume 9B of the *Notice to Applicants* suggests that this should be a brief section. In practice, this is one of the most useful parts of the PSUR. In general all the other sections are not much more than listings with or without standardised coding, thus allowing little room for clarifications. Consequently, due attention should be given to this section. Too often it is reduced to a standard text which is then slightly adapted in relation to the specific circumstances of the particular PSUR submission. This should be avoided. While the cases are well known to the QPPV who is compiling the PSUR, this will not always be the case for the regulatory assessor, who will have to form his or her opinion based on these listings.

It is a good approach to start this section with a detailed description of the product and how it is used in daily practice, highlighting all the useful details such as local veterinary techniques, disease patterns and changes in breeding techniques over the reported periods. Against this background the reported cases can then be positioned and qualified. This will ensure that the PSUR is properly assessed and remove the risk that evaluation and possible conclusions are based on a simple application of incidence rate calculations and trigger values.

This section is also the most appropriate place to elaborate further on the reports that are included in the 'other report' section. Sometimes these are so difficult to discuss and subsequently assess in a simple listing and they may lead to questions that can be avoided by a good narrative explanation.

Third country reporting

Third country reporting comes into the scope of both expedited and periodic reporting. However, since it is mainly a pass-through activity for the QPPV it is briefly addressed under a separate heading.

The obligation for third country reporting, meaning the submission in the EU of adverse event reports for the same product in countries not belonging to the EEA, is foreseen in the EU legislation and also in the VICH texts. However, the EU was the driver behind this. The actual definitions of what have to be reported and when are described in the legal texts and are even partially tabularised, so they will not be repeated here. In spite of this apparent simplicity, several companies struggle with this legal requirement. This may be partly from a purely organisational point of view and partly due to local interpretations and the interest shown by the different competent authorities within the EU to reports from third countries.

A prerequisite is that the MA holder is capable of receiving the reports as per the expected time lines. This implies actually that the network that the QPPV has to build and work within exceeds the EU area. For multinational operations, this may be beyond the QPPV's influence. In practice it has been demonstrated that working with commercial databases has an advantage since the worldwide pharmacovigilance data can be entered into the database and retrieved as and when necessary. This area is complicated by the fact that products with similar or even identical names may be different across geographical boundaries. A product with a specific name and active ingredient may be totally different from the same company's product with the same name in another country.

The VICH GL24 draft guideline contains a concise definition. While the text is still in draft and therefore non-binding, it is probably the most reasonable solution to apply in defining the status of products:

- The 'same biological VMP'[2] is defined as originating from the same MAH being responsible for pharmacovigilance of this/these VMPs with the same manufacturing specifications.
- The 'same pharmaceutical VMP' is defined as originating from the same MAH being

[2] Veterinary medicinal product.

responsible for pharmacovigilance of this/these VMPs with the same formulations.

- A 'similar pharmaceutical VMP' is defined as:
  - originating from the same MAH being responsible for pharmacovigilance of this/these VMPs;
  - the same active ingredients;
  - major excipients with the same or similar pharmaceutical function;
  - at least one common registered species.

This definition still leaves room for interpretation. In case of doubt on whether something is reportable or not, the best approach, as it is for any event, is to report the case and to leave it to the competent authorities to decide whether it is relevant or not. A suitable explanatory note may be of assistance. It is better to have a degree of over-reporting than being accused of withholding information.

Specific product classes

There are two product classes that should be discussed specifically since they are not addressed specifically in the EU legislation, and they are in an unclear position with respect to pharmacovigilance obligations.

Pre-mixes for medicated feeding stuffs and other products for mass treatments

The authorities deemed it necessary to attract special attention to these kinds of products and indicate that in case of an adverse event, the necessary controls must be undertaken on feed composition, milling processes, storage, feed consumption and other related issues. This is actually fairly obvious. However, these remarks should not be limited to medicated pre-mixes as they can be expanded to any type of group or mass medication, such as drinking water application. Special attention should be given to products administered with a drenching device or injection gun technique. In practice it often happens that the person administering the product adjusts the device to a standard animal weight. When treating a whole flock this may often result in both over- and under-dosing, resulting in a lack of expected efficacy or tolerance and residue problems.

Feed additives

Products such as coccidiostats and histomonostats registered as feed additives do not generally fall under the scope of veterinary pharmaceutical legislation since they are regulated under Regulation 1831/2003. Hence, they do not fall under the scope of its pharmacovigilance provisions. Despite this, it is necessary for a number of good reasons to give them some attention.

First of all, within the frame of the VICH process it should be noted that in other geographical areas, especially the USA, they are regarded as veterinary pharmaceutical products and so do fall within the scope of local pharmacovigilance obligations. Furthermore, for EU renewal applications as feed additives, the following requirements in the new guidelines on data to be presented are foreseen:

> 'Evidence should be presented that in the light of the current knowledge the additive remains safe under the approved conditions for target species, consumers, workers and the environment. A safety update for the period since the authorisation for putting into circulation, or the last renewal with information on the following items should be presented:
>
> - reports on adverse effects including accidents (previously unknown effects, severe effects of any type, increased incidence of known effects) for target animals, users and the environment. The report on adverse effects should include the nature of the effect, number of affected individuals/organisms, outcome, conditions of use, and causality assessment;
> - reports on previously unknown interactions and cross-contaminations;

- data from residue monitoring, where appropriate;
- any other information concerning the safety of the additive.'

This very brief section actually summarises the duties of pharmacovigilance as it is applied to veterinary medicines. No further detailed guidelines for veterinary medicines exist and it is not even a legal obligation to have a formal pharmacovigilance system or a QPPV. However, it is probably the best and easiest thing to apply the veterinary pharmacovigilance system, or at least a comparable or duplicated system, for collecting and handling data for these products if they are present in the portfolio of the MA holder. This should ensure that the necessary data are available at the renewal. It should be noted that there is no duty on expedited reporting or PSUR submissions at well-defined intervals for these products.

It should also be mentioned that in some member states the national authorities appear to handle expedited cases reported on coccidiostats from the field within their national veterinary pharmacovigilance system. Whether this is deliberate for convenience or just a case of confusion is not always clear. PSURs are not expected for these products.

Identification of critical parameters in the prevention of crises

Pharmacovigilance is an area that is prone to being affected by crisis situations (see Chapter 29). So when a system is being developed, this aspect should be taken into account. Crises all have something in common: they cannot be predicted. The most practical approach is to try to identify where the major risks lie and to work around this. This is in fact just a part of the risk: benefit assessment which any pharmacovigilance activity should be conducting as a matter of routine.

It is essential that the system is operational at all times and capable of receiving and channelling data in an emergency situation. As discussed above and apart from the elements discussed further, it may be a good idea and good practice to validate the pharmacovigilance system through the use of mimicked cases. The inclusion of a worst case situation, corresponding to a crisis involving the company's products, could be included in such an approach.

No two companies are identical and this is also valid for their approach to and concerns regarding their pharmacovigilance issues. Clearly, not all risk scenarios can be foreseen, but taking the time to consider a few specific parameters should make it possible to foresee periods and conditions where there may exist an increased risk of unexpected situations and the development of a crisis. Steps should be put in place to address a crisis if and when it occurs.

Importance of the product portfolio

A major parameter to be taken into account when setting up a veterinary pharmacovigilance system is the composition of the MA holder's product portfolio. Some classes of products are likely to cause more or less reporting than others, regardless of the intrinsic qualities of the product. It was emphasised in the section on the handling of cases that adverse reaction reporting in animals is triggered by those responsible for the animals, not by the animals themselves, and this can have an impact on the nature of reporting. The make-up of the product portfolio is important in this respect.

New versus existing products

First, it is necessary to differentiate between products containing new active substances or having new claims for existing substances or any other innovative feature and established or 'old' products. Anyone involved in innovative product development and the introduction of such products to the market place will be aware that such products may cause peaks of reporting in the first months after introduction. In principle this can

be due to intrinsic attributes of the product coming to light after exposure to a broader patient population, but often it is simply due to a learning process which the users of the products have to go through in combination with some natural prudence for novel situations.

For those involved in this class of products, the pharmacovigilance system should be designed and validated by way of a mimicked scenario so that it can handle such post-introduction peaks. This should be seen not merely from a reporting point of view but also from the perspective of follow-up and technical support for the users and patients. However, this does not fall within the legal duties of the QPPV. Nevertheless, this close support is important since the pattern observed on the introduction of the product is often illustrative of events that might be seen during its whole life span. So the sooner the necessary information is gathered and assessed, the better one is prepared for further follow up and support for future cases.

Non-innovative products tend to lead to less reporting because users are normally better acquainted with the products, their ingredients and the ways in which they are used. These products normally have an established and accepted safety profile, making it unlikely that many new unexpected adverse events will occur.

Companion versus food-producing animals

Another relevant point is the distribution in the product portfolio between products for companion animals and products for food-producing species. Within the food-producing animals a further difference has to be made between individual and mass medication products.

In general it is expected that a comparable product in companion animals will lead to more case reports than in food-producing animals. Since there is no physiological background for this observation, it is probably due to two main factors. First, companion animals are normally more closely observed than food-producing animals by their respective owners. Second, it appears there is a difference in social acceptability of levels of suffering animals have to go through. A comparable event (e.g. a local swelling after injection) can be considered by a dog owner as an unacceptable event, resulting in a complaint to the veterinarian, leading to a pharmacovigilance report, while a similar swelling in a single pig might pass almost unnoticed and will probably never be reported even when observed; it will generally be regarded as trivial.

Individual treatments versus group treatments

The preparedness to report threshold tends to decline further with group treatments on animals. This is partly recognised by the legislation which makes a distinction between single animals and animals treated in groups. There is even a different interpretation on what constitutes a 'serious' event. Quite correctly, the definitions of severe adverse events are different from the standard cases. In fact, serious adverse events only apply in situations where there is increased incidence of mortality rate, a severe clinical symptom, or where there is an excessive variation in expected production rates. In principle, only fish, poultry and bees are by law considered to be animals kept in group, and under this restricting condition that they are indeed kept in a group. Individually kept animals should always be considered as such and for them the normal definitions of 'serious' will apply.

In real life, several other species are also kept and medicated in groups and they will be observed by their owners that way, resulting in a comparable reporting pattern. It is obvious that for these products there will be less reporting. However, this is not always appreciated by some regulatory authorities which have indicated there is significant under-reporting for this kind of product, and thus attention will be focused on the reporting behaviour of the concerned MA holders. Furthermore, while an MA holder dealing with these products will receive relatively few reports, one should realise that when something does go wrong, it can quickly become a major crisis because of the numbers of animals potentially involved. Large medication volumes

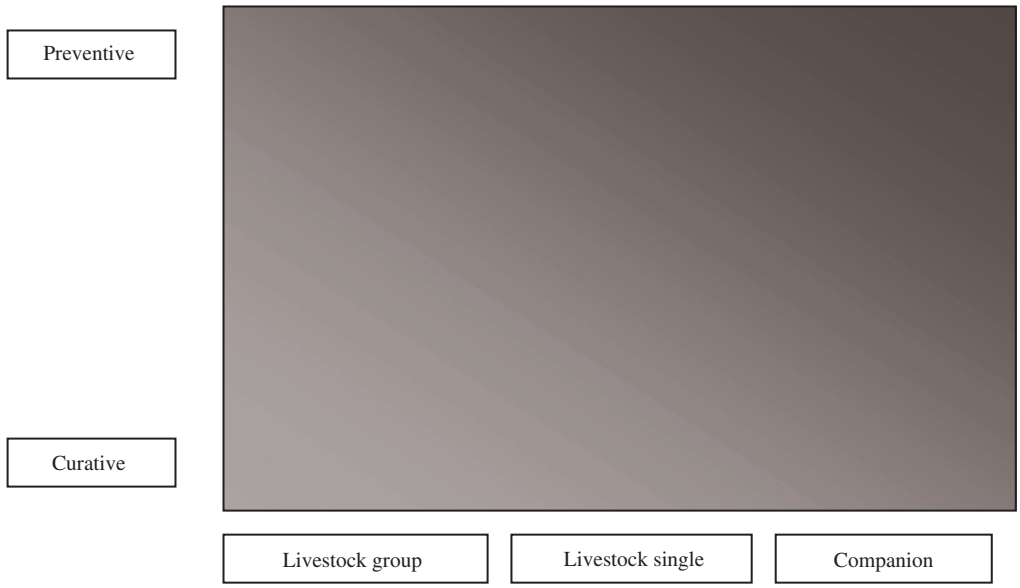

Fig. 10.1 Animal type, product type and pharmacovigilance reporting.

are used and so any error (e.g. in food or water mixing) may affect a large number of animals. MA holders should have crisis management plans in place for these types of products.

Preventive versus curative treatment

While the general differences between food-producing and companion animals apply both to curative and preventive treatments, there is a tendency for preventive treatments to be more prone to adverse events reports. Preventive in this context should be understood as any treatment administered to animals that at the time show no apparent symptoms. It is not limited to vaccines, but also applies to treatments such as routine worming. There is probably a double explanation for this phenomenon. Preventive treatments are normally administered to healthy animals. Owners seem in general less tolerant of adverse effects in healthy animals treated prophylactically than they are of adverse effects in sick animals treated therapeutically. Furthermore some side effects in sick animals are more or less masked by the disease itself and are not always recognised as a side effect of a treatment.

The relationship between animal type and product type with respect to likelihood of pharmacovigilance reporting can be represented with a simple diagram as shown in *Figure 10.1*, where the darker area shows the likelihood of reporting being highest with preventive treatments with companion animals, and the lighter area shows it lowest with curative treatments for livestock.

Conclusions

There is a complex raft of veterinary pharmacovigilance in the EU governing virtually every aspect of adverse event reporting – from the way that reports are written and submitted to regulatory authorities, to the use of electronic reporting systems and the operation and maintenance of databases. These regulatory activities are not necessarily confined to the EU but may extend far beyond its boundaries, ranging from the maintenance of computer hardware and software facilities on other continents, to third country reporting of adverse drug reactions. The majority of these tasks and responsibilities, some quite onerous, fall to the responsibility of the QPPV. This person has the ability to ensure the operation of an efficient and compliant pharmacovigilance system or he/she can turn it into a

ruinous legal liability. Whatever, it is clear that the QPPV requires the full support and assistance of numerous departments within a company, ranging from information technology, and technical services, to sales and marketing and finance, if he/she is to operate properly. It is clear that the QPPV deserves the respect and full support of all of those who work in animal health companies.

Veterinary pharmacovigilance has seen an exponential growth over recent years. This is particularly true of the requirements, obligations and responsibilities placed on the Qualified Person, whose tasks have become extremely complex. Moreover, the actual implementation of these requirements across the EU has become, if anything, disharmonised. Recently, there are signs that veterinary pharmacovigilance is entering a period of consolidation, rationalisation and harmonisation, which is to be welcomed. This gives rise to hopes that the qualified person will soon be able to work under much clearer and well-defined pragmatic systems. This will make it easier to use the available resources for what is probably now the most challenging task – the improvement of the quality and frequency of adverse drug reaction reports from the field.

11 Veterinary pharmacovigilance in an industry setting – the European Union

M. O'Gorman

Introduction

Unfortunately, a disclaimer is required with regard to the content of this chapter since it is based on experience of pharmacovigilance practice in accordance with Part II of Volume 9 of *The Rules Governing Medicinal Products in the EU*. For veterinary medicines, this document is to be replaced by Volume 9B, the publication of which has been subject to considerable delay and has been imminently expected for at least a year at the time of writing. (The corresponding document dealing with medicines for use in humans, Volume 9A, was published in January 2007.) At present, one section of Volume 9B, *Guideline on Monitoring of Compliance with Pharmacovigilance Regulatory Obligations and Pharmacovigilance Inspections for Veterinary Medicinal Products*, has been published, prefaced by a statement to the effect that in all else Volume 9 must be adhered to until its replacement appears. This is currently available in mid-2008 as EMEA/CVMP/PhVWP/430286/2007 – draft 13. It is, of course, possible that the publication of this book will coincide with the much-awaited appearance of the new guidelines (expected late 2008 or early 2009), in which case, parts of this chapter may become instantly obsolete.

This possibility is symptomatic of a system that is at present in a transitional phase, as recent changes in the underlying EU legislation increasing the emphasis on pharmacovigilance are in the process of being implemented. Unfortunately, the rate and degree of implementation vary across the member states of the EU. As a consequence of this, it seems reasonable to make a major theme of this chapter the collision between the ideal, represented by legislation and guidelines, and the more muddled reality of a system involving multiple human and corporate actors with possibly divergent, and often poorly defined, aims.

Whilst it is in the interests of consumers (veterinarians, farmers and owners of companion animals), animal health companies and regulatory authorities to maintain a system whereby therapeutic or prophylactic products are appropriate in their range of actions, safe for use by humans and in animals, and freely available, though subject to control to avoid misuse, the correct balance between these factors may be a matter of dispute between the various actors. It might be thought that the wishes of the regulatory authorities would trump all others (and one certainly gets the impression at times that this is their opinion), but they are constrained both by the legal framework under which the whole

system operates and, as government agencies, by political pressures which may indirectly represent the interests of other groups.

For the pharmacovigilance practitioner in industry the challenge is to negotiate a path through the thicket of pressures generated by conflicting views of the function. Obviously the aim should be to comply with the legislation, both in its aims and its details, while maintaining the supply of needed products, without bankrupting the company. Ultimate responsibility for this rests on the Qualified Person for Pharmacovigilance (QPPV). The existence and duties of this post are laid down by the legislation, although rather poorly defined in places. The holder is theoretically in a position of great power, but is also the point at which the buck stops.

There is an inevitable tendency in the commercial functions of many, if not all, companies to regard pharmacovigilance solely as an additional cost in the maintenance of a product in the market place. However, I would argue that there are a number of ways in which pharmacovigilance can properly be regarded as a contributor to competitive advantage for animal health companies. In practice, pharmacovigilance in the animal health industry involves the marketing authorisation holder (MAH) on a number of different levels. At the base is the legal requirement to provide the data necessary for post-authorisation surveillance by the regulatory authorities of the safety and efficacy of licenced products. This requirement is now based in European Union law, transposed into national regulations and guidelines. The system has traditionally focused on safety, although in recent legislation there is increased emphasis on also monitoring efficacy. At the same time, because the MAH is a commercial enterprise, the processes of pharmacovigilance involve interaction between the MAH and its customers, thus also becoming a customer relations issue. On a further level, aspects of quality control within the processes of manufacture and delivery of the products may be involved. Considered from these three points of view, the desired responses may, in certain circumstances, differ or even conflict.

In terms of post-authorisation surveillance of product safety, the primary aim is to establish whether or not there is a causal relationship between the suspected adverse reaction (SAR) and the product. Where the observed signs are already known to be associated with the product, little investigation may be required, but in other cases, clinical examination of affected animals, laboratory tests or post-mortem examinations may be necessary. Because of the time taken to establish causality, this process may be interpreted by the owner of the animal(s) as an attempt to delay or avoid acceptance of 'responsibility' by the MAH. Investigation of quality control issues at the manufacturing level may take even longer.

For this reason, there may be a tendency for those directly involved with the customer to be too ready to assume a causal relationship between a reaction and the product in order to maintain good commercial relations. Within animal health companies there is a continual need for the pharmacovigilance function to educate their commercial colleagues to regard the system as an important part of maintaining confidence in the company and its products, rather than inconvenient red tape or a glorified complaints system.

The legal basis of veterinary pharmacovigilance in the EU

As described in Chapter 2, the present system of pharmacovigilance in the EU has its basis in Directive 2001/82/EC, as amended by Directive 2004/28/EC, and Regulation (EC) 726/2004. These set out the basic framework and refer to published guidelines for the detailed application of these rules. These guidelines were published as Volume 9 of *The Rules Governing Medicinal Products in the EU*, with veterinary products being covered in Part II. Administration of the system, apart from when dealing with products authorised through the Centralised Procedure (CP), is through the member states' national regulatory authorities (referred to as National Competent

Authorities or NCAs). Whilst marketing authorisations (MAs) may apply to a single country, as national authorisations, to several countries via the mutual recognition procedure (MRP) and the decentralised procedure (DCP) or to the whole of the EEA through the centralised procedure (CP), all products are covered by the same pharmacovigilance requirements (see Chapter 2). Thus one might expect a reasonably straightforward system with a single set of guidelines to be followed.

Unfortunately, the implementation of Directive 2004/82/EC has not been smooth, leading to several anomalies and a degree of confusion and a distinct lack of a harmonised approach. Following the entry into force of the amendments contained in the Directive in November 2005, Volume 9 was to be updated and split into Volume 9A dealing with products licensed for use in humans, and Volume 9B dealing with veterinary products. The former was published in January 2007, whilst the latter has yet to appear, with the exception of the *Guideline on Monitoring of Compliance with Pharmacovigilance Regulatory Obligations and Pharmacovigilance Inspections for Veterinary Medicinal Products*. Although the cover page of this document states that until Volume 9B is published, Volume 9 remains in force, the text refers repeatedly to the need to comply with the content of Volume 9B.

In place of the previous requirement for 5-yearly renewals of the MA, the new Directive established a single renewal 5 years after initial authorisation leading to the granting of an authorisation of unlimited duration. Further monitoring of the product is to be primarily through pharmacovigilance, particularly through the submission of Periodic Safety Update Reports (PSURs). At present there is a discrepancy between the timetable for PSURs contained in Volume 9 and that contained in the amended Directive.

In addition, a revised draft of the guidance on pharmacovigilance for MAHs was produced in 2004 (EMEA, 2004), ostensibly to conform with the content of Volume 9B, but this version has not been accepted by the European Commission (among other things, the authors appear to have been unaware of the changes affecting MA renewals and PSURs contained in the 2004 Directive). However, some NCAs have decided to act as if the revised guidelines had in fact been accepted. To compound the confusion, a draft guideline for regulatory authorities on the assessment of PSURs, issued for consultation in 2007 (EMEA, 2007), refers in its text to the PSURs complying with Volume 9, ignoring Volume 9B entirely.

NCAs of course publish local regulations, which, since they are intended to give effect to the Directive, logically should comply with it and the guidelines to which it refers. It is worth noting that whereas some EU directives allow national authorities the scope to introduce national measures that go beyond those contained in the Directive, there is no such provision in the directives concerning veterinary pharmacovigilance. However, it is not unknown for NCAs to be unable to resist the temptation to introduce additional requirements, either in anticipation of new EU legislation or guidelines, or due to treating the EU requirements as a baseline to be 'gold-plated' at the national level. A degree of wilful ignorance of the primacy of EU law over national law may become apparent if this is raised with the NCA.

So instead of a relatively simple reporting system, overseen by the EMEA and administered by the NCAs, requiring the provision of a common accepted set of information for products across the EU, there are currently two guidelines, one of which we cannot see, and conflicting instructions from different authorities as to which to follow. Since the reporting requirements for individual cases are unlikely to undergo any major changes in the new guidelines, the main area where this may become a problem is in the production of PSURs. Particularly with regard to MRP products, this means that the form and content of PSURs may vary according to the choice of Reference Member State (RMS). From the industry point of view, the risk is that certain NCAs will demand extra information, which then becomes accepted as standard even though not required by law, thus increasing the

regulatory burden on the MAH. Given that the underlying legislation aims at the approximation of regulatory measures across the EU in order to facilitate the free movement of goods, this would be a rather disappointing outcome.

The basic process of pharmacovigilance practice

Turning now to the basic processes of pharmacovigilance practice in industry, it is as well to set out the ideal as embodied in the legislation and related guidelines. One can then comment on the ways in which the process is complicated by contact with the real world.

The ideal

Pharmacovigilance in industry consists, in practical terms, of a series of steps. These consist of receiving and recording reports of suspected adverse reactions (SARs), cases of suspected lack of expected efficacy (SLEEs) or cases of suspected violation of the EU maximum residue limits (MRL) in animal products, with further investigation being required in some cases, assessing the likely causality, and reporting the reactions to the appropriate regulatory authority. Provision must also be made for action to be taken, if necessary, based on these reports.

Receiving and recording reports

The vast majority of reports of SARs or of SLEEs are received as spontaneous reports, although a small number may be reported as part of post-authorisation trials of products for new indications or in new species. The route by which these are received varies in different member states of the EU. In the United Kingdom, most reports are received directly from veterinary surgeons, farmers or companion animal owners. Depending on the product, others may be received via wholesalers, agricultural merchants or the MAH's sales force. A relatively small proportion of reports arrive via the regulatory authority, the Veterinary Medicines Directorate (VMD), having been reported via the Yellow Card scheme. This is in contrast to the situation in, for example, France, where the majority of reports are submitted to the Agence Française de Sécurité Sanitaire des Aliments, Agence Nationale du Médicament Vétérinaire (AFSSA/ANMV) and then forwarded to the MAH.

The primary requisite at this stage is that the basic data required to comply with the legislative definition of an SAR report is collected and accurately recorded. This can be achieved either by using specifically designed computer software which is structured to prompt the recorder to collect the appropriate data, or by using printed forms with the required fields completed manually. When reports are received by telephone, this can be relatively simple, as the reporter can be questioned in a structured way following the form or computer program. With written reports, which are often composed as letters of complaint, relevant details are often missing, and follow up, either by telephone or by letter, enclosing a questionnaire, is required. The yellow form produced by the VMD is designed to capture the relevant data, provided that it is completed properly. It would be expected that the MAH would have standard operating procedures in place to cover both the initial recording of data and the process of following up to obtain missing details, including timetables for these operations.

In addition to the recording of a case narrative, certain data should be recorded according to a specific list of terms. These include the VEDDRA list of clinical terms for reporting SARs in animals contained in EU guidelines EMEA/CVMP/413/99-Final–Rev. 2 and EMEA/CVMP/PhVWP/1752/05, and EMEA/CVMP/189/04 listing terms to be used for reporting adverse reactions in humans to veterinary medicinal products. A further document, EMEA/CVMP/553/03, lists species and breeds for electronic reporting of SARs in animals.

Investigation

Many reports will be straightforward involving clinical signs listed in the product Summary of Product Characteristics (SPC), therefore requiring little or no investigation. A typical example would be an injection site swelling following the use of a vaccine containing an adjuvant. In other cases, the signs may not be commonly associated with reactions to the product, or they may be possibly indicative of an unrelated problem. Diarrhoea in a pup occurring 2 days after the initial injection of a primary vaccination course would be an example of a report requiring further investigation. This would be undertaken firstly as a matter of the MAH's obligation to investigate SAR reports, but also, in terms of customer relations, it indicates to the reporter that the matter is being taken seriously. This investigation may take the form of clinical examination by a veterinary surgeon, laboratory tests or post-mortem examinations in the case of deaths. In some cases, unused product may be returned for either potency testing or sterility testing.

Causality assessment

Once the reported SAR has been recorded, an assessment must be made of the likelihood of a causal relationship between the product and the observed signs. This should then be recorded using the ABON classification[1] (see Chapter 27). In many cases the classification can be decided on the basis of the initial data, but in others follow-up information may be required before an assessment can be made. The results of further investigation may either confirm or lead to a change in the classification.

Again, an EU guideline, EMEA/CVMP/552/003, exists which attempts to set out a framework for considering the data in reports so as to harmonise the assessment of causality across different member states by both NCAs and MAHs. At present, this framework is not proposed as an algorithm for causality assessment, but the document recommends reappraisal of the guideline after 3 years' experience, i.e. in October 2007, to consider whether such an algorithm might be developed. Pragmatically, the guideline accepts that even if an algorithm is established, there needs to be scope for its results to be overridden by professional judgement in certain cases.

Reporting

Once received, the report must be assessed with regard to the possible requirement to submit it to the regulatory authority as an expedited report. Suspected adverse reactions occurring within the EEA that are serious or that involve human exposure to products must be reported to the regulatory authority of the member state in which they occur within 15 days of receipt by the MAH. Reports received in 'third countries', i.e. countries outside the EEA, must be submitted as expedited reports if they are serious and unexpected, involve human exposure or involve the transmission of an infective agent via a veterinary medicinal product. A standard form agreed by the CVMP is used for these reports, and may be either completed manually or generated automatically by the pharmacovigilance software.

Article 1 (12) of Directive 2001/28/EC defines a serious adverse reaction as one

> '. . . which results in death, is life threatening, results in significant disability or incapacity, is a congenital anomaly/birth defect or which results in permanent or prolonged signs in the animals treated'.

Volume 9 modifies this definition with regard to intensively farmed animals such as poultry, fish or bees which are usually medicated as a group. Here an increased mortality above the 'normally probable' death rate, severe clinical signs or exceeding variations in expected animal production rates qualify the report as serious. However, individual deaths in livestock such as cattle, sheep, pigs and goats warrant an expedited report, even if they are reared intensively, as do deaths in companion animals[2]. An unexpected

[1] A – probably product related, B – possibly product related, O – unclassified, N – unlikely to be product related.

[2] Volume 9, Part II, S1, ss5.

reaction is defined in Volume 9 as one 'the nature, severity and outcome of which is not consistent with the summary of product characteristics[3].

A distinction should be drawn between severity and seriousness of suspected reactions. For example, a large injection site reaction is more severe than a small one, but not more serious, unless the smaller results in prolonged morbidity.

These reports should be included along with reports of non-serious SARs, MRL violations, SLEE reports, extra-label use and adverse environmental effects in the PSURs, submitted in accordance with the timetable set out in Directive 2004/28/EC. For the first 2 years from the date of issue of the Marketing Authorisation (MA) for the product, a PSUR must be submitted every 6 months. For the next 2 years the reports are submitted annually, and thereafter every 3 years. Pharmacovigilance data covering the whole period since authorisation must also be included in the documents submitted for the renewal of the product's MA 5 years after the original authorisation. Since these documents must be submitted 6 months before the renewal is due, and the PSUR data should include SARs that occur up to a data lock point (DLP) 60 days before the submission date, this means that a further PSUR is required 4 months after the sixth report in the normal sequence (the second annual report). According to Volume 9, this should be a bridging report as an addendum to the previously submitted reports, and does not affect the normal sequence of periodic submissions. However, this has been considered impractical by some regulatory authorities since it would lead to the existence of duplicate reports of the SARs received during the 4-month period.

Action

The most likely action required in response to accumulated pharmacovigilance data would be an amendment or addition to the SPC of a product. Once a product is in commercial use, the number of animals exposed to it will (hopefully) be far larger than the numbers involved in preclinical or clinical trials. It is therefore possible that SARs with a low incidence may be observed that were not identified prior to authorisation, necessitating additional warnings in the product literature. With less severe reactions, the need for action may be identified after the submission of PSURs or at the renewal of the MA. More serious reactions may be identified through expedited reports. In extreme cases SARs may be of sufficient seriousness or severity to warrant curtailment of the use of the product or even withdrawal. Where necessary, therefore, the MAH must be able to recall a product from the supply chain and notify users of the requirement to return unused product.

Practical complications to the ideal

The impact of the real world complicates the orderly and relatively simple theoretical process of pharmacovigilance at all stages, but especially at the very first stage.

Receiving and recording reports

The information required to constitute a reportable case is set out in Volume 9:

1. MAH case reference number (+ country where incident reaction occurred if different to the country of the member states concerned, or if Community authorised product).
2. Date(s) of treatment(s)/date(s) of vaccination(s).
3. Was the product used as recommended?
4. Date of adverse reaction.
5. Number of animals treated.
6. Species.
7. Age(s).
8. Number of animal(s) reacted (approximate).
9. Number of animal(s) dead.
10. Other products used concurrently.
11. Clinical signs/diagnosis.
12. MAH comments and causality assessment (A, B, O, N code).

[3] Volume 9, Part II, S1, ss5.2.

As a list of data to be collected, this seems eminently reasonable and proportionate to the purpose for which it is being collected. However, only the first and last entries on the list are directly under the control of the recorder of the report. For the other parameters the MAH is dependent upon the reporter. For a variety of reasons, the reporters of SARs may be remarkably resistant to providing this information. In part this may be due to a misunderstanding of the nature of pharmacovigilance reporting, a matter of which the general public, or farmers for that matter, might be expected to have a rather vague conception. Less excusable is the fact that many veterinary surgeons and nurses also appear to consider these reports more as a matter of customer complaints than of post-authorisation surveillance of licenced products.

As described above, telephone reports can provide a good opportunity to extract the relevant facts through a structured questioning process, but unfortunately the caller may not be prepared for this and therefore not have the relevant details to hand. This is particularly likely where the reporter has seen more than one case of a reaction over a period of time before deciding to report it. In these cases, dates in particular may be vague, but also the numbers involved, ages, outcomes and even the clinical signs or product name may be unclear. For the MAH, this poses the problem, particularly where an expedited report may be warranted, of obtaining the missing information within a time frame specified by either an internal company SOP or that set by the regulatory guidelines.

Whilst pet owners are often keen to complete a follow-up questionnaire promptly, one occasionally encounters reporters who appear convinced that the less information they provide, the more difficult it will be for the company to launch the cover-up which they assume to be the default response of pharmaceutical companies. Farmers and veterinary surgeons can also pose something of a challenge in terms of acquiring the full range of data required. They are, of course, generally busy people, and often rather paperwork averse, but sometimes the attitude appears to be that they have done their bit by making the initial report. In other cases, the initial report seems to have been made in a state of annoyance, which has abated (possibly in response to the professional manner in which their complaint has been handled) by the time the questionnaire arrives, and there is a reluctance to make any more fuss. Occasionally, after the initial contact the reporter may have realised that they had misused the product and therefore prefer to forget about the matter, so declining to provide further details.

Reporters within the company can also, usually through a desire to be helpful, cause problems in collecting data. The scenario that I am envisaging here is that of the field operative who has a good relationship with a customer, receives the initial report, and 'sorts things out to everyone's satisfaction'. Of course they may have done a very good job of discussing the signs observed and the likely causality, explaining the incidence of such reactions and discussing any possible product misuse, resulting in a happy, or at least mollified, customer. However, in the process they may not have actually collected the relevant data. This then leaves the pharmacovigilance operative to go back and 'pester' the customer to obtain the necessary information. The response to this can be variable, to say the least.

Although the list of data required is generally not contentious, there is one parameter where the differing interpretations of different regulatory bodies can cause problems for a multinational company. This is the 'number of animals reacted' figure in SLEE reports. In reporting to the regulatory authorities in the USA, this figure is given as zero, since there is no adverse reaction, and this also seems to be acceptable to the EMEA. However, at least one European NCA insists that a figure should be entered, on the grounds that the lack of reaction is a reaction. Apart from the semantic difficulties associated with this statement, SLEE reports are not included in the calculation of incidence of SARs as set out in Volume 9, so the relevance of the figure is questionable. This would seem to be an example of the willingness of some NCAs to introduce extra requirements above those set out in the EU legislation.

In spite of these problems, there is usually sufficient data to enter some form of report in the record and it is preferable to err in the direction of recording incomplete reports rather than to reject them. Certainly, if a regulatory authority suspected that significant numbers of reports were being discarded due to incomplete data, they would require both a convincing explanation and prompt corrective action.

Investigation

The main aim of investigation should be to obtain sufficient information to confirm or disprove, as far as possible, a causal connection between the product and the SAR, but for various reasons, the level of investigation may be affected by factors more related to the reporter than the product. Apart from establishing causality, investigation may be necessary just to establish the facts of the case. For example, in some reports, usually received from members of the public, the initial description of the signs may be so vague that investigation is required just to determine what is actually being reported. Initial reports in other cases may raise a suspicion that the description of the signs has been affected by an assumption on the part of the reporter as to the causality. Such reports are not usually deliberately misleading, but often stem from a reporter with a limited knowledge of possible adverse effects trying to make sense of what is going on by interpreting what they observe before they report it.

Unfortunately, one does also occasionally receive reports from veterinary surgeons where an over-hasty diagnosis has been made and the description of the signs appears to be to some extent adapted to fit this. In many cases multiple signs may be present, and the weight to be placed on each is a matter of clinical judgment, which can sometimes be distorted by a narrow focus on a particular possible cause. One would hope in cases like these that evidence provided by further investigations will lead to refocusing of attention on the most significant signs, thus allowing a correct diagnosis, thereby aiding causality assessment.

As stated in the previous section, many reports will not justify further investigation, since the signs are listed in the SPC or are expected from the pharmacology of the product. There may, however, be pressure from the attending veterinary surgeon to perform some form of investigation. In some cases this may stem from a desire to placate their client by showing that something is being done, and a simple clinical examination may be sufficient, whilst in others, the veterinarian may be conforming to a culture that emphasises 'working up' a case. At times one might suspect that the latter situation is affected by the perception that pharmaceutical companies have deep pockets. That said, it should also be accepted that the ethos of some veterinary practices is based on higher than usual levels of diagnostic investigation, and this is what their clients expect. In both these situations, it may be worthwhile for the sake of goodwill to fund some investigation, regardless of the likelihood of producing meaningful results.

Other reports may involve signs either possibly attributable to other causes or simply unlikely to be related to the product, for example, due to the time lag between exposure to the product and the appearance of signs. In the latter case, it might be argued that investigation is unnecessary and a waste of resources, but again there may be customer relations reasons to investigate. Where the observed signs are compatible with an adverse reaction to the product, but other possible causes also exist, investigation is generally warranted to establish, if possible, the actual cause. However, because of delays in reporting SARs to the MAH, the opportunity may be lost. This is particularly true if the report arrives via a regulatory agency, especially if the agency has the practice, as some do, of forwarding reports to the MAH in batches rather than individually as received.

Some types of reaction may not provide scope for investigation, for example a transient non-fatal anaphylactoid reaction will leave no ongoing signs, particularly if symptomatic treatment has been given. Treatment for other suspected reactions may also hamper investigation due

to the effects of the drugs used on blood, for instance, blood biochemistry. In other cases, such as reports of the MRL for an antibiotic used for dry cow mastitis prevention being exceeded in milk after the end of the withdrawal period, investigation is dependent upon the availability of retained samples, which are not always forthcoming. In cases of sudden death in livestock, the carcass may have been disposed of before the report is received, preventing a post-mortem examination being carried out. Similarly, if a death occurs in a companion animal the owner may be reluctant to agree to a post-mortem examination which they regard as intrusive. Rather frustratingly, in some reports received via the regulatory agency the reporter may have requested that their contact details should be deleted, which they have a right to do, but it does mean that follow-up investigation is impossible.

Thus in some circumstances the investigations carried out may be greater than truly warranted, whilst in others attempts to reach a definite conclusion as to causality may be hampered by actions carried out after the reaction has occurred. In the majority of cases of suspected adverse reactions cases, no one would argue that treatment should be withheld in cases where it interfered with later investigation, but it is certainly frustrating to be confronted with a report where the causality is uncertain and the evidence has been discarded or destroyed.

Causality assessment

Causality assessment poses a number of problems, some of which relate to the previous sections. Obviously, deficiencies in the data arising either from difficulty in collecting basic facts or from problems in carrying out further investigations will affect the ability to make an accurate assessment of the possible connection between observed signs and the product. It is also important where the results of investigations contradict the original assessment that the assessment is changed in the records, particularly if the case involved an expedited report. In addition there is the fact that the ABON system of classifying causality is a rather blunt instrument.

The difficulty in definitively assigning causality is recognised in the ABON system in the sense that it categorises reports on a scale of probability, so that the strongest statement of causality is 'probably', rather than 'definitely' product related and the weakest 'unlikely to be', rather than 'definitely not' product related.

Guideline EMEA/CVMP/552/03 sets out a series of subsidiary questions to be answered with a view to establishing the strength of the data in providing answers to six basic questions:

1. Is there a reasonable association in time and/or anatomical site between administration of the product and the occurrence of the reaction?
2. Is there a reasonable association with the known pharmacological/toxicological profile, the allergic profile of the product, and/or a dose–effect relationship?
3. Are any characteristic clinical or pathological phenomena present, and are there any objective confirmatory factors such as laboratory or post-mortem examination results?
4. Is there previous knowledge of similar reactions to this product, from the SPC, published literature or previous SAR reports?
5. Is there another possible cause for the signs observed, including the animal's health status, the disease for which it is being treated or other products administered concurrently, and is that cause more likely than an SAR to this product?
6. Is the reported information insufficient, and is there reason to doubt the reporter or the information?

Without going into too much detail, the different causality assessments require, as a minimum, the following combinations of answers to these questions:

- A: 'probably product related'
  – positive answers to (1) and (2) and negative answers to (5) and (6).

- B: 'possibly product related'
  – positive answers to (1), (2) and (5), plus a negative answer to (6) *or* a positive answer to (1) *or* (2) combined with positive answers to (4) and (5), plus a negative answer to (6).
- N: 'unlikely to be product related'
  – a positive answer to (5) and negative answer to (6).
- O: 'unclassifiable'[4]
  – a positive answer to question (6) regardless of the answers to the other questions.

From this, it is apparent that the answer to (6), i.e. the quality of the data, is of the greatest importance if one is to avoid assessing everything as 'O'. In practice, there are factors that may interfere with the ability to answer the other questions, but hopefully not to the extent that this becomes the critical factor in too many cases.

As previously discussed, the data necessary to answer question (1) is usually, though not always, available. However, certain types of adverse reaction, such as teratogenicity or carcinogenicity, may be poorly correlated to the time and site of exposure. By itself, a positive answer to (1) may be insufficient, for example the administration of an antibiotic intravenously to an animal *in extremis* may be followed shortly after by its death, without any adverse reaction being involved. Questions (2) and (4) are relatively independent of any deficiencies in the data reported, provided that the reporter is able to describe the reaction reasonably accurately, which is not a foregone conclusion if dealing directly with a member of the public. (A colleague in clinical veterinary practice once told me that he had used every term he could think of, from the technical to the obscene, in the course of a consultation, and had still been unable to discover from the client whether or not their dog was suffering from diarrhoea.)

The confirmatory data referred to in question (3) may be missing either due to the time lag between the reaction and its being reported or because of lack of co-operation by the reporter in collecting it, for example by discarding samples or disposing of a carcass. Question (4) can only affect the causality assessment if the answer is positive. The fact that a reaction has not previously been reported should not be used to dismiss it as unrelated to the product if other factors indicated a possibility of a causal relationship.

Both the accuracy of the description of the signs and the completeness of the data (with regard to the reason for use of the product, the animal's state of health and the concurrent use of other products) will affect the answer to question (5). It should also be considered that the experience of the person assessing the causality may affect the answer to this question. A particular cause of certain signs might be sufficiently rare that only a clinical specialist or a general practitioner of considerable experience is likely to have encountered it. Many general practitioners might not be familiar with it and it might not appear in general textbooks. Even if it were identified, the problem would then be to decide whether the rare medical condition is more likely than an adverse drug reaction. It should be borne in mind that the apparent rarity of the condition may be due to under-diagnosis, which in turn might be affected by the frequency with which the signs are attributed to a drug reaction.

In addition, the pattern of disease may change over time, so that the balance of probabilities may change. An example of this might be diabetes mellitus in cats, which was rarely diagnosed about 20 years ago, partly because it was not tested for; certainly it was under-diagnosed. This condition can be triggered by exposure to anti-inflammatory corticosteroids or steroidal hormones, both of which were commonly used on a long-term basis for the treatment of allergic skin disease in cats. However, the relevant diagnostic tests are now routinely performed, and the condition is recognised as common in elderly cats, even without exposure to these drugs. In fact,

[4] Both EMEA/CVMP/552/03 and EMEA/CVMP/345/98-Rev.1-FINAL, *Guideline on Procedures for Competent Authorities for Pharmacovigilance Information for Veterinary Products*, suggest the splitting of the O category into subcategories: O1 – inconclusive (where other factors prevent a conclusion being drawn, but a product relationship cannot be discounted); and O2 – unclassified (where insufficient or unreliable information does not allow any conclusion to be drawn). However, this suggestion does not appear to have been put into practice, even by those NCAs that seem keen to extend requirements beyond those required by the legislation.

the incidence of the disease appears to be rising, even though the use of steroids has declined.

Reporting

Although the 2001 Directive set out the aim of introducing electronic reporting across the EU, progress in this has been slow, and at present only limited functionality exists. In addition, some member states have proceeded with their own computerised systems, which are not necessarily compatible with each other or with the EudraVigilance system developed by the EMEA. However, the basic details of reporting SARs will remain the same whether reported manually or electronically.

Reporting of SARs is divided into expedited individual reports and periodic aggregated reports. The former involve suspected serious reactions, according to the definitions in the Directive and Guideline noted above, plus adverse reactions in humans. The requirement for the MAH to submit these reports within 15 days of first being informed can cause problems, particularly if the original report is incomplete, since it is difficult to be sure that a follow-up questionnaire will be returned within that time. There may therefore be a tendency to submit more expedited reports than are really warranted, since the follow-up information may indicate that the SAR does not in fact meet the criteria to be defined as serious. However, since failure to submit these reports is viewed seriously by the regulatory authorities, it is better to err on the side of caution, and submit a follow-up report clarifying the situation later. Even if the time limit were 30 days there would be no guarantee that a completed questionnaire would be returned in time. It is not unknown, where the report involves a product used seasonally by farmers, for the completed questionnaire to surface at the start of the season in the following year.

Some problems exist with regard to the definition of which SARs are serious in the use of terms that are themselves undefined. The reference to 'significant disability or incapacity' requires interpretation, as does the term 'permanent or prolonged signs'. For instance, the degree of lameness that would cause a significant incapacity would be different for a racehorse than for a sheep. It may also be difficult or impossible to know whether signs are going to be permanent or prolonged within 15 days. A long-haired dog that developed a small area of alopecia following treatment is certainly likely to take quite some time to return to its normal condition, but the reaction could hardly be classed as serious (unless of course it is a pedigree dog, in which case the owner may well be reaching for a lawyer, claiming that the dog would have been Supreme Champion at Crufts and sired an enormous number of extremely valuable pups.) Some NCAs also produce guidelines to flesh out the EU definitions, but unfortunately they are not necessarily consistent with each other.

Another area where clarity is lacking is with regard to reports of suspected lack of expected efficacy where the animal dies. Examples would include the sudden death of a bullock vaccinated against clostridial disease or the death of a calf with respiratory disease in spite of antibiotic treatment. In the Directive and guidelines SLEEs are referred to separately from SARs, and the expedited reporting requirements clearly refer to SARs. However, some NCAs, as noted above, fail to distinguish between the two types of reports, and so it may be deemed expedient to submit expedited reports in these cases, even though other regulatory authorities may not actually want them.

Serious and non-serious SARs are aggregated for periodic submission (PSURs) in accordance with the timetable set out in Directive 2004/82/EC, which replaced that set out in Directive 2001/28EC from 30 October 2005. According to the previous timetable, PSURs were submitted every 6 months for 2 years after the MA was granted, then annually for 2 years, and then with the MA renewal application 9 months later (for renewal of the authorisation at the 5-year point) and then at 5-yearly intervals with successive MA renewals. The new Directive requires only a single MA renewal, with the application submitted 6 months in advance of the renewal date, and shifts the function of safety monitoring to 3-yearly PSURs after the first 4 years. As set

out, the renewal application requires copies of previous PSURs plus a bridging report covering the next 4 months, which is separate from the regular PSUR timetable. The next PSUR would then be due 7 years after authorisation and would include the data submitted in the bridging report.

However, at present the system does not work quite like that. As part of the transition to the new system it was decided that each product should undergo one MA renewal after the introduction of the new timetable, at the date that it would have been due under the previous system, but also that every product should be the subject of at least one PSUR before the third anniversary of the new regime, i.e. by 30 October 2008. For products that have already had one or more MA renewals, there would be no bridging report, since the last PSUR will be 5 years old.

Another complication involves MRP products, where the PSUR timetable may be based on the date of day 90 of the MRP, while the MA renewal date is based on the date of the original MA in the Reference Member State (RMS). In addition, in order to avoid overlapping sets of data, the NCAs seem to prefer the first PSUR after the renewal to run for 3 years from the end of the report submitted with the renewal. Quite a number of products are therefore caught in a transitional state, and the simplest thing to do seems to be to ask the NCA of the RMS when they expect the next PSUR following a renewal.

Proposals have also been put forward to synchronise the submission of PSURs for all products containing the same active ingredient or combination of active ingredients across the EU. Whilst this will eventually lead to greater transparency in terms of comparing products, superimposed on the other transitional measures it is likely to complicate matters in the short term. There is also a proposal to synchronise PSURs for vaccines according to species, although how this would work for vaccines authorised in more than one species is unclear.

The PSUR should include all SARs reported during the period covered, including those in third countries, in tabular form as line listings. The line listing includes the standard information set out in Volume 9 as necessary to constitute a report. Those that have previously been submitted as individual expedited reports or were received from NCAs should be identified, with the authority reference number if available. Suspected adverse reactions in humans should also be listed separately.

In the past, for repeat MA renewals for nationally authorised products, NCAs were prepared to accept the line listing plus a calculation of the incidence rate for reactions to be sufficient for a PSUR, although some also required formal statements that the overall safety and efficacy were satisfactory. However, Volume 9 does set out additional information to be included, and most NCAs now require this information both for MRP and nationally authorised products, and the EMEA requires it for CP products. This includes a brief narrative review of the cases reported in the line listings and any published SAR reports, together with a bibliography of the latter. A critical overview of the safety profile should also be provided and opinion on the benefit:risk analysis for the product should be provided, specifically addressing the following:

- evidence of increased toxicity;
- increased frequency of known toxicity;
- product interactions;
- overdose and its treatment;
- suspected adverse reactions associated with extra-label use;
- human adverse reactions.

Lack of significant new information in any of these categories should also be reported.

The quality of reports may cause problems in the preparation of the narrative review, due to missing information. This may be particularly true in older reports and those from third countries where reporting is not a legal requirement. Unfortunately, even a few years ago within the EEA, pharmacovigilance had a much lower priority than it has now, as a minor function attached to other jobs, and one gets the impression that in some cases it was considered sufficient that a

report had been recorded at all. There may also have been a tendency to overuse the 'O' category, or to leave the causality assessment blank (possibly on the grounds that the regulatory authorities are better qualified to make the assessment). To paraphrase Dr Johnson, the prospect of having to produce a sensible narrative for a PSUR certainly concentrates the mind when recording an SAR.

One of the most important parts of the PSUR is the calculation of the incidence of SARs. This figure includes all SAR reports coded A, B or O, but excludes SARs classified N, SLEEs and cases involving human exposure. In some cases, the MAH may disagree with the causality assessment in the original report, but although this can be commented on, the assessment cannot be changed in the PSUR. However, it is reasonable to include reports in the incidence calculation where either no causality assessment is made or an assessment as N has been made that seems to be unreasonable. It should, though, be borne in mind that the original assessment may have been made on the basis of more information than is present in the report as recorded. The inclusion of cases involving extra-label use may be problematic, particularly if they involve use in a non-target species. It hardly seems reasonable to assess the safety of a product authorised for use in cattle on the basis of adverse reactions in sheep. Therefore it may be necessary to perform two incidence calculations, one excluding the extra-label use.

The incidence is calculated by dividing the number of animals affected by SARs by the number of doses administered during the period. For convenience it is assumed that all the total volume sold in the period is administered, although commercial factors such as sales promotions might in fact mean that this is not true. This can produce occasional anomalies, for example where sales of a product are discontinued in a particular country or where the product may be returned by wholesalers for commercial reasons, leading to zero or even negative sales. However, SARs may still be reported related to product sold earlier in the period or towards the end of the previous reporting period. It is also assumed that no product is wasted or allowed to go out of date (i.e. beyond the end of its shelf-life).

While the number of doses sold can be easily calculated for some products, for others only an estimate can be provided. In order to standardise the calculations, Volume 9 suggests the following approach:

- vaccines to be expressed in numbers of doses;
- liquid to be expressed in litres;
- powder to be expressed in kilograms;
- tablets to be expressed in numbers of tablets;
- sprays to be expressed in litres or kilograms;
- flea collars to be expressed in numbers of collars;
- paste to be expressed in kilograms.

Unfortunately, this system, while offering an objective measurement of the volume used, gives figures that are by and large unrelated to the numbers of animals treated. Therefore it is not very helpful for calculating the probability that exposure will be related to a SAR. Whilst, some vaccines require only a single dose, many require two doses for an initial course, with a single booster dose at regular intervals thereafter. The situation becomes more complicated if the product is licensed in more than one species at different dose rates, particularly if it is sold in a number of countries with different patterns of agriculture and hence different mixes of the target species. Equally, the use of litres of liquid or kilograms of powder or paste gives very little indication of the likelihood of an SAR in an animal exposed to a normal dose of the product, such as 20 ml of antibiotic injection or a 37.5-g tube of oral antibiotic paste. Even in terms of tablets, the use of a single tablet as the reference may not be helpful, since the variation in size of companion animals means that many will receive either fractions of tablets or more than one tablet per dose.

The question also arises if, for example, the intention is to compare incidences between a long-acting antibiotic injection and tablets:

Should the number of tablets used be a single dose or a course that gives a similar duration of effect to that of the injection? In the case of products for which PSURs have been produced in the past, there is a strong case for retaining whatever measurement was used previously, in order to allow comparison of reaction incidences over time, but this may make comparison between products difficult.

In an attempt to deal with this problem, the International Federation for Animal Health (Europe) (IFAH-Europe) has published a list of standard weights for different species to be used in calculating the number of doses (IFAH-Europe, 2004). Whilst this is helpful, if the product is used in both adult and young stock, estimation of the proportions used in each may still be necessary. Volume 9 also states that if reliable data are available the incidence for each authorised target species may be calculated. For many products it is doubtful that such data would be reliable.

Incidence calculations should be performed for each member state where the product is authorised, as well as for the EU as a whole. There is no requirement to calculate the incidence for third countries, but it may be felt that to include third country reactions in the line listing without putting them in the context of sales figures risks giving an impression that adverse events are more common than is in fact the case. One feature of the national incidence calculations is that it does highlight the variation in reporting between member states.

Action

Pharmacovigilance data from either expedited reports or PSURs may give rise to concerns relating to the safety of a product, leading the regulatory authorities to require appropriate action by the MAH. This may be due to a series of reports of unexpected and serious SARs, reports of expected reactions that are more severe or have longer-term sequelae than previously described, or a significant increase in the reporting rate of serious adverse reactions. As stated above, the most likely action to be required is a change in the warnings or contraindications contained in the SPC, which will usually be handled through the normal procedure for variations in the authorisation. Other changes to the SPC that might be required include a change in the recommended dose rate, restriction of the indications or restriction of availability. The MAH may also be required to inform animal health professionals of these changes by letter and communications published in appropriate journals, and in some cases also the public. Changes to the PSUR reporting schedule may be required, either to allow closer monitoring of the suspected adverse reactions causing concern or to assess the effects of changes in the SPC.

In rare cases, withdrawal of a product may be required, either in terms of a particular batch or in total. This should be relatively straightforward at the wholesaler and veterinary practice level, but may be more problematic with regard to a product that has already reached the public. For seasonal products this may be particularly true as they may lurk in dark corners of farm buildings or household cupboards for at least another year. It is notable that the publicity surrounding the withdrawal of one product may have a knock-on effect with regard to other products, even if the connection between them is tenuous. A number of years ago a spot-on flea treatment was withdrawn from the UK market due to safety concerns traced back to a problem in the manufacturing process. Since a large amount of the product was already in the hands of the public, the withdrawal was widely publicised to encourage return of unused product. Not only did this product fail to regain its sales once the manufacturing problem had been corrected, but also for a considerable time afterwards many pet owners were reluctant to use spot-on flea preparations from other MAHs, even though they contained completely different active ingredients.

The Qualified Person for Pharmacovigilance

A key feature of the legislation is the requirement for each MAH to have a designated Qualified

Person for Phamacovigilance (QPPV) resident in the EU and 'permanently and continuously at his disposal' (see Chapter 10), the name and contact details of whom must be communicated to the authorities. This person is responsible for establishing, maintaining and managing the pharmacovigilance system of the MAH within the European Community. However, although not mentioned in the legislation, Volume 9A acknowledges the role of an alternative to the QPPV, since no individual can be expected to be literally available continuously, and this provision is likely to appear in Volume 9B. In addition, NCAs often require a national contact within their own territory, although the QPPV would still bear ultimate responsibility.

Although the QPPV is required to be appropriately qualified, there is at present no definition of what constitutes appropriate qualifications. This may be elucidated in Volume 9B, but one would assume that a degree in veterinary medicine, pharmacology, pharmacy or a life science or an appropriate equivalent qualification would be required. If the QPPV is not a veterinarian, he or she must have access to a safety expert with appropriate qualifications. Despite this, and in a further display of lack of harmonisation, some EU countries insist that the QPPV is either a veterinarian or a pharmacist. In line with the wording of the Directive, it is not necessary for the Qualified Person to be an actual employee of the MAH, and therefore the position could be contracted out to a pharmacovigilance specialist outside the company. However, the responsibility for fulfilment of pharmacovigilance obligations still resides with the MAH, including ensuring that any such arrangement will guarantee this.

Particularly in a large multinational company, the QPPV would not be expected to personally attend to all the minutiae of recording and reporting suspected adverse reactions across all authorised products. Specific tasks may be delegated to suitably qualified and trained individuals within the company. It is important that this delegation is clearly documented as it may be investigated during a pharmacovigilance inspection by the regulatory authority. The Qualified Person is, however, expected to maintain oversight of both the pharmacovigilance system in terms of structure and performance and of the safety profiles of all products. A specific requirement for the QPPV to ensure prompt compliance with requests for additional information (apart from the legally required reports), including sales figures, is also included in Article 74 of the Directive.

In order to perform these functions, the Qualified Person requires sufficient authority within the company to be able to implement changes in the pharmacovigilance procedures and possibly other reporting mechanisms where required to maintain or improve compliance. He/she also requires the authority to implement any changes required due to safety issues identified through the pharmacovigilance system The MAH is responsible for ensuring that sufficient resources are available to support this. In theory, the QPPV has

> '. . . unimaginable powers within a company, and hypothetically can overrule the CEO in matters of safety'
> (O'Rourke, 2007).

Again these are matters that need to be clearly addressed if the position is contracted out. Within a company, there is potential for conflict in terms of competition for scarce resources and competing spheres of management influence. This is likely to be exacerbated where the pharmacovigilance system is regarded solely as an extra cost centre with no influence on revenue generation. If, as I hope to show below, pharmacovigilance is to be regarded as part of establishing the quality of products and a contributor to customer satisfaction, the scope for conflict may be less.

Pharmacovigilance inspections

The increased emphasis on pharmacovigilance for monitoring veterinary medicinal products has been accompanied by the introduction of pharmacovigilance inspections. These have their

legal basis in Article 80 of Directive 2001/82/EC and Article 44(1) of Regulation (EC) 726/2004, but only began to be carried out from the end of 2006. As noted above, a guideline dealing with the monitoring of compliance with pharmacovigilance regulations and pharmacovigilance inspections was published in March 2007 which will form part of Volume 9B. Although the guideline states that procedures for inspections will be prepared and published, this has not yet happened, and at present there seems to be a degree of variation from one member state to another in the conduct of inspections.

Partly these reflect the involvement in some states of agencies involved in the regulation of human medicines, due to the lack of experience in this field of the veterinary regulatory agencies. There is some indication that these agencies, being accustomed to dealing with human pharmaceutical companies, have approached inspections without considering the much more limited resources available in animal health companies. In other countries, the variations may be due to the veterinary regulatory agency 'learning on the job', so that the conduct of more recent inspections has been modified in the light of experience gained in the earlier ones. There is also a stated intention by some NCAs to tailor the content of the inspection to some extent to the size of the company as measured by the number of MAs held.

The guideline states that the competent authority for inspection of the MAH's pharmacovigilance system will be the NCA of the country in which the QPPV resides. Where the MAH's pharmacovigilance database is situated in a different EEA country from the residence of the QPPV, it is expected that the database would be inspected by the NCA in that country, but if it is situated outside the EEA, the responsibility would fall on the original NCA. Although the guideline states that there should be collaboration between NCAs in order to minimise duplication, some companies have already had inspections of more than one national subsidiary by the corresponding national agencies.

Pharmacovigilance inspections are broadly divided into routine and targeted inspections, although, since one of the triggers for a targeted inspection is that the MAH has not previously been inspected, at present all inspections could be considered as targeted. Other triggers not related to concerns about specific products or compliance include the placing of the MAH's first product on the market in the EEA, the involvement of the MAH in a merger or takeover, or significant changes in the MAH's pharmacovigilance system, e.g. a new database system or contracting out of pharmacovigilance functions.

Triggers may also be related to specific concerns over product safety or compliance, including:

- delays in expedited or periodic reporting;
- incomplete reports;
- inconsistencies between reports and information from other sources;
- delays in carrying out or failure to carry out specific obligations;
- lack of follow up with regard to product safety identified at the time that a marketing authorisation was granted.

This list is not exhaustive, and in fact the guideline states both that inspections may be triggered by other issues and that the presence of a trigger may not lead to an inspection. This seems to leave a very wide discretion to the regulatory authorities. It is envisaged that the majority of inspections will be announced, giving the MAH time to prepare, although unannounced inspections could take place if the authority felt it appropriate.

In general, the prior announcement of an inspection allows the NCA and the MAH to agree a mutually convenient date and the NCA would provide a draft plan of the inspection to allow the MAH to ensure the availability of key personnel. Although the QPPV is ultimately responsible for the pharmacovigilance system, the inspector is likely to wish to interview members of staff involved in the system to assess the suitability of their training and experience to their role. This

may include interviewing members of the field sales force who are likely to be the initial recipients of many reports from veterinary practices, wholesalers or farmers. The MAH would also be expected to provide in advance a detailed description of their pharmacovigilance system (unless the NCA already has a copy of this submitted as part of a recent MA application), an outline of the structure of the organisation with the names of the relevant personnel, and copies of all SOPs or other written documents relevant to pharmacovigilance within the company. Prior arrangement of the inspection date also allows the MAH to ensure that its system will be active and running at the time of the inspection, i.e. not offline due to maintenance or other IT issues.

The main areas of focus for inspections are likely to be:

- the awareness of the QPPV and other staff of their responsibilities with regard to pharmacovigilance and whether they have the appropriate level of training in this. This would include the arrangements made with other MAHs where distribution or co-marketing agreements exist;
- the processing and recording of reports of SARs and SLEEs in particular, but also other reports such as residue violations or adverse environmental effects;
- the reporting of serious SARs within the appropriate time frame, and the correspondence between the MAH's records of serious reports and those of the NCA, including missing reports and duplicates;
- the quality of PSURs, including the sales figures for different presentations of the product, the inclusion of NCA reference numbers where available and the completeness of the line listings of SARs and SLEEs, including reports in published literature;
- evidence of internal auditing of the pharmacovigilance system by the MAH.

In general, this information should be reasonably easy for the MAH to provide, as it is basic to compliance with the legal requirements. Evaluation of the quality of recording and reporting may involve analysis of a number of previously submitted PSURs and comparison of NCA lists of expedited reports with those included in the PSURs. Some problems may arise if the PSURs chosen are relatively old due to changes from a paper-based system or proprietary database to a more modern windows-based system, which may lead to migration issues. In addition, for example, in a 5-year PSUR submitted in 2005, the records will extend back to 2000, and it must be admitted that the quality of SAR recording, in terms of detailed case notes or causality assessment, may well be below present standards. Although these are historical problems, they do serve as a reminder of the need to audit the quality of new reports and to be aware of possible software problems during systems upgrades.

At the end of an inspection a report will be produced detailing the findings and requirements for any remedial action, which is made available to the CVMP and the MAH. (Although this is not specified in the guideline, in the UK the VMD allows 30 days for the MAH to reply or accept the report. However, it does commit itself to accepting or even discussing points made in the reply.) Where non-compliance with the pharmacovigilance obligations is detected, the action taken by the authority will depend upon the potential negative effect of the non-compliance on human or animal health. For the less serious cases, the MAH will be informed of the non-compliance and advised on how to correct it. Time limits will be set for this action and the MAH expected to confirm to the NCA when the deficiency has been corrected.

In more serious cases, further inspection may be warranted either to determine the extent of the non-compliance or to confirm that compliance has been achieved, or a formal warning may be issued to the company. The guideline has provision for the authorities to publish a list of MAHs that are seriously or persistently non-compliant with their obligations. In the worst case scenario, evidence might be found during an inspection of

safety issues sufficiently serious to warrant restrictions on the sale of a product, amendments to the SPC, or suspension or revocation of the marketing authorisation.

Pharmacovigilance and the commercial operation

Within an animal health company, pharmacovigilance cannot operate in a vacuum. It interacts with the commercial operations of the company in a number of ways. Some of these have financial implications, but it also affects intangibles such as the company's reputation, customer goodwill, etc. The most basic level at which it impacts commercial operations is in the requirement that now exists for a description of the applicant's pharmacovigilance system to be included in the application for a marketing authorisation.

A distinction has been drawn between 'qualifiers' and 'order winners' in manufacturing which is applicable to the animal health industry. The former are factors that are necessary for a product to even be considered by a buyer, while the latter are those where the company's performance relative to its competitors determines whether or not the product will be bought (Hill, 2000). In this sense, the pharmacovigilance system is a 'qualifier, since the MA will not be granted unless this system is adequate, and therefore the product would not be in the market without it. However, the practice of pharmacovigilance within a company is also capable of contributing to order winning by the effect it has on the company's reputation and its relationship with its customers.

All company employees are potential sources of reports of SARs, either as animal owners or through their contacts, both business and social, with the public. There should be a company policy to make them aware of the company's obligations in this area and training should be provided to make them aware of the basic information required and the contact points for pharmacovigilance within the company. The ability of individuals to collect information will vary; one would not expect a payroll clerk to handle a report to the same extent as a veterinary advisor. However, non-technical staff should be aware of how to contact those responsible for pharmacovigilance within the company and at least be able to pass on information, allowing the originator of the report to be contacted. It is important that SOPs are established, including timelines for the communication of information to the pharmacovigilance function that will permit data collection and onward reporting to regulatory agencies within the time limits for expedited reports if these are required.

Animal health companies, of course, exist for the same reason as other commercial enterprises: to 'maximise the net present value of future cash flows' as they say in business schools, i.e. to make money. This is done by ensuring that there is a difference between the inward costs of goods and the values of outward sales. However, the reference to *future* cash flows should be noted; maximising the difference between costs and income in the short term may damage the ability to do so in the future. Since pharmacovigilance costs money, there may be a temptation to regard it solely as a cost burden, whilst it should more properly be considered as an investment. Apart from the legal requirement for its existence, the potential exists for the pharmacovigilance system to contribute to the company's reputation for quality and to increase customer satisfaction, thus boosting sales in the long term.

Most of the direct customer contact in animal health companies is with either the veterinary profession or farmers and other professional animal keepers rather than the general public. In recent years, consolidation into larger units in both the veterinary profession and the farming industry has increased the levels of scientific input expected by both. Although this does not apply to all veterinary practices and farmers, in general the ones to whom it does are the most promising sources of revenue growth for the animal health industry. If pharmacovigilance is presented properly to consumers like these, it can become another facet of the scientific support

that the company provides along with its physical product.

As with other products, pharmaceuticals have a product life cycle:

- introduction
- growth
- maturity
- decline.

A large part of the commercial function consists of managing this cycle and, if possible, extending it. The first two phases may be exciting to the sales and marketing departments, but are not necessarily profitable. Development costs large sums of money prior to product launch and the growth phase requires high levels of marketing support. The real money is made in the maturity phase when the product has an established reputation. Even the decline phase can be profitable if a niche market exists that requires the product, but which is too small for a competitor to justify developing an alternative.

Then there is also the option of restarting the cycle by extending the product to new areas – in the case of veterinary pharmaceuticals, new species or new indications. True 'blockbuster' drugs that are so superior to the other offerings on the market that they practically sell themselves are extremely rare, and most companies would recognise that overreliance on such a product will eventually lead to problems. Either newer products will appear or medical opinion may change, and eventually the patent protection runs out, allowing competition from generic copies. If one company can identify a market, then so can its competitors; therefore in major clinical areas there will be a number of competing products, which may be at different stages of their life cycle.

One would expect that the newer products will have a better profile with regard to efficacy, safety, environmental effects, residue levels, etc., but this will usually be accompanied by a higher price tag. Some of these benefits will not be particularly obvious to the user, or insufficiently valued to justify the increased cost. Although price sensitivity (the price elasticity of demand) varies for different types of product, there will always be some trade-off to be made between the volume of unit sales and the price (and hence the profitability of the product to the company).

In such competitive situations, factors other than price can play an important part in differentiating one product from the others. One of these is the reputation of the company for quality and integrity. Although beloved by marketing departments, a range of notepads, novelty pens and calendars is far less effective in supporting sales to science-based business customers than the provision of reliable technical support. A desktop toy may amuse the receptionist, but her role in selecting the antibiotic that the practice uses to treat canine pyoderma is likely to be very limited. What is more likely to influence the veterinarian who does make such decisions is contact with sales staff that are able to back the product with up-to-date scientific information with regard to its use, which should include information derived from the pharmacovigilance system.

It should be obvious to all concerned that some level of adverse events is to be expected with any pharmacologically active product, but unfortunately there is little attention paid to adverse event reporting in veterinary education, and so awareness of this fact in the veterinary profession is lower than it should be. Although there may be a natural tendency to avoid unpleasant subjects in dealing with customers, it is better in the long run for the company's reputation for honesty that the potential for adverse reactions should be acknowledged. Most adverse reactions will already be included among those listed in the product data sheet (labeled adverse reactions), but quite a number of veterinary surgeons may not actually read the whole of this document and are therefore taken by surprise when they occur.

It is important that sales staff are made aware of the likely types, severity and incidence of adverse reactions to the products they sell. Failure to do this leaves the company vulnerable on several counts:

- The company's reputation for competence suffers if staff are unaware of a reaction,

particularly if it is either relatively common or at least has been reported in professional journals.
- If they are unaware of the incidence rate of a reaction they may give the impression that it is more common than it really is, which may harm the reputation of the product and also reduce confidence in the quality of information previously provided by the company.
- There is also the danger that they will accept at face value a report of an SAR which in fact is not product related, leading to an impression that the company is being devious when the report is later classified as 'unlikely'.

Lack of understanding of the function of pharmacovigilance can lead to a reluctance by commercial staff, from sales teams to brand managers, technical services, veterinarians and higher management, to become involved in its operation. This stems from two misconceptions:

1. that it is an extra piece of government red tape; and
2. a tendency to regard it as an excessively complicated customer complaints system.

Both of these attitudes work against getting the most advantage from the system. In the first case, the tendency will be to collect the minimum information necessary to satisfy legal requirements, thus potentially missing the opportunity to reach definite conclusions as to the causality of reported SARs. This may weaken the company's position with regard to whether changes in the SPC are required in response to these reports. It would also seem logical that it is safer for the MAH to know as much as possible about its products in view of the modern consumer's taste for litigation.

The second attitude leads to an emphasis on placating the customer rather than investigating reports. Payments towards the cost of investigating or treating animals following SARs are a legitimate way of maintaining the goodwill of the customer. Supplying product free of charge may also preserve goodwill but tends to distance the process from scientific support for the product. There is a danger that these sort of payments will be seen as some form of compensation, implying lack of confidence in the product or that it was in some way faulty. This may amount to buying a temporarily placated customer at the expense of the company's reputation and may even lead to further, possibly less justified, complaints. It is important that those dealing with SAR reports remember that they are reports of the reaction of one or more animals *to* a product, not complaints *about* a product.

It should be emphasised that the existence of an appropriate pharmacovigilance system and its use to improve knowledge and awareness in the sales force is not purely defensive. Since the incidence of SARs in authorised products is generally very low, this knowledge should enable them to promote products with increased confidence in their efficacy, safety and quality. It also allows management of the customer's expectations with regard to types and incidence of possible adverse events, and may help to identify problems that are not product related. Back-up by technical and professional staff with detailed knowledge of previous SARs allows the company to advise and support customers when a reaction occurs. In addition, a systematic approach to recording and investigating reported SARs demonstrates to the customer that the company takes the customer's concerns seriously.

Conclusion

The veterinary pharmacovigilance system within the EU is intended to produce a regime for monitoring the safety and efficacy of authorised veterinary medicinal products that is harmonised across the member states in order to facilitate the free movement of goods. As such, there are advantages to MA holders in the standardisation of requirements and the possibilities for coordination of submission of reports.

The changes laid down in legislation in 2004, with an increased emphasis on 3-yearly pharmacovigilance reports in place of 5-yearly authorisation renewals, are potentially beneficial by

reducing the regulatory burden that was involved in the repeated renewal process. However, the partial and piecemeal implementation of the changes, together with the failure to produce the guidelines that the legislation requires for the detailed working of the system, has led to a degree of confusion and uncertainty. In the meantime some NCAs have pressed on with certain parts of the changes faster than others or introduced national rules based upon an interpretation of what the guidelines might say in future. Combined with directions from the EMEA to adhere to the previous guidelines, the effect has so far been to reduce harmonisation and increase confusion. In addition, proposals to harmonise submission of PSURs for products containing the same active substance are under development. Again, these will be beneficial once fully implemented, but further confusion seems likely as these are introduced into a system in the process of transition from one regulatory timetable to another.

In terms of the actual practice of pharmacovigilance in the recording, assessment and reporting of SARs, there is at times a mismatch between theory and practice. Due to a number of factors, including quirks of human nature and misunderstandings about the nature of pharmacovigilance, data collected may be incomplete or investigation may be hampered and therefore assessment of possible causal links between the signs observed and the product administered is made more difficult. This may be particularly true in the case of serious SARs where the time limits for submission of expedited reports may preclude thorough investigation before an initial report is submitted. Follow-up reports will then be required which may contain a different conclusion as to causality, a potential cause of confusion.

One of the most important features of periodic reports is the calculation of incidence rates, i.e. the number of animals affected by SARs compared to the number that received the product. For many products this figure will not be completely objective because of the need to make assumptions about the size or age of the animals treated and/or the split between species in which it is authorised when calculating the number of doses administered. In some cases there may be a choice between maintaining comparability with previous reports on the same product or using another basis for the calculation of dose numbers to allow comparison with other products. These comparisons in turn may affect decisions as to whether action such as amendments to the SPC are required.

With the increased emphasis on pharmacovigilance to monitor the safety and efficacy of veterinary medicinal products, the need to in turn monitor the systems used has led to the introduction of pharmacovigilance inspections. These are conducted by the NCAs, and this so far has led to a degree of variability in the inspections, related to the lack of experience in the regulatory agencies in this field. Once again, procedures for these inspections are to be prepared and published, but are not available yet. In general, inspections cover the personnel, data collection and processing procedures, and the databases used in the pharmacovigilance function. Evidence is also likely to be required of internal auditing of the function between inspections and of procedures for taking action if required in response to safety concerns.

An important feature of the present system is the requirement for each MAH to have a permanently available QPPV resident in the EU. This person has overall responsibility for maintaining the pharmacovigilance system of the MAH, and should be suitably qualified (although this term is not defined). This responsibility includes ensuring that sufficient resources are available for the system and also implementing any actions required to deal with concerns over safety and efficacy of products. There is therefore a potential for conflict if the value of the pharmacovigilance function is insufficiently appreciated by other functions within the company.

If pharmacovigilance is considered solely as an additional cost, there is likely to be resistance from the commercial arm of the company to providing resources and co-operating in both information collection and responses to safety

concerns. However, the alternative is to recognise that the main potential for revenue growth in the animal health industry lies in supplying larger and consolidating businesses in the veterinary profession and farming. In this market the 'extended product offering' that includes scientific support along with the product has a marketing advantage over the product alone. An effective pharmacovigilance system, together with education of staff who regularly deal with customers in the range of adverse reactions likely to be associated with particular products and the significance of incidence rates, should be part of that support. This will increase the confidence of company staff in the product they sell and their ability to offer appropriate support to customers. In turn, this will enhance the company's reputation for quality, integrity and customer service, leading to increased sales and profitability.

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12 Pharmacovigilance in the US – an industry perspective

T.M. Hodge

Introduction

Pharmacovigilance refers to the collection, investigation, maintenance and evaluation of spontaneous reports of suspected adverse events associated with the use of marketed veterinary medicinal products. Veterinary medicinal products include therapeutic agents, biologics, vaccines, agents used in disease diagnosis or agents otherwise administered or applied to an animal for protective, therapeutic or diagnostic effects or to alter physiological functions (Novotny, 2003).

An adverse event is an undesired effect or lack of a desired effect for claimed indications. In addition, adverse events include human exposures to animal products, product defects, potential residue complaints in food-producing animals and environmental contamination reports (Hampshire *et al*., 2004). The regulatory requirement for reporting adverse events and the responsible regulatory agency differ depending upon the product type. Adverse events associated with pharmaceutical products are reported to the United States Food and Drug Administration – Center for Veterinary Medicine – Office of Surveillance and Compliance (FDA-CVM-OS&C), biological product adverse events are regulated by the United States Department of Agriculture – Animal and Plant Health Inspection Service – Center for Veterinary Biologics (USDA-APHIS-CVB) and pesticide product adverse events are reported to the United States Environmental Protection Agency (EPA). The regulatory requirements for each of these agencies will be discussed in a later section.

The greater emphasis on monitoring the safety and efficacy of marketed products has been an ongoing trend in recent years, partly encouraged by greater consumer awareness of safety issues and information available through internet access. In recent years the increased public demand for more transparent safety processes has occurred as a result of high-profile issues including the removal of Vioxx from the market (Solomon and Avorn, 2005) due to its association with a greater risk of myocardial infarctions. Many safety concerns were raised following the withdrawal of this widely used product that was successfully marketed for over 5 years. On the veterinary side, the recent voluntary recall of ProHeart®6 created some uncertainty in the public mind about regulatory agencies' abilities

to ensure the safety of marketed products (Curry-Galvin, 2005). The debate over the safety of ProHeart®6 is still not resolved even after a Veterinary Medicine Advisory Committee (VMAC) heard arguments from both sides of the debate. The most recent large-scale issue has been the pet food recall related to melamine-tainted wheat gluten imported into the United States from China. Contaminated pet food was responsible for the illness and death of numerous cats and dogs primarily from acute renal failure (see http://www.fda.gov/oc/opacom/hottopics/petfood.html).

Added to the availability of information through the internet, the access to prescription drugs through the internet, and the complexity of global product manufacture, including the purchase of ingredients produced outside the US, safety issues are becoming an area of concern for consumers, industry and regulatory agencies. Changing global product manufacture, distribution and use have created new demands on the regulatory agencies, which are already resource poor, and on animal drug companies to put adequate processes in place to monitor product safety. More emphasis is placed on the risk and benefit balance of each product, and as a result, veterinarians are expected to discuss these issues with their clients prior to the administration or dispensing of medications. In addition, the increased expectation of animal owners for quality pet care has resulted in the launch of medications to treat complex medical conditions and these innovative products require more attention to benefit:risk analysis and enhanced pharmacovigilance.

In this changing environment, the animal health companies becomes the greatest stakeholders in assuring that their products maintain a safe and effective product profile on the market. In this chapter, the regulatory requirements for adverse event reporting will be discussed, as well as the tools necessary to maintain regulatory compliance. Also discussed will be additional internal methods of data mining, report generation and signal detection used to monitor product safety and efficacy.

Adverse event reporting requirements

Animal health company employees are trained to understand that if they become aware of an adverse event associated with one of their company's products they are required to report it to the appropriate person within their company, with subsequent regulatory reporting as required. It is considered an individual's 'corporate responsibility' to report any adverse events that he or she becomes aware of. Employees are trained on their corporate reporting responsibilities by various safety training mechanisms.

The four criteria required to identify an adverse reaction are:

- an identifiable *reporter* of the adverse event;
- an identifiable *patient* or animal that has received the product;
- an identifiable *product*; and
- a *description* of the event.

Adverse events are received from end users, pet owners, ranchers, producers, distributors and veterinarians, and by various methods including telephone, fax, mail and e-mail. Most marketed products contain the manufacturer's phone number and address on the product label to facilitate the reporting of adverse events to the company.

FDA-CVM

The Center for Veterinary Medicine (CVM) is the part of the Food and Drug Administration (FDA) that regulates pharmaceuticals for use in animals. The pre-approval process is a phased submission and review process that takes into account each study performed on target animals/species. The pre-approval process is the responsibility of CVM's Office of New Animal Drug Evaluation (ONADE) and includes the review of the target animal safety (TAS) studies, safety and efficacy clinical trials, human food safety studies, user safety and 'all other information' components of the technical section.

Once an animal health product is approved it is assigned an NADA (new animal drug application) number, and marketed with an approved label. Adverse events for this marketed product are submitted to the CVM Office of Surveillance and Compliance (OS&C) and in compliance with 21 CFR PART 510.80 (Department of Health and Human Services, Food and Drug Administration, 2003a). According to this regulation, an adverse drug experience is defined as any adverse event associated with the use of an animal product whether or not considered to be drug related, and whether or not the new animal drug was used in accordance with the approved label. This means that all events are reported to CVM whether or not the company's causality assessment deems that the event was related to the product administration. In addition, even if the product was used in an extra-label manner, including the wrong dosage, wrong species and wrong route of administration, or used for an off-label indication, the adverse event is still reported.

A serious adverse drug experience is defined as an adverse event that causes one or more of the following:

- fatality
- a threat to life
- professional intervention
- abortion
- stillbirth
- infertility
- congenital anomaly
- prolonged or permanent disability
- disfigurement

(Department of Health and Human Services, Food and Drug Administration, 2003b).

Professional intervention is defined as any veterinary treatment or hospitalisation. Professional intervention is not part of the definition of serious in other markets outside of the US. This means that any adverse event that is seen by or treated by a veterinarian is considered serious by CVM definition.

Unexpected adverse drug experiences are defined as adverse events that are not listed in the current labelling for the new animal drug. They can also include any event that may be related to an event listed on the label but which differs due to severity or specificity.

These definitions are important when making the determination of whether to submit an adverse event as an expedited or periodic report.

Three-day field alert reports

A 3-day field alert is a report of a product defect that may result in a serious adverse drug event. These reports are submitted to the appropriate FDA District Office within 3 days of the animal health company first becoming aware that a serious product defect may exist. The information may initially be provided by the company to the District Office by telephone or other telecommunication, with prompt written follow up using FDA Form 1932: Veterinary Adverse Drug Reaction, Lack of Effectiveness, Product Defect Report (available at http://www.fda.gov/cvm/forms/forms.html; see *Figure 12.1*).

While the regulations are not specific, most companies choose the FDA District Office located in the area where the company is registered, particularly in the case where multiple company locations exist. This part of the regulation is open to individual interpretation as to what would constitute a product defect that could cause a serious adverse event, but clearly any defect that could result in a product overdose, or administration of the incorrect product or contaminated or adulterated product would be included. Some common examples of 3-day field alerts include missing product labels, wrong tablet size in the bottle or mixed tablets in the bottle, defective metering devices to measure out oral paste products or chemical or microbial contamination of a product.

When notifying the FDA District Office of a 3-day field alert it is also important to notify the product manufacturing site at the same time so they can begin their quality investigation of retention samples if a lot number is known. Depending on the product and whether or not the field sample is available this may also be sent

Section 9a

FOOD AND DRUG ADMINISTRATION 7500 Standish Place (HFV-210), Room N403 Rockville, MD 20855	**VETERINARY ADVERSE DRUG REACTION, LACK OF EFFECTIVENESS, PRODUCT DEFECT REPORT** *(Forward to address at left. Attach all correspondence that pertains to this reaction)*	**Form Approved: OMB No. 0910-0284** **Expiration Date: January 31, 2010**

Public reporting burden for this collection of information is estimated to average 2 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to:

Food and Drug Administration
7500 Standish Place (HFV-210), Room N403
Rockville, MD 20855

An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number.

NOTE: This report is required by law (21 CFR 510.300). Failure to report can result in withdrawal of approval of the application.

1. REPORT SOURCE AND ADDRESS *(Mfr., Distr.)*

2a. DATE REPORT RECEIVED

b. DATE SENT TO FDA

c. NUMBER OF DAYS BETWEEN 2a AND b:

3a. TYPE OF REPORT
☐ 3-day Alert ☐ 15-day Alert
☐ Periodic Report

3b. ☐ Initial Report
☐ Follow Up Report Of *(Give Date)* _____

4. NAME, ADDRESS AND PHONE NO. OF ATTENDING VETERINARIAN *(In confidence)*
Name:
Street Address:
City: State: ZIP:
Phone No. (____) ____-____

5. NAME OR CASE IDENTIFICATION OF OWNER *(In confidence)*

6. TRADE NAME AND GENERIC NAME(S) OF ACTIVE INGREDIENT(S) *(Include dosage form and strength - Ex., tab, 500 mg.)*

7a. NAME OF MANUFACTURER

b. NADA NO.

8. LOT NUMBER(S)

9. DOSAGE ADMINISTERED AND ROUTE *(Ex. 250 mg., q 12 h, p.o.)*

10. DATE(S) OF ADMINISTRATION

11. ILLNESS/REASON FOR USE OF THIS DRUG

12. DRUG WAS ADMINISTERED BY
☐ VETERINARIAN, STAFF ☐ OWNER, OTHER

13. NUMBER OF ANIMALS IN THIS INCIDENT

a. TREATED WITH DRUG	b. REACTED	c. DIED

14. REACTING ANIMALS

a. SPECIES	b. BREED
c. AGE	d. WEIGHT

e. SEX
☐ FEMALE ☐ MALE ☐ PREGNANT ☐ NEUTERED

15. CONCOMITANT MEDICAL PROBLEMS

16. OVERALL STATE OF HEALTH AT TIME OF REACTION
☐ GOOD ☐ FAIR ☐ POOR ☐ CRITICAL

17. DID ANY NEW ILLNESS DEVELOP OR DID INITIAL DIAGNOSIS CHANGE AFTER SUSPECT DRUG STARTED?
☐ NO ☐ YES *(Explain)*

18. **CONCOMITANT DRUGS ADMINISTERED**

NAME OF DRUG	ROUTE	DOSAGE REGIMEN	DATE(S) OF ADMINISTRATION

FOR FDA USE ONLY

1. _____ ☐ D ☐ NAI
2. _____ ☐ PR ☐ AI
3. _____ ☐ PO ☐ AP
4. _____ ☐ R ☐ AL
5. _____ ☐ NC
6. _____
T. _____

☐ I.L. ☐ CR ☐ CONT

COMMENT

FORM FDA 1932 (1/07) PSC Graphics: (301) 443-1090 EF

Fig. 12.1 FDA Form 1932.

REACTION DATA

19. DESCRIBE SUSPECTED ADVERSE REACTION: INCLUDE ALL SIGNS, RESULTS OF PERTINENT LAB TESTS, NECROPSY RESULTS, POSSIBLE CONTRIBUTING FACTORS, ETC. ALSO, INCLUDE IN THIS SECTION PRODUCT INEFFECTIVENESS AND PRODUCT DEFECTS SUCH AS CRACKED TABLETS, CLOUDY SOLUTION, ETC.

20a. ATTENDING VETERINARIAN'S LEVEL OF SUSPICION THAT DRUG CAUSED REACTION

☐ HIGH ☐ MEDIUM ☐ LOW ☐ NO ATTENDING VET.

20b. WAS THERE EXTRA LABEL USE (ELU) INVOLVED?

☐ NO ☐ YES *(Explain)* _____

21. LENGTH OF TIME BETWEEN LAST ADMINISTRATION OF SUSPECT DRUG AND ONSET OF REACT

22. DATE OF ONSET *(Mo., day, yr.)*

23. DURATION OF REACTION *(Hrs., days, etc.)*

24. WAS THE ADVERSE REACTION TREATED?

☐ NO ☐ YES *(Describe treatment)*

25. OUTCOME OF REACTION TO DATE

☐ DIED *(Give date)* _____
☐ REMAINS UNDER TREATMENT
☐ ALIVE WITH SEQUELAE
☐ RECOVERED
☐ UNKNOWN

26. WHEN REACTION APPEARED, TREATMENT WITH SUSPECT DRUG:

☐ HAD ALREADY BEEN COMPLETED DISCONTINUED
☐ DUE TO THE REACTION DISCONTINUED,
☐ REPLACE WITH ANOTHER DRUG DISCONTINUED,
☐ REINTRODUCED LATER CONTINUED AT ALTERED
☐ DOSE
☐ OTHER *(Explain)*_____

AND THE REACTION

☐ CONTINUED
☐ STOPPED
☐ RECURRED
☐ OTHER *(Explain)* _____

27. HAD ANIMAL(S) BEEN PREVIOUSLY EXPOSED TO THIS DRUG?

☐ NO ☐ YES ☐ UNKNOWN

28. DID ANIMAL(S) PREVIOUSLY REACT TO THIS DRUG?

☐ NO ☐ YES ☐ UNKNOWN

29. HAD ANIMAL(S) PREVIOUSLY REACTED TO OTHER DRUGS?

☐ NO ☐ YES ☐ UNKNOWN

(If yes, give drug(s) and reaction if known)

30. HAS THE ATTENDING VETERINARIAN SEEN SIMILAR REACTIONS TO THIS DRUG IN ANY OTHER ANIMALS?

☐ NO ☐ YES *(Describe treatment)*

31. NAME AND TITLE OF INDIVIDUAL RESPONSIBLE FOR ACCURACY OF REPORTED INFORMATION *(Type or print)*

32. SIGNATURE OF INDIVIDUAL RESPONSIBLE FOR ACCURACY OF REPORTED INFORMATION

FORM FDA 1932 (1/07)

Fig. 12.1 *Continued*

to the manufacturing site for evaluation. Once the 3-day alert report has been received by the FDA District Office, they will communicate this information to the FDA-CVM Office of Surveillance and Compliance (OS&C).

Fifteen-day expedited reports

Fifteen-day alert reports, otherwise known as 'expedited' reports, are adverse events that are assessed as serious and unexpected regardless of the source of the information and whether or not there is an association with the product and the event. These reports take the form of Form FDA 1932 and must be submitted within 15 working days of the company first receiving the information. The company is required to promptly investigate all 15-day adverse drug events, and if the investigation reveals significant new information, a follow-up report must be submitted within 15 working days of the company receiving this follow-up information. If no additional information is obtained after an effort is made to obtain it, a follow-up report is required explaining why no further information is available within 3 months of the initial report.

With the future implementation of the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH): *Guideline 24 Pharmacovigilance of Veterinary Medicinal Products: Management of Adverse Event Reports (AERs):VICH GL24*, it is likely that adverse events that are serious *or* unexpected will be required to be submitted as 15-day alert reports to CVM (VICH, 2007a). Currently, as described above, only cases that are both serious *and* unexpected are required to be submitted as 15-day reports to CVM. In the past, there was some advantage for companies to submit as few 15-day reports as possible, with the remainder of adverse event reports submitted as periodic reports. This meant that fewer reports were reviewed by CVM quickly and that fewer reports would appear on the CVM ADE website in an expedited manner (see http://www.fda.gov/cvm/adetoc.htm). With the anticipated launch of electronic reporting, which will mean faster review of all submitted reports by CVM and changes that have been made to the CVM ADE website which has removed the report numbers associated with various clinical signs, the timing of the submission to CVM is less of an issue. In fact, some companies submit all adverse events regardless of seriousness or expectedness as 15-day reports to avoid the extra effort of creating large periodic submissions for products generating large numbers of adverse event reports.

Periodic Drug Experience Report

Adverse event reports that have not previously been submitted as 3-day or 15-day reports are submitted to CVM as part of the Drug Experience Report (DER). Currently, these include serious and expected adverse events, and non-serious reports both expected and unexpected. These adverse event reports are provided as with the 15-day reports, on an FDA Form 1932. A DER must be submitted every 6 months for the first 2 years following approval of a product, and yearly thereafter. The six-month DERs must be submitted within 30 days following the end of the 6-month reporting period. The yearly DER reports must be submitted within 60 days of the anniversary date of the product approval.

For yearly DER reports, the company may petition CVM to change the date of submission or the frequency of reporting. This is important if a company is attempting to harmonise reporting requirements between different global regulatory agencies or if a product has been on the market for a long time and very few adverse events are received for that product. The DER must be accompanied by a completed FDA Form 2301 *Transmittal of Periodic Reports and Promotional Material for New Animal Drugs* (available at http://www.fda.gov/cvm/forms/forms.html; see *Figure 12.2*).

The DER contains other components in addition to adverse event case reports including:

- distribution data;
- labelling;

- non-clinical laboratory studies;
- a summary report of increased frequency of adverse drug experience;
- advertisements and promotional labelling.

The distribution data must include the total number of distributed units of each size, strength or potency. This must include the quantities distributed domestically and the quantities exported. Since there is also a requirement to report adverse events globally for identical products, where the distribution data reveal that the identical product was distributed outside of the US (with a US label), all adverse events received for that identical product that have occurred outside the US must also be reported to CVM and included in the DER if not already submitted.

The current package labelling and package inserts must also be submitted along with a summary of any changes in labelling made since the last DER report.

Copies of in vitro studies and other non-clinical laboratory studies are included in the DER along with copies of published clinical trials, including clinical trials on safety and effectiveness, and clinical trials on new indications and reports of clinical experience. These are often obtained by doing a literature search at the time the DER is compiled. The adverse events also include product defect reports that have not been previously submitted as 3-day field alerts. Reports of adverse drug experiences in the literature must also be noted in the DER. A bibliography of pertinent references must be included with the DER report. The summary report of increased frequency of adverse drug experiences must also be included.

The company is required to review the frequency of adverse event reports to determine if there has been an increased frequency of serious expected or serious unexpected events. This review must take place at least as frequently as the DER reporting period. If there is an increase in frequency then the company must submit a summary of this increase in a narrative format. Observed increases in the frequency of serious adverse events can often be explained by an increase in product distribution data, differences in reporting practices from one time period to another or label changes including new indications for the product. Occasionally, a more detailed analysis of the data is needed to explain an increase in adverse event frequency.

Inactive or discontinued products

The terms inactive or discontinued are synonymous in meaning that the product is no longer being marketed but the company still retains the NADA number. The adverse event reporting obligations for these products continue as long as the product is still in use in the field. By convention, companies may choose to be responsible for these products for a reasonable period of time (1 year) past the expiry date of the last manufactured lot. Since there are unlikely to be any adverse events received for these products past this time period, it is customary to query the database at yearly intervals for all the inactive products, to be certain that no adverse events are pending submission to the regulatory authorities.

Third party products

Third party product is a term used to describe products that are manufactured by one company and marketed and/or distributed by another company. Either one of these companies may be the holder of the product licence and therefore considered to be the market authorisation holder or (MAH). This is synonymous with the terms described in 21 CFR 514.3: *applicant* is the owner of the NADA and *non-applicant* is the company whose name appears on the label and who is engaged in the manufacturing, packing, distribution or labelling of the product.

In the case of a third party product it is the responsibility of the two companies to have a contract in place which stipulates how adverse event data will be collected and submitted to the regulatory agency. The agreement on adverse event reporting can be part of the general contract describing the sales and marketing terms established between the two companies or a

Section 2301

TRANSMITTAL OF PERIODIC REPORTS AND PROMOTIONAL MATERIAL FOR NEW ANIMAL DRUGS
(See Instructions on Back)

1. NADA NO. or ANADA NO.

Form Approved: OMB No. 0910-0284
Expiration Date: January 31, 2010
See OMB statement on page 3.

Note: Required by 21 CFR 514.80. Failure to make the reports is a basis for withdrawal of the NADA/ANADA.

2. NAME OF APPLICANT	3. DATE REPORT SUBMITTED	4. DATE REPORT DUE
5. DRUG TRADE NAME	6. GENERIC NAME	
7. COMBINED REPORT *(List NADA numbers involved. See Instructions.)*	8. REPORT PERIOD (MO) (YR) FROM — (MO) (YR) TO	
	9. TYPE OF REPORT *(Check one)* ☐ 6 MONTH ☐ ANNUAL ☐ FOLLOW-UP ☐ OTHER *(Specify)* _____	

10. **INFORMATION REQUIRED - PERIODIC AND SPECIAL**
(See CFR 514.80. Check Column A if "None." Always complete Column C, for items "(d)" & "(e)".)

NONE A	ITEM B	DESCRIPTION *(Volume Number(s), Tab(s), Pages of Report)* C			
☐	(a) ADVERSE EXPERIENCES	(1) TOTAL NO. OF REPORTS	(2) NO. OF PRODUCT DEFECTS	(3) NO. OF COMPLAINTS AFFECTING ANIMALS	(4) NO. OF ANIMALS REACTED
☐	(b) CLINICAL DATA *(Animal Experience)*				
☐	(c) MAILING PIECES AND/OR ADVERTISING MATERIAL				
	(d) CURRENT PACKAGE LABELING				
	(e) QUANTITY MARKETED				

11. **INFORMATION REQUIRED - PROMOTIONAL MATERIAL ONLY**

DATE OF ISSUANCE A	TYPE OF MATERIAL B	IDENTIFICATION *(Code No., etc.)* C

12. NAME / TITLE OF RESPONSIBLE OFFICIAL / AGENT *(Type or print)*	Amount Marketed	Stability Data	Clinical Data	Labels	Promotional Material
13. SIGNATURE OF ABOVE OFFICIAL / AGENT					
14. RETURN ADDRESS OF APPLICANT / AGENT					
15. TELEPHONE & FAX NUMBER OF APPLICANT / AGENT					

PREVIOUS EDITION IS OBSOLETE

PSC Graphics: (301) 443-1090 EF

Fig. 12.2 FDA Form 2301.

INSTRUCTIONS FOR COMPLETION OF FORM FDA 2301

Copies of this form may be obtained by writing to:

Department of Health and Human Services
Public Health Service
Food and Drug Administration (HFV-12)
7519 Standish Place, Room 3508
Rockville, MD 20855

1. Enter the NADA number assigned to the drug. If fewer than six digits, add leading zeros.

7. A combined report may be submitted for NADAs or ANADAs [See 514.80 (c)]. Whenever an applicant is required to submit a periodic drug experience report under 514.80(b)(4) with respect to more than one approved NADA or ANADA for preparations containing the same new animal drug so that the same information is required to be reported for more than one application, the applicant may elect to submit as a part of the report for one such application (the primary application) all the information common to such applications in lieu of reporting separately and repetitively on each. If the applicant elects to do this, the applicant must do the following:

   (1) State when a report applies to multiple applications and identify all related applications for which the report is submitted by NADA or ANADA number.

   (2) Ensure that the primary application contains a list of the NADA or ANADA numbers of all related applications.

   (3) Submit a completed Form FDA 2301 to the primary application and each related application with reference to the primary application by NADA/ANADA number and submission date for the complete report of the common information.

   (4) All other information specific to a particular NADA/ANADA must be included in the report for that particular NADA/ANADA.

9. Check this box if report is a follow-up to one previously submitted or is a response to an FDA request.

   Reports for all NADA/ANADA involved should be submitted on the anniversary date of the earliest approved NADA/ANADA involved (primary application).

10(a). Adverse drug experience is any adverse event associated with the use of a new animal drug, whether or not considered to be drug related, and whether or not the new animal drug was used in accordance with the approved labeling (i.e., used according to label directions or used in an extralabel manner, including but not limited to different route of administration, different species, different indications, or other than labeled dosage). Adverse drug experience includes, but is not limited to:

   (1) An adverse event occurring in animals in the course of the use of an animal drug product by a veterinarian or by a livestock producer or other animal owner or caretaker.

   (2) Failure of a new animal drug to produce its expected pharmacological or clinical effect (lack of expected effectiveness).

   (3) An adverse event occurring in humans from exposure during manufacture, testing, handling, or use of a new animal drug.

10(a)(1). Enter total number of complaints being reported. Each complaint may involve one or more adverse drug reactions. A complaint is defined as a report involving one situation or incident and may involve one or more animals.

10(a)(4). Enter total number of animals experiencing reactions involved in item 10(a)(3).

10(e). Report the quantity marketed in units of highest concentration and the largest marketing package size. In the case of a dosage form product, e.g., tablets which are formulated on body weight range basis, give the quantity marketed of specific strength and package size separately without converting into highest concentration and the largest marketing package size unit.

Submit two copies of the report to:

Department of Health and Human Services
Public Health Service
Food and Drug Administration (HFV-199)
7500 Standish Place, Room N403
Rockville, MD 20855

Public reporting burden for this collection of information is estimated to average 30 minutes per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to:

Department of Health and Human Services
Public Health Service
Food and Drug Administration (HFV-199)
7519 Standish Place, Room 3508
Rockville, MD 20855

An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number.

Fig. 12.2 *Continued*

separate document solely dedicated to adverse event reporting. These adverse event reporting agreements can vary from one or the other company taking full responsibility for all adverse event reporting, or one company only submitting expedited reports (3-day and 15-day) to CVM and the other company preparing and submitting the DER report. Both companies must be informed of all adverse events occurring with these shared products and be certain that they are in compliance with the submission of these reports to the regulatory agency.

Third country reports

Third country reporting refers to the submission of adverse event reports occurring outside of the country where the submission is being made. In the case of the FDA-CVM in the US, this would refer to the submission of adverse events occurring in countries outside of the US that involved the US product. 21 CFR 514.80 does not give a detailed explanation of what third country reports are expected. It only states that:

> 'Applicants and non-applicants must submit data, studies, and other information described in this section from domestic, as well as foreign sources'.

Personal communication with CVM OS&C clarified that this means adverse events associated with *identical products (same pharmaceutical)*, which is defined by VICH GL24 as a product originating from the same MAH with the same formulation. CVM clarified that for adverse event reporting purposes in the US, *identical product* means that the product is sold with a US label.

The US is also responsible for submitting adverse event reports that have occurred in the US for *similar products* to regulatory agencies in other countries where the product is marketed by the same MAH. A *similar product* is defined by VICH GL24 as originating from the same MAH, with the same active ingredients, with major excipients with the same or similar pharmaceutical functions and having at least one common registered species (VICH, 2007b). In other words, the labels do not have to be identical in order for there to be reporting obligations to regulatory agencies outside the US.

This is particularly true for Centrally Registered Products in the EU where a summary report of the third country reports is included in the PSUR (Periodic Safety Update Report) of the product submitted to the European Medicines Agency (EMEA). Other countries may request US pharmacovigilance data for re-registration or submission purposes. These data are most commonly provided in a case line-listing format with or without a written summary report.

Adverse event reporting from research studies

21 CFR 514.80(b)(4)(iii)(C) states that:

> 'Descriptions of completed clinical trials conducted by or for the applicant must be submitted no later than 1 year after completion of research'.

CVM, together with cooperation from the Animal Health Institute (AHI), an animal industry group, drafted guidelines on reporting adverse events arising from Investigative New Animal Drug (INAD) research studies involving an approved product (Animal Health Institute, 2005). The following points of clarification were discussed regarding the reporting of adverse events associated with an approved product used in an INAD controlled research study.

Reporting of adverse events should not hinder, alter or invalidate the study. For instance, the research study should not be un-blinded for the purposes of reporting adverse events. In un-blinded studies, serious and unexpected adverse events should be submitted to CVM within 15 days of the company becoming aware of the event. All other adverse events for the approved drug involved in these studies should be submitted in the DER. In a blinded study, serious and unexpected adverse events should be submitted to CVM within 15 days of the study being un-blinded.

This is a protocol-driven process and the investigator can define in the protocol what is an expected and unexpected adverse event and what will be considered serious in terms of what constitutes 'professional intervention' with regards to an investigational study. 'Active professional intervention' in research studies would be defined as active treatment or therapy administered by a veterinarian (or other trained professional) above and beyond routine preventive measures or common first aid. If a disease or other abnormal condition exists prior to administration of the approved product, then the presence of that disease or condition after administration will not be considered an adverse event. Adverse events will either be submitted to ONADE or OS&C or both depending on the type of study. See *Table 12.1* for a summary of adverse event reporting responsibilities for marketed products involved in research studies.

Interpretation of Form 1932

Consistency in populating the fields of Form 1932 allows for greater data accuracy. There are no published guidelines on the interpretation of Form 1932 and for most users it is a trial and error process with occasional feedback from CVM. For animal health companies that report a large number of adverse events, it is advantageous to train their staff recording adverse event data to use consistent processes to facilitate product trending. In addition, it is important to remember that care in collecting and reporting adverse event data to CVM on Form 1932 can often mean the difference between being able to mount an effective product defence if safety or efficacy issues are raised.

As databases become more complex and the process of populating Form 1932 with data is automated, it becomes easier to lose sight of what data fields appear on the form and what data are submitted to CVM.

Fields 2a, b and c refer to the dates that the adverse event was received by the animal health company (2a), the date of submission of the report to CVM (2b) and the difference between 2a and 2b (2c). Field 2c is used to ensure that the company is submitting adverse event reports within the limits of 3 days, 15 days and 365 days for 3-day alerts, 15-day reports and periodic reports, respectively. When follow-up reports are received, field 2a must reflect this new date.

Field 6 should contain the brand name and active ingredient, dosage form and strength of the product involved in the adverse event report. If more than one company product is associated with the adverse event and both are considered to be suspect, then a report will need to be submitted for each company product and the product being submitted for will appear in field 6. The reason that a case is submitted multiple times if it contains more than one company suspect product is because CVM distributes these forms to reviewers assigned to particular products.

Field 7a should contain information on the location of manufacture, not just the name of the NADA holder.

Field 11 contains the illness/reason for use of this drug and should agree with field 20b (was there extra-label use involved?). If the drug was used for an off-label indication then this should be reflected in field 20b. This is also true if there was an extra-label route of administration, dose, species, duration of treatment, etc. If a database is used to populate the form, then a drop-down list could be used to populate the second part of field 20b to add the extra-label use reason.

Field 15 is of importance in capturing underlying disease or medical conditions. If care is taken in putting appropriate data in this field, it can go a long way toward explaining an adverse event occurring because of an ongoing or underlying disease condition.

Likewise, field 16 should be answered with care in order to reflect the animal's state of health immediately preceding the adverse reaction. If an animal was treated for a disease condition with the suspect product or had an underlying disease condition, then 'good' should not be chosen to describe the animal's prior state of health.

Table 12.1 Summary of research scenarios and ADE reporting and final report submission requirements (ONADE, Office of New Animal Drug Evaluation; TAS, arget animal safety).

Scenario	*CVM division/office reported to*	*Type of report or report form*	*Time frame*
Post-Marketing Studies Scenarios 1–3: approved drug used for safety and/or efficacy evaluation or as a concomitant medication in non-comparative or comparative studies: a) Serious and unexpected ADEs b) Summary of all ADEs	a) Division of Surveillance b) Division of Surveillance	a) Form FDA 1932 b) Summary along with description of the study	a) 15 working days b) DER or special DER within 1 year of completion of study or whichever is first
INAD Research Study Scenarios 1 and 2: approved drug is used as a positive control or as a concomitant medication: a) Serious and unexpected ADEs; product defects b) Summary of all ADEs Investigational drug: c) Summary of all ADEs	a) Division of Surveillance b) Division of Surveillance c) ONADE	a) Form FDA 1932 b) Summary along with description of the study** c) Final study report	a) 15 working days* b) DER or special DER within 1 year of completion of study or whichever is first c) After study completion
INAD Research Study Scenarios 3–5: new indication, new indication with disease challenge or TAS study at higher dose, in approved species: a) Serious and Unexpected ADEs; product defects b) Summary of all ADEs Additionally, report to ONADE: c) Summary of all ADEs	a) Division of Surveillance b) Division of Surveillance c) ONADE	a) Form FDA 1932 b) Summary along with description of the study c) Final study report	a) 15 working days* b) DER or special DER within 1 year of completion of study or whichever is first c) After study completion
INAD Research Study Scenario 6: TAS study in new species: a) Summary of all ADES b) Summary of all ADEs	a) ONADE b) Division of Surveillance	a) Final study report b) Summary with study description†	a) After study completion b) Periodic DER

*If the adverse event is both 'serious' and 'unexpected', submit within 15 working days after unblinding (if blinded) or within 15 working days of the sponsor becoming aware of the event if the study is not blinded.

**A description of the study and a summary of all adverse events involving the approved new animal drug should be submitted to the Division of Surveillance, either in the DER or in a special DER, within 1 year of completion of research.

†If development of the indication is terminated, a summary should be submitted to the Division of Surveillance.

Field 17 refers to new illnesses or diagnoses that were made after the product was initiated. This field is also very helpful in demonstrating that the observed adverse event may be due to a newly diagnosed disease condition. For instance, this could be the diagnosis of diabetes after an antibiotic was used to treat polyuria with no success.

Field 18 is populated with concomitant drugs that were administered to the animal. Some of

these may be company products, but due to the nature of the adverse event, they were selected as 'concomitant medications' instead of 'suspect' drugs. An example of this would be an injection site reaction with an injectable product as the 'suspect' drug appearing in field 6 and an oral antibiotic product given at the same time to treat an unrelated condition. This medication would appear in field 18 as it would be considered a concomitant medication and not associated with the adverse event.

Field 20a is the attending veterinarian's causality assessment. This is the only place on the form where any type of case causality is provided and the attending veterinarian's 'level of suspicion' may or may not be correct. This assessment can be balanced by providing an in-depth medical investigation and providing this information in the event narrative. As with all the fields on the form, as new information is received, the data fields should be updated. For instance if the attending veterinarian's level of suspicion changes following further diagnostics, then this should be reflected on the follow-up form.

Field 21 (time between administration and reaction), field 22 (reaction onset date), field 23 (reaction duration), field 24 (reaction treatment) and field 25 (reaction outcome) are all fields used to assess the likelihood that the adverse event was associated with the product and how long the reaction usually lasts if it is a result of the product administration and also what treatment if any was used and the outcome. If at all possible, the follow-up of the case should be maintained until the outcome is known. A case that is closed to follow up with an outcome marked as 'remains under treatment' raises questions about the disposition of the animal and, if associated with product administration, the safety of the product.

Field 26 is designed to assess the results of dechallenge and rechallenge. A positive dechallenge would be answered by the fact that the drug was discontinued and the adverse reaction stopped, and a positive rechallenge would be indicated by the fact that the drug was discontinued and reintroduced later and the adverse reaction recurred. Dechallenge and rechallenge are important indicators of an association between the administered product and the adverse reaction.

Fields 27, 28, 29 and 30 are aimed at collecting information on the animal's previous exposure to the product. A product that the animal has received multiple times in the past is less likely to be causally associated with an adverse reaction in that animal unless field 28 reveals that the animal had problems with the drug in the past or had a history of having adverse reactions to similar products. Field 30 collects data on the veterinarian's experience with the product in the past.

Field 19 (reaction data), sometimes referred to as the 'agency summary' or 'event narrative', is the free text description of the adverse event. This is a vitally important field to enter case data, clinical signs, laboratory tests, contributing factors and opinions from the technical staff on what may have caused the adverse event. It is critical to provide all the needed information for the regulatory agency to make a critical assessment of the case in this section.

Follow-up investigation is essential in order for the agency and the animal health company to make an appropriate assessment of the case. One of the biggest mistakes that is made is not to update all of the fields of Form 1932, including field 19 when new information is obtained on a case.

Clarification of reporting requirements for various products

Special agreements can be made between the company and CVM to clarify what type of reports need to be submitted for specific case types or for specific products. Often, certain types of reports may be requested after a product has initially launched, that may no longer be necessary after the product has been on the market for a period of time. An example of this might be asymptomatic overdoses of a companion animal product. Another example might be lack of efficacy reports when the reported event is taking place beyond

the labelled duration of effect for the product. These agreements with CVM should be kept on file within the pharmacovigilance department and the cases duly recorded in the database in case there are any questions regarding discrepancies in what is being reported. It is also not mandatory to report asymptomatic human exposures to CVM. However, it is important to record these cases in the database, not only in case there is a problem at a later date with a particular case, but also because it can be useful to be able to compare the symptomatic and asymptomatic human exposures for safety reasons.

Relationship with manufacturing sites

Pharmacovigilance groups need to maintain communication with the manufacturing sites where their responsible products are produced. Manufacturing sites need to be aware of product complaints that are received and, as appropriate, perform quality investigations on retention samples or request field samples be returned to them for a visual examination. In addition, the 21 CFR Food and Drugs Good Manufacturing Practice 211.198 Complaint files section a states that:

> 'Written procedures describing the handling of all written and oral complaints regarding a drug product shall be established and followed. Such procedures shall include provisions for review to determine whether the complaint represents a serious and unexpected adverse drug experience.'
> (http://www.fda.gov/cder/dmpq/cgmpregs.htm)

This regulation has been interpreted to mean that company manufacturing sites need to have visibility to all product complaints and, following review of the cases, may elect to initiate a product quality investigation, if one has not been already requested. A global pharmacovigilance database with linkage to the manufacturing sites not only allows the manufacturing site to have visibility to cases but also allows the manufacturing site to record their quality investigation within the case. The investigation results are copied to Form 1932 as follow-up information and submitted to CVM.

USDA

In the US, animal vaccines and most biological products are regulated by the United States Department of Agriculture (USDA) under the Virus-Serum-Toxin Act. 9 CFR 116.1 states:

> 'If, at any time, there are indications that raise questions regarding the purity, safety, potency, or efficacy of a product, or if it appears that there may be a problem regarding the preparation, testing, or distribution of a product, the licensee, permittee, or foreign manufacturer must immediately notify the Animal and Plant Health Inspection Service concerning the circumstances and the action taken, if any'
> (Department of Agriculture, Animal and Plant Health Inspection Service, 2005)

Compliance with the requirement for 'Immediate' notification is considered to be met by notification to CVB within 3 business days (Center for Veterinary Biologics Notice No. 05–24). At present, routine reporting of suspected adverse events to USDA is not required, although some form of adverse event collection is assumed to be in place for all animal health companies that market and distribute veterinary biological products. While adverse event reporting is not required on a routine basis, the CVB has the right to request adverse event reports for any licensed products. These requests are generally prompted by the direct reporting of an adverse event to CVB from a veterinarian or animal owner or by questions originating from within CVB.

States within the US that license a biological product often require that adverse event reports be submitted to the state veterinarian at agreed-upon intervals. One example might be a bi-annual report of adverse events associated with rabies vaccines. Another example would be adverse event reporting associated with conditionally licensed vaccine products. APHIS may issue a conditional veterinary biological product licence

in order to meet an emergency condition, limited market, local situation or other special circumstance, such as an unmet medical need. These licences are granted under expedited procedures that ensure purity, safety and a reasonable expectation of efficacy. Each conditionally licensed product has a termination date and it is expected that progress will be made by the company for completion of the requirements necessary for full licensure (USDA Veterinary Services, 1999). Examples of recent conditionally licensed products include the West Nile virus vaccine for horses, Melanoma vaccine for canines, avian influenza killed vaccine and the Porphyromonas vaccine for canines. For conditionally licensed products, the USDA and the states with licences to sell the product can request adverse event reports and/or distribution data at variable intervals, generally no more frequently than monthly. These data help to ensure the safety and efficacy profile of the product prior to full licensure.

APHIS has proposed amending the Virus-Serum-Toxin Act to require veterinary biologic licensees and permittees to record and submit reports to APHIS concerning adverse events associated with the use of all biological products they produce or distribute (Department of Agriculture, Animal and Plant Health Inspection Service, 2005). This proposed rule is similar to the requirements for adverse event reporting of veterinary pharmaceuticals, with a few exceptions. The rule would require the animal health companies to not only record reports of all adverse events that they receive but also submit a summary of the reports on an annual basis for products licensed for more than 1 year and on a semi-annual basis for products licensed for less than 1 year. The summary reports would include copies of the individual adverse event case reports, and the number of doses, or the average number of doses, of the product in distribution channels.

The proposed rule does not require differential reporting requiring expedited or periodic submissions of adverse events based on seriousness or expectedness of the event. In 9 CFR 101.2 an adverse event is defined as:

> 'any observation in animals, whether or not the cause of the event is known, that is unfavourable and unintended and that occurs after any use (off label or on label) of a biological product. Included here are events related to a suspected lack of expected efficacy. For products intended to diagnose disease, adverse events refer to anything that hinders discovery of the correct diagnosis.'

The rule also defines the data fields that would be required to be submitted for each adverse event. These are listed in 9 CFR part 116.9 and include:

- the date of the report;
- the identification of the person initiating the report;
- the product code number;
- the product trade name;
- the serial numbers of the product, if available;
- a description of the adverse event;
- a description of the animal(s) involved, including:
  - numbers dead
  - numbers affected
  - numbers exposed to the product
  - species
  - breed
  - age
  - sex
  - physiological status
- the opinion (probably, possible, unknown, unlikely, no assessment) of the person making the report as to product-event causality;
- the route and site of vaccine administration;
- the identity of the person administering the product;
- the date of the event;
- the eventual outcome of the event.

The Adverse Event Report case form is found on the APHIS/USDA website at www.aphis.usda.gov/vs/cvb/forms/adverseeventreportform.pdf and a copy of the form is provided in *Figure 12.3*.

Adverse Event Report

Pharmacovigilance
United States Department of Agriculture
Center for Veterinary biologics
510 South 17th Street, Suite 104
Ames, IA 50010
Phone: (515)232-5785 FAX: (515)232-7120

*Required Fields

Product information

List ALL immunobiological products used.

*Brand Name or Generic Name	*U.S. Vet. License (ESt. No.) or Manufacturer Name	Serial (lot) Number	Type of Product[1]
1			
2			
3			
4			

1. Type of Product (select one for each product) = Viral, Bacterial, Combination, Antibody, Coccidia, Immunomodulator, Protozoa, Recombinant, Rickettsia, Other, or Do Not Know.

Administration of products

Dose	Route	Site	Needle Size	Date Reconstituted
1				
2				
3				
4				

Administered by:[2]	*Date of Product Use (MM/DD/YYYY):
Concurrent Drugs or Procedures:	

2. Administered by (select one) = Veterinarian or Veterinary staff or Nonveterinarian

Event Information

*Event description:[3]
Explain the event description and treatment in a concise paragraph:

3. Event description (select one) = Anaphylaxis-hypersensitivity, autoimmune, birth defect, lack of expected efficacy, local, neoplasia, reproductive, systemic, other

Onset (How long after product use did the event begin?) : (Specify whether units are in mins,hrs, days, wks, mos, yrs)

Fig. 12.3 USDA Adverse Event Report Form.

Attending veterinarian's level of suspicion that product caused event: High Medium Low Not Listed	*Outcome: (Select One) Recovered without treatment Recovered with treatment Did not recover Died Other

Animal Information

Case identification number:		
*Species[4]	Breed:	Age (i.e., 2 yrs or 2 mos):
Sex: (male, female , not listed) Neutered: (yes, no, not listed)	For animals handled in a group (herd, litter, etc) Number in group: _____ Number vaccinated:_____	Number affected:_____ Number dead: _____

4. Species (Select One) = Porcine, Bovine, Canine, Feline, Ferret, Ovine, Caprine, Equine, Exotic, Fish, Poultry, or Other

History and Enviroment (e.g., acquisition, vaccination, and medical histories; housing, diet, contacts, etc)

Personal Information

Veterinarian	Owner
*Name:	Name:
Address:	Address:
City: State Zip:	City: State: Zip:
*Phone: FAX: E-mail:	Phone: E-mail:

Submitter's information

This event has been reported to the manufacturer(s): (Select one) = yes or no	
*Submitter's first name:	*Submitter's last name:
*Submitter's phone number:	* Today's Date:
Relationship to animal:[5]	

5. Relationship to animal (select one) = veterinarian, owner, other, not listed)

For internal use:

Product Code	Other comment(s):
1.	
2.	
3.	
4.	

Fig. 12.3 *Continued*

EPA

Most products used topically for the control of ectoparasites and insects on animals are regulated by the US Environmental Protection Agency (EPA) under the Federal Insecticide, Fungicide and Rodenticide Act (USA Code of Federal Regulations, 1999). For purposes of reporting to the EPA, adverse events in domestic animals and humans must be placed in one of ten categories in order of decreasing severity. The categories are:

H-A (human death);
H-B (human major);
H-C (human moderate);
H-D (human minor);
H-E (human exposure only);
D-A (domestic animal death);
D-B (domestic animal major);
D-C (domestic animal moderate);
D-D (domestic animal minor);
D-E (domestic animal exposure only).

Registrants of pesticide products are required to submit to the EPA reports of adverse events.

- Category H-A adverse events are required to be submitted no later than 15 days after the company becomes aware of a human death associated with an EPA registered product.
- Categories H-B and H-C must be submitted to EPA within 30 days of the company becoming aware of the adverse event, with a grace period of 30 days.
- Category H-D through D-E cases are submitted quarterly to the EPA with a grace period of 60 days.

Other EPA categories include:

- W-A and W-B (adverse events affecting fish or wildlife);
- P-A and P-B (adverse events affecting plants);
- ONT (adverse events affecting non-target organisms including beneficial insects);
- G-A, G-B and G-C (water contamination);
- PD-A, PD-B and PD-C (adverse events causing property damage).

More guidance on the classification of adverse events into categories can be found in the Pesticide Registration Notice 98–3. Adverse events are submitted to the EPA as an aggregate report (see *Figure 12.4*) which includes the time period covered by the report and a count of the number of incidents (cases) for each exposure and severity category listed by product registration number. The 'EPA Summary' part of the table is a link to the case numbers that are represented in the table. More detailed information must be provided to the EPA for cases classified as H-A, H-B, D-A and W-A cases in the form of a case report. *Figure 12.5* illustrates the overall flow of the reporting process of adverse events to the EPA.

The 20 May 1998 update *Information Regarding the Use of the Voluntary 6(a)(2) Incident Reporting Forms* (Anonymous, 1998) contains a sample case report form that was developed in cooperation with industry trade organisations, registrants, professional groups and the EPA in order to facilitate individual case reporting to the EPA. Copies of these Voluntary Industry Reporting Forms are provided in *Figure 12.6*. The different sections are completed depending on whether or not the incident involves a human, domestic animal, fish, wildlife, plants or other non-target organism, surface water, groundwater, residue in food and feed or property damage.

Requirements for reporting to regulatory authorities

In order to facilitate the submission of adverse events to the regulatory agencies there must be a means for the users of the product to report adverse events. End users can report directly to the regulatory agencies, but most commonly reports are made to the animal health company.

As stated previously, on the literature for most products there is an 800 telephone number (a free-phone number) or some other means for

PV WORKS

Aggregate Adverse Event Report
For Period 01-Jan-2004 through 31-Dec-2007

Registration Number	D-A	D-B	D-C	D-D	D-E	H-A	H-B	H-C	H-D	H-E	LOE	Not Categorized	Not Reportable	Total
XXXX-AA	0	0	1	0	1	0	0	0	0	0	6	1	4	13
XXXX-BB	1	1	0	1	0	0	0	0	0	0	4	0	0	7
12345-67-8910	1	1	9	7	3	0	0	0	12	3	11	0	7	54

EPA Summary

Product(s): Report run for the following list of products: Product A, Product B, Product C.

Note: The field 'Not Categorized' indicates that the regulatory user needs to go into the system and assign the case to an EPA category
LOE (lack of efficacy) reports are not required to be reported but are counted in this table. 'Not Reportable' is a company assigned category for those cases not meeting the minimum requirements for reporting.

* - Products with the same registration number appear more than once in some cases.

Fig. 12.4 Sample of EPA Aggregate Report Form.

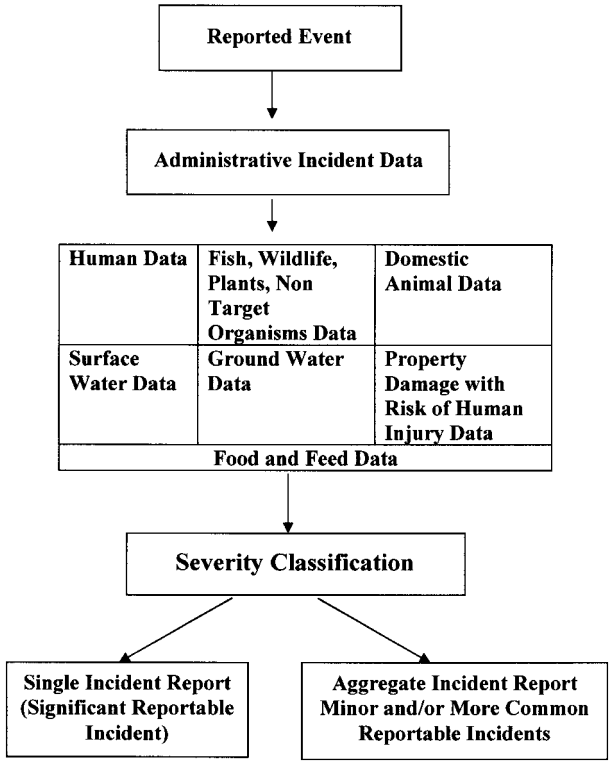

Fig. 12.5 Flow of adverse event reporting process to EPA.

contacting the animal health company for product information or to report an adverse event. These calls are received by a call centre which is either part of the regulatory pharmacovigilance group or part of a customer service group or outsourced to another company that provides product inquiry information and records adverse event information for subsequent submission to the regulatory agencies by a designated group within the animal health company.

For small animal health companies, the function of adverse event call receipt and recording and subsequent submission to the regulatory authority is often handled by the technical services group which has customer services, marketing, regulatory and pharmacovigilance responsibilities. For larger animal health companies, these responsibilities are often divided into different reporting structures. For animal health companies that are part of a larger company that includes a human health counterpart, these functions are often kept separate to avoid the 'conflict of interest' that can occur when adverse event reporting functions fall under a marketing or sales reporting structure.

In addition to a dedicated group responsible for answering product inquiries and recording adverse event reports, there needs to be a database or some other pharmacovigilance system that allows for the recording of adverse events and the ability to query the system for reports needing submission to regulatory authorities. In addition, the system must be able to be queried to fulfil internal data requests and requests from regulatory authorities regarding the safety and efficacy of the product. Due to enhanced requirements for third country regulatory submissions and global product trending, it is of great advantage to have a pharmacovigilance data capture and reporting system that will provide global support for companies with global product marketing.

In the past, many companies have used various systems including Microsoft Access databases, AS/400, 'Sentinel-Vet', company-developed systems (home grown) or human pharmaceutical databases modified to handle animal health complaints. Often there was more than one animal health system in place, one for the US and one for the rest of the world (ROW). These systems rarely communicated with each other and this made third country reporting and global trending difficult. With an increase in global pharmacovigilance requirements from regulatory agencies and company internal customers, it became a necessity for global animal health companies to have a pharmacovigilance database that would facilitate global regulatory reporting as well as global product trending.

The majority of large animal health companies at this time are using PV Works (Vet) as their pharmacovigilance database system (PV Works (Vet), Assured Information Systems Ltd, http://www.assured.co.uk/pv-works-vet.htm). In addition to the majority of animal health companies adopting PV Works, CVM OS&C has recently acquired PV Works as their database for review of adverse events and the USDA has been review-

ing the functionality of PV Works for future implementation pending the adoption of the proposed rule on adverse event reporting.

PV Works (Vet) is a commercially available software application that may be configured by a particular animal health company to meet specific business needs. Interfaces with other company programs can be developed to enhance the functionality of PV Works (Vet). These can include product dictionaries, customer relationship management databases (CRM) which can include customer contact databases, sales representatives, and sales databases, product manufacturing databases which can include lot and serial number databases.

In addition to interfaces that allow for information to populate the pharmacovigilance system, there must be a way to retrieve data from the system for regulatory report submissions, data requests and product trending. These additional tools can include Business Objects (or a similar tool) for data export and report creation, Crystal reports and data mining software. Pharmacovigilance systems are required to be 'validated' systems with documented evidence that provides a high degree of assurance that the system will consistently function according to predetermined specifications and quality attributes. Documentation to support validation must be archived and available for review. In addition, if the pharmacovigilance system that is chosen is 21 CFR part 11 compliant, it allows for electronic signatures and facilitates data security, data integrity and data confidentiality (http://www.21cfrpart11.com/pages/library/index.htm).

Electronic submission of adverse event data

Currently, all regulatory agencies worldwide either require electronic transmission of adverse event reports (e.g. the EMEA in the EU) or are working to initiate this requirement. The FDA-CVM will begin testing PV Works in the second quarter of 2008. While it is not a requirement to have a pharmacovigilance system with electronic reporting capabilities, it is critical if large quantities of adverse event reports are submitted. PV Works (Vet) includes an electronic reporting module, which can export cases to an XML format.

A Cyclone Electronic Data Interchange (EDI) gateway is used to transfer cases once extracted and converted into an XML electronic record. This gateway meets the International Conference on Harmonisation Electronic Standards for the Transfer of Regulatory Information (ICH ESTRI) recommendations that allow for secure electronic data transmission with the EMEA and other competent authorities (http://estri.ich.org/index.html).

FDA-CVM will be using HL7 (Health Level Seven) as the basis for their schema for electronic reporting of adverse events and product labelling. HL7 is one of the American National Standards Institute (ANSI) accredited Standards Developing Organisations (SDOs) used by health care industries including hospitals, pharmacies and insurance companies to transfer and communicate clinical data (available at http://hl7.org/about/).

Since the HL7 schema was designed for human health care data, modifications to the schema are underway by CVM in order to be applicable for animal health data. The Individual Case Safety Report (ICSR) is that portion of the HL7 schema that relates to data that are captured for an adverse event report. This is the portion of the HL7 schema that needs to be modified to capture species, breeds, more than one patient on a single record and herd information, among a few of the differences between human adverse event reporting and animal event reporting. VICH GL 35 gives recommendations for the methods and standards of electronic submission of data (VICH, 2007c) and references VICH GL 42 which describes the data elements necessary to exchange information on spontaneous adverse event reports (VICH, 2007d).

In addition to all the requirements listed in the above sections needed to capture and report

Voluntary Industry Reporting Form for 6(a)(2) Adverse Effects Incident Information

Provide all known, required information. If required data field information is unknown, designate as such in appropriate area. Page# of

Row 1 Administrative Data	Reporter Name	Submission date.	Contact person (if different than reporter)	Internal ID
	Address		Address	
	Phone #		Phone #	
	Incident Status: New__ Update__ If update, include date of original submission.	Location and date of incident. (City, County, State)	Date registrant became aware of incident.	Was incident part of larger study? Y___N___U___
Row 2 Pesticide(s) Involved	EPA Registration # (Product 1)	EPA Registration # (Product 2)	EPA Registration # (Product 3	
	A.I. (s)	A.I. (s)	A.I. (s)	
	Product 1 name	Product 2 Name	Product 3 Name	
	Exposed to concentrate prior to dilution? Y N U NA	Exposed to concentrate prior to dilution? Y N U NA	Exposed to concentrate prior to dilution? Y N U NA	
	Formulation	Formulation	Formulation	
Row 3 Incident Circumstances	Evidence label directions were not followed? Yes___No___U___ Intentional misuse___	Incident site: (examples include home, yard, school, industrial, nursery/greenhouse, surface water, commercial turf, building/office, forest/ woods, agricultural (specify crop) right-of-way (rail, utility, highway)).	Situation (act of using product): (examples include mixing/loading, reentry, application, transportation, repair/ maintenance of application equipment, manufacturing/ formulating).	
	Applicator certified PCO? Yes__No__U__			
	How exposed: (examples include direct contact with treated surface, ingestion, spill, drift, runoff)	Brief description of incident circumstances.		

Fig. 12.6 EPA Voluntary Industry Reporting Form.

Voluntary Industry Reporting Form for 6(a)(2) Incident Information Involving Humans

Provide all known, required information. If required data field information is unknown, designate as such in appropriate area. Page# of

Demographic information: Age_____ Sex_____ Occupation (if relevant)	Exposure route: Skin___ Eye___ Oral___ Respiratory___ Unknown___ Other:	Was adverse effect result of suicide/homicide or attempted suicide/homicide?	Was protective clothing worn (specify)?
If female, pregnant? Yes___ No___ Unknown___	Was exposure occupational? Yes___ No___ Unknown___ If yes, days lost due to illness:	Time between exposure and onset of symptoms:	
Type of medical care sought: (examples include none, clinic, hospital emergency department, private physician, PCC, hospital inpatient).	List signs/symptoms/adverse effects		If lab tests were performed, list test names and results (If available, submit reports)
Exposure data: Amount of pesticide: Exposure duration: Victim weight: ____lb ____kg ____unknown			
Human severity category___			
This box can be used to provide any explanatory or qualifying information surrounding the incident. (add additional pages if necessary)			
			Internal ID #

Fig. 12.6 *Continued*

Voluntary Industry Reporting Form for 6(a)(2) Incident Information Involving Fish, Wildlife, Plants or Other Non-Target Org.
Provide all known, required information. If required data field information is unknown, designate as such in appropriate area. Page# of

List species affected and number of individuals per species.	
List symptoms or adverse effects.	
Magnitude of the effect: (Examples include miles of streams, square area of terrestrial habitat).	Pesticide application rate, intended use site (examples: corn, turf), and application method
If plant, plant type: (Examples include crop, forest, forage, orchard, home garden, ornamental).	
If lab test(s) performed, list name of tests and results (submit laboratory report(s) if available).	
Description of the habitat and the circumstances under which the incident occurred.	
Distance from treatment site.	Fish, wildlife, plant, other non-target organism severity categories:_____:_____:_____ Include all categories that apply, ex. W, P, ONT
This box can be used to provide any explanatory or qualifying information surrounding the incident (add additional pages if necessary).	
	Internal ID#

Fig. 12.6 *Continued*

Voluntary Industry reporting form for 6(a)(2) Incident Information

If incident involves domestic animals use this form to collect information to be reported on the aggregate form. Page# of

Type of animal: (Examples include livestock, bird, fish, poultry, pet (specify)).	Breed/species (name, no./adv.Effect)	Exposure route: (Examples include skin, eye, oral, respiratory, unknown).
Domestic animal severity category___		Time between exposure and onset of symptoms:
List sign/symptoms/adverse effects. Was animal treated (optional)?		
If lab test(s) performed, list name of tests and results (submit laboratory report(s) if available)		
This box can be used to provide any explanatory or qualifying information surrounding the incident (add additional pages if necessary).		Internal ID#

Fig. 12.6 *Continued*

Voluntary Industry Reporting Form for 6(a)(2) Incident Information
Detections of Pesticides in Surface Water

Provide all known information. If required data field information is unknown, designate as such in appropriate area. Page# of

Pesticide/degradates analyzed for, methods of analysis, corresponding detection limits and amount detected:

Pesticides	Degradates	Method of analysis	Detection limit	Amount detected

Sampling times/frequency	Sample type: (Grab, composite, other)
If raw water samples, water bodies sampled and approximate locations in each water body.	If raw water samples, proximity of sampling locations to drinking water supply intakes and identities of systems supplied
If finished water samples, water supply systems sampled	If finished water samples, percent surface water source by specific surface water sources to water supply system(s)
Water severity category	

Additional space for answers or explanatory information in this box.

Internal ID#

Fig. 12.6 *Continued*

Voluntary Industry Reporting Form for 6(a)(2) Incident Information
Detections of Pesticides in Groundwater
Provide all known information. If required data field information is unknown, designate as such in appropriate area. Page # of

Pesticide/degradates analyzed for, methods of analysis, corresponding detection limits and amount detected:				
Pesticides	Degradates	Method of analysis	Detection limit	Amount detected

Date sample collected	Depth to groundwater	Well use/well identifier

Screened interval	Soil series and texture: (sand, clay, silt, other)	Latitude/longitude

Aquifer description: Confined ____ Unconfined ____	Hydrologic group.	Hydraulic conductivity.

pH of water.	Organic matter/organic carbon (percent).	Maximum rainfall and date

Annual cumulative rainfall	Cumulative irrigation (inches).	Years Pesticide used.

Application frequency per year.	Application method

Date of last application	Water severity category

Additional space for answers or explanatory information in this box.	
	Internal ID#

Fig. 12.6 *Continued*

Voluntary Industry Reporting Form for 6(a)(2) Incident Information

Incident involving property damage with risk of human injury. Page# of

Describe property damage (if any).	PD - A

Additional space for answers or explanatory information in this box	
	Internal ID#

Fig. 12.6 *Continued*

Voluntary Industry Reporting Form for 6(a)(2) Incident Information
Unauthorized Residue in Food and Feed

Provide all known information. If required data field information is unknown, designate as such in appropriate area. Page# of

Pesticides/degradates analyzed for and corresponding detection limits	
Amount of Pesticide detected	
Sample type	Sampling times/frequency
Method of analysis	Tolerance Level
This box can be used to provide any explanatory or qualifying information surrounding the incident (add additional pages if necessary).	
	Internal ID#

Fig. 12.6 *Continued*

adverse events to the regulatory authorities, there needs to be a process (automated or manual) that ensures that all cases in the database are submitted within the appropriate time period. If an automated process is used, the software needs to record within the case when the submission was made for both initial reports and follow-up reports. It also needs to record submissions that are made when the case is submitted to multiple regulatory agencies. For a case containing multiple products, the submission needs to be recorded at the product level, as some products may be reported to different regulatory agencies depending on the regulatory requirements for reporting. In addition, regulatory reports must be archived and retained in compliance with the regulatory agency guidelines and corporate record retention policies.

Organisation of data

To facilitate data retrieval, trending and case submission to regulatory agencies, data are organised by case identification, case type, product problem type, reportability and assessment. Whenever possible, free text entry of data is kept to a minimum and code lists are used. The following represents examples of data organisation that may vary somewhat between companies.

Case identification refers to general case categories including:

- reportable;
- non-reportable;
- inquiry;
- erred.

These categories allow case information to be entered into the system and tracked, but they do not have regulatory submission requirements. Case types are:

- animal complaints;
- human complaints;
- product defects;
- inquiry.

Assigning case types allows for efficiency and accuracy of data collection. For instance, if the case type is a human complaint, the patient data collection screen will be relevant for humans. If the case type is an animal complaint, then the patient screen will have drop-down menus with code lists related to animals, including the breed and species code lists, animal production types, etc. If the case type is a human complaint then a human patient screen will be populated with relevant human data.

The product problem type organises the adverse event at the product level. So for each product listed on the case, a different problem type can be chosen. Product problem types can include:

- product defect;
- adverse reaction;
- lack of efficacy;
- residue violation;
- extra-label use;
- ecotoxicity.

One product can have more than one problem type associated with it. It is important to remember that these case types and problem types do not appear on the single case regulatory forms and are used to organise, sort and query the data.

For purposes of electronic reporting, a clinical sign must be reported for every case. The accepted clinical sign dictionary for reporting to CVM as well as other regulatory agencies worldwide is the Veterinary Dictionary for Drug Regulatory Affairs (VEDDRA) (see Chapter 2). VEDDRA was developed in the EMEA by the Committee for Veterinary Medicinal Products (CVMP; now the Committee for Medicinal Products for Veterinary Use) and its Pharmacovigilance Working Party to facilitate the electronic reporting of adverse events. VEDDRA is organised in a hierarchy of four levels ordered by increasing granularity:

- SOC (system organ class);
- HLT (high level term);

- PT (preferred term);
- LLT (lower level term).

Most internal reports are designed to report SOC and PT. Due to the structure of VEDDRA, cases can be searched for using any level VEDDRA term. The adoption of VEDDRA by regulatory agencies worldwide allows for a common language in the reporting and analysis of adverse events. Training on the dictionary is advised within a company to enhance consistency and the ability to query the database for similar cases occurring globally, with accuracy. While there are no written guidelines on the use of VEDDRA, it is possible to extrapolate from the much more complex system used in the pharmacovigilance of human medicinal products, MEDDRA.

It is important when coding a case with VEDDRA clinical sign terms to keep in mind how this information will be used to query the data. For instance, it is helpful to record only those clinical signs that occurred following the result of the administration of the medication, not the clinical signs that were pre-existing and due to an underlying disease condition that the product was being used for. Another rule of thumb is not to code clinical signs with such granularity that they need to be combined when querying the data. An example of this is to record all the clinical signs observed following an anaphylactic reaction instead of simply choosing the preferred term (PT) of anaphylaxis.

There is also a VEDDRA human version that can be used as the code list for symptomatic human cases following exposure to veterinary medicinal products. For those problem types without clinical signs, such as lack of efficacy or product defect cases, a VEDDRA sign of 'lack of efficacy', 'no sign' or 'uncoded sign' is chosen for these cases in order to facilitate electronic reporting.

Many companies have 'company specific' VEDDRA terms that they use to trend safety and efficacy issues. They need to keep in mind that these terms will not transmit during electronic submissions and must be mapped to an existing VEDDRA term for submission purposes. New VEDDRA terms can be submitted to the CVMP Pharmacovigilance Working Party VEDDRA subgroup which meets six times a year, with new additions made to the VEDDRA dictionary once a year, usually at its spring meeting (see http://eudravigilance.emea.europa.eu/veterinary/standardList01.asp).

The seriousness of the adverse event is assessed at the case level, and expectedness of the event is assessed at the product level based on the product label. The reportability of the case is based on the seriousness of the case and the expectedness at the product level. When more than one product is present for a case, an adverse event may be expected based on one product label and unexpected based on another product label. This is also reflected in the causality assessment which is also at the product level. For cases containing multiple suspect company products, a causality assessment must be entered for each product. There is no place on Form 1932 to capture the animal health company's assessment.

Once CVM OS&C receives an adverse event submission, their own product safety reviewers asses the case and assign a causality based on the algorithm derived from Kramer (Kramer *et al*., 1979). Using the Kramer algorithm, each clinical sign that is reported to be associated with a product is assessed individually and a summary score is assigned to that clinical sign and entered into the CVM database (STARS; http://www.fda.gov/cvm/kramer.htm). The scores range between –9 and +7, with scores ≥0 being considered possibly, probably or definitely drug-related. The following criteria are used to evaluate product causality associated with each reported clinical sign:

- previous experience with the drug;
- other possible causes for the clinical sign;
- the temporality of the event;
- evidence of overdose;
- dechallenge and rechallenge.

Other countries require that the animal health company assess the adverse event and report this assessment to the regulatory agencies.

The most commonly used causality approach is that outlined in the CVMP *Guideline on Harmonising the Approach to Causality Assessment for Adverse Reactions to Veterinary Medicinal Products*, which was finalised on 15 April 2004 (EMEA, 2003). This guideline developed a causality assessment questionnaire which was designed to reduce the bias and inconsistency in using the ABON system. The ABON causality system assigns cases in category A if the event is 'probably' related to the product, category B if the event is 'possibly' related, category O if the event is 'unclassifiable' or 'unassessable' due to lack of information, and category N if the event is 'unlikely' to be due to product administration. Note that the ABON system of causality is applied to the entire adverse event at the product level, not at each clinical sign level as is the case with the Kramer system.

There are six major questions that are asked in the CVMP causality algorithm. These are:

1. Is there a reasonable association in time and/or anatomical site?
2. Is there a reasonable association with the known pharmacological/toxicological profile, the allergic potential of the drug and/or a dose–effect relation?
3. Are additional data (laboratory tests, pathological findings) confirming clinical plausibility?
4. What about the consistency of the reported reaction – is it already described in literature or SPC, or has it been reported before?
5. Is there any other explanation for the adverse event (confirmed, possible, no other explanation)?
6. Is the reported information insufficient/is there reason to doubt the reporting source/information?

Based on the answers to these questions, a causality category of A, B, O or N can be assigned to the adverse event. Even though causality assessment is not required or reported to CVM, this information is required for US cases submitted as third country reports outside the US and needs to be captured in the pharmacovigilance database. The causality assessment is also an important criterion to be assessed during product safety and efficacy trending.

Since there are no specific requirements for reporting adverse events to USDA, cases involving biological products are assessed with the same criteria for seriousness and expectedness as pharmaceutical products. This will enable future adverse event reporting as well as facilitate product trending using the same criteria for reviewing the data.

Product trending and signal detection

Product trending should be performed for the life of the product. The most critical time for in-depth product trending is immediately following a new product launch and whenever there is a change to the product label, addition of a new claim or change in the product formulation. The aim of product trending during the post-marketing period is to ensure that adverse event signals of clinical impact are detected and safety hazards are minimised, in spite of the inherent limitations in spontaneous adverse event reporting (Goldman, 1998).

There are many factors that influence the number of adverse events that are received for a particular product. The 'Boeing phenomenon' refers to the fact that drugs commonly used by large numbers of individuals are more likely to generate increased numbers of adverse events than less frequently used medications. This phenomenon, named after Boeing aircraft, explained why Boeing aircraft had the highest number of accidents – because they outnumber other kinds of aircraft in the civil aviation market, not because they are less safe (Amery, 1999). This is important to remember when evaluating the number of serious expected and unexpected adverse events that are reported to CVM in the DER reports. While an incidence or prevalence rate is not a

reliable calculation for adverse event reporting or product trending, it does help to put the number of adverse event reports received in relation to the number of doses sold or distributed.

The main reason why these rates are of little use in pharmacovigilance is that the number of reports of adverse events received or the numerator is under-reported and the reporting practices vary by company, and by country. Likewise, the denominator or animals treated, doses sold or doses distributed are most often a best guess. The denominator may be most accurate for biological products where one dose does generally equate to one animal treated and due to the limited shelf life, because biological products are not stocked on the shelves for long periods of time.

Data displaying adverse reaction numbers and distribution data for biological products are best represented by presenting the adverse reaction number and the doses sold or distributed as separate lines on a graph in order to look for trends. See *Figure 12.7* for an example of a very high-level trending of a vaccine product. In this example it can be seen that the number of adverse reactions follows a similar pattern to that of the doses distributed. Generally the adverse reactions lag about a month behind the doses distributed the previous month.

The 'secular trend' in adverse event reporting describes the fact that the total number of reports for all pharmaceutical products in the US have been increasing on a yearly basis. The reason for this trend is not fully understood, but may be related to a number of factors, including the litigious nature of our society, increased media attention, consumer advocacy group activities and the availability of medical information (Bortnichak and Dai, 1999). The 'Weber effect' describes the decrease in the number of adverse event reports that occurs after the first 5 years a product is on the market. This probably reflects the familiarity that practitioners develop with potential side effects of a medication the longer it is available on the market (Amery, 1999).

Taking into account all of these factors, along with the regulatory adverse event reporting obligations, it seems reasonable to apply enhanced pharmacovigilance product trending practices to

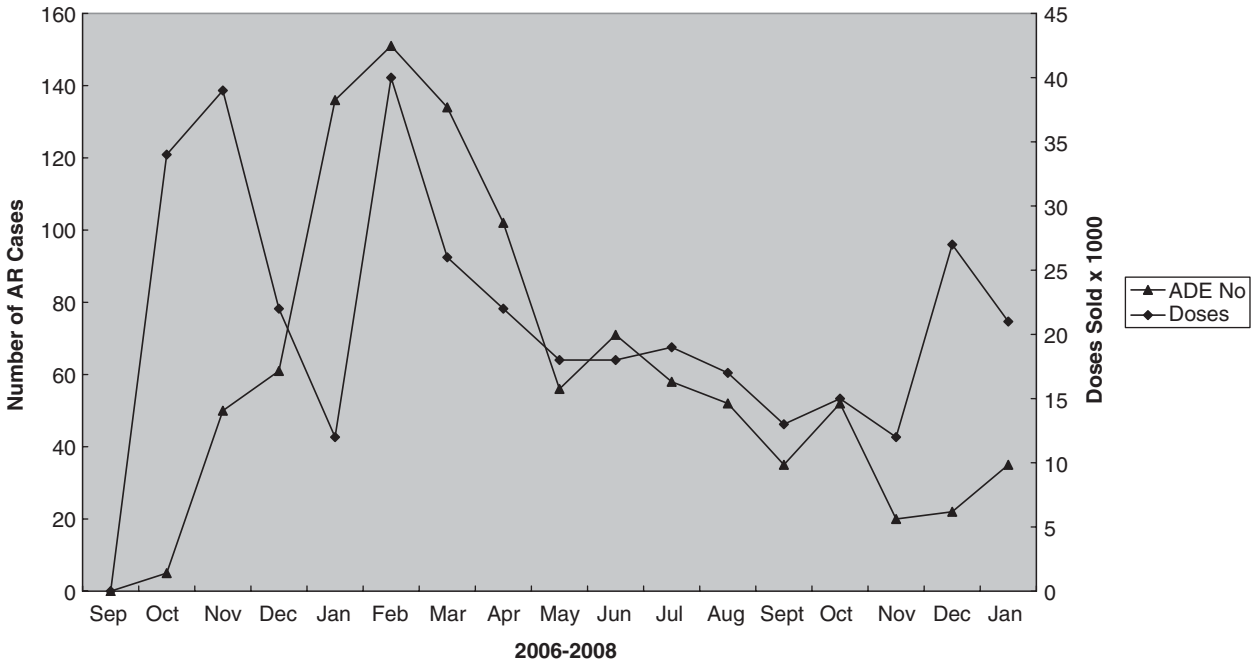

Fig. 12.7 Vaccine adverse reactions (AR) by doses distributed by month. Product × vaccine adverse reactions and doses sold.

newly launched products for the first 2–3 years on the market and then to apply routine surveillance. By the time a product has been on the market for 5 years, it is expected that the safety and efficacy profile of the product should be well defined, unless there has been a new indication or usage approved.

Detailed trend reports (individual case review)

Individual case review remains the gold standard for product trending. By reading and analysing each case and assessing the association (causality) of the product with the event, the best conclusions can be made. Unfortunately, this is not a viable method when many cases and many products require product trending for safety and efficacy signals. Individual case review and analysis still remains the best way to assess new products on the market or assess safety signals identified by other methods of analysis.

Detailed trend report is the term used to report cases in depth and summarise any safety or efficacy issues over a given time period. Similar adverse event cases can be described using a case description and the frequency of this type of case can be monitored for an increase in frequency. For instance, adverse events resulting in gastrointestinal disturbances associated with the administration of a particular antibiotic may be presented in a case description which would describe the duration, severity, outcome and treatment needed if any to bring the case to resolution. This becomes helpful information used to differentiate an event associated with the product versus one more likely to be caused by an underlying disease condition and information that can be disseminated to practitioners.

During individual case review, exclusion criteria are used to eliminate cases from analysis (personal communication with Safety Evaluation and Epidemiology, Pfizer Inc). These can include the following:

- There are duplicate reports of the same case.
- The drug in question was not taken or the treatment had not been started at the time the adverse event was reported to have occurred.
- The adverse event occurred greater that 5–7 half lives after the last reported dose of the drug was taken.
- The adverse event appears to be associated with a pre-existing condition or disease and is not temporally associated with the drug.
- The temporal association with the adverse event is more suggestive of the use of a concomitant drug treatment.

Except in the case of a duplicate report or when the drug was not actually involved, all of the above exclusion criteria would only apply to a detailed product trend report and all cases regardless of causality would still be reported to the regulatory agency.

Crystal reports

Crystal reports are reports that query the data for various parameters that can be chosen by the user, such as time period, product, country and case type. These reports return data in a predefined report that can be used to review data quickly. One method for reviewing data is the use of 'report rates', which is a proportional comparison using the number of times a given system organ class, clinical sign, product defect type or lack of efficacy reason was reported divided by the total number of cases for that species, presented as a percentage.

Figure 12.8 is an adverse reaction report that displays the data by the system organ class, clinical sign, species, report numbers, report rate and report numbers classified by ABON causality. This allows the reviewer to quickly look at all the clinical signs reported for a particular product and species for a given time period and also view the causality assessment of these clinical signs.

Figure 12.9 shows a presentation of an adverse reaction cumulative report for four consecutive

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AR Report Rate
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Species	Product	System	Sign	Reports	Reports/Cases	Causality Assessment A	B	O	N
Canine									
	Product X								
		Application site disorders		**18**	**3.44 %**	**0**	**1**	**8**	**9**
			Application site haemorrhage	1	0.19 %	0	0	1	0
			Application site hair change	1	0.19 %	0	0	0	1
			Injection site abscess	1	0.19 %	0	0	0	1
			Injection site alopecia	1	0.19 %	0	0	1	0
			Injection site erythema	1	0.19 %	0	0	0	1
			Injection site lesion	1	0.19 %	0	0	0	1
			Injection site necrosis	1	0.19 %	0	0	1	0
			Injection site oedema	4	0.76 %	0	0	3	1
			Injection site pain	5	0.96 %	0	1	2	2
			Injection site pruritus	1	0.19 %	0	0	0	1
			Injection site ulcer	1	0.19 %	0	0	0	1
		Behavioural disorders		**61**	**11.66 %**	**0**	**21**	**27**	**13**
			Aggression	4	0.76 %	0	3	0	1
			Anxiety	3	0.57 %	0	1	2	0
			Behavioural disorder NOS	14	2.68 %	0	3	7	4
			Disorientation	6	1.15 %	0	2	3	1
			Grooming disorder	1	0.19 %	0	0	1	0
			Hyperactivity	12	2.29 %	0	5	5	2
			Inappropriate defecation	2	0.38 %	0	1	0	1
			Inappropriate urination	3	0.57 %	0	1	1	1
			Polyphagia	5	0.96 %	0	2	2	1
			Sleep disorder NOS	1	0.19 %	0	0	1	0
			Vocalisation	10	1.91 %	0	3	5	2
		Blood and lymphatic system disorders		**187**	**35.76 %**	**0**	**66**	**89**	**32**
			Anaemia NOS	41	7.84 %	0	14	21	6
			Aplastic anaemia	1	0.19 %	0	1	0	0
			Blood and lymphatic system disorder NOS	1	0.19 %	0	1	0	0
			Blood dyscrasia NOS	1	0.19 %	0	0	1	0
			Eosinophilia	1	0.19 %	0	1	0	0
			Haemolytic anaemia	1	0.19 %	0	0	0	1
			Haemorrhage NOS	3	0.57 %	0	0	3	0
			Hypoproteinaemia	16	3.06 %	0	10	4	2
			Leucocytosis	36	6.88 %	0	12	17	7
			Leucopenia	6	1.15 %	0	1	4	1

Fig. 12.8 Adverse reaction report rate – extract.

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Species	Product	System	Sign	Reports	Reports/Cases	Causality Assessment A	B	O	N
			Lymphadenopathy	5	0.96 %	0	2	2	1
			Lymphocytosis	3	0.57 %	0	0	1	2
			Lymphoma	3	0.57 %	0	1	2	0
			Lymphopenia	5	0.96 %	0	1	4	0
			Neutropenia	1	0.19 %	0	0	1	0
			Neutrophilia	11	2.10 %	0	2	7	2
			Other coagulation abnormality	12	2.29 %	0	6	3	3
			Red blood cell disorder NOS	5	0.96 %	0	0	5	0
			Spleen and reticulo-endothelial system disorder NOS	2	0.38 %	0	0	2	0
			Splenomegaly	2	0.38 %	0	1	1	0
			Thrombocytopenia	28	5.35 %	0	13	10	5
			Thrombosis	3	0.57 %	0	0	1	2
		Cardio-vascular system disorders		**29**	**5.54 %**	**0**	**6**	**15**	**7**
			Arrhythmia	4	0.76 %	0	1	1	2
			Bradycardia	1	0.19 %	0	0	0	1
			Cardiac arrest	3	0.57 %	0	0	3	0
			Cardiac disorder NOS	3	0.57 %	0	0	2	1
			Cardiac enlargement	1	0.19 %	0	0	1	0
			Cardiac tamponade	1	0.19 %	0	0	0	1
			Circulatory shock	1	0.19 %	0	1	0	0
			Endocarditis	1	0.19 %	0	1	0	0
			Murmur	4	0.76 %	0	2	2	0
			Tachycardia	8	1.53 %	0	1	5	2
			Valvular disorder	1	0.19 %	0	0	1	0
			Vasculitis	1	0.19 %	0	0	0	0
		Digestive tract disorders		**467**	**89.29 %**	**15**	**271**	**136**	**45**
			Abdominal cavity disorder NOS	5	0.96 %	0	2	3	0
			Abdominal pain	17	3.25 %	0	9	5	3
			Ascites	4	0.76 %	0	2	2	0
			Bloat	2	0.38 %	0	2	0	0
			Colitis	2	0.38 %	0	0	2	0
			Diarrhoea	77	14.72 %	2	45	24	6
			Digestive tract disorder NOS	14	2.68 %	0	6	5	3
			Digestive tract haemorrhage NOS	10	1.91 %	0	4	2	4
			Distension of abdomen	2	0.38 %	0	0	1	1
			Emesis	199	38.05 %	9	120	54	16
			Enteritis	2	0.38 %	0	0	2	0

Fig. 12.8 *Continued*

Species	Product	System	Sign	Reports	Reports/Cases	Causality Assessment A	B	O	N
			Eructation	1	0.19 %	0	0	1	0
			Flatulence	3	0.57 %	0	1	2	0
			Gastric dilation	1	0.19 %	0	0	0	1
			Gastric ulcer	5	0.96 %	0	4	1	0
			Gastritis	4	0.76 %	0	1	3	0
			Gingival disorder	1	0.19 %	0	0	1	0
			Haematemesis	19	3.63 %	1	16	2	0
			Haemorrhagic diarrhoea	36	6.88 %	1	24	8	3
			Hypersalivation	5	0.96 %	0	1	1	3
			Intestinal stasis	4	0.76 %	0	1	1	2
			Intussusception	1	0.19 %	0	1	0	0
			Involuntary defecation	1	0.19 %	0	1	0	0
			Melaena	26	4.97 %	2	15	8	1
			Oral haemorrhage	2	0.38 %	0	1	1	0
			Pancreas disorder	4	0.76 %	0	2	2	0
			Pancreatitis	6	1.15 %	0	3	2	1
			Peritonitis	1	0.19 %	0	1	0	0
			Rectal haemorrhage	4	0.76 %	0	4	0	0
			Retching	1	0.19 %	0	0	1	0
			Small intestine ulcer	3	0.57 %	0	3	0	0
			Stomatitis	1	0.19 %	0	0	0	1
			Tenesmus	2	0.38 %	0	1	1	0
			Tongue disorder	1	0.19 %	0	0	1	0
			Tooth disorder	1	0.19 %	0	1	0	0
		Ear and labyrinth disorders		**9**	**1.72 %**	**0**	**3**	**6**	**0**
			Internal ear disorder	6	1.15 %	0	1	5	0
			Otitis externa	3	0.57 %	0	2	1	0
		Endocrine system disorders		**7**	**1.34 %**	**0**	**5**	**1**	**1**
			Diabetes mellitus	1	0.19 %	0	0	1	0
			Hypothyroidism	6	1.15 %	0	5	0	1
		Eye disorders		**27**	**5.16 %**	**0**	**2**	**14**	**11**
			Blindness	4	0.76 %	0	0	1	3
			Conjunctivitis	3	0.57 %	0	1	1	1
			Corneal disorder NOS	1	0.19 %	0	0	0	1
			Eye disorder NOS	7	1.34 %	0	0	4	3
			Eye redness	1	0.19 %	0	0	0	1
			Eyeball protrusion	1	0.19 %	0	0	1	0

Fig. 12.8 *Continued*

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Species	Product	System	Sign	Reports	Reports/Cases	Causality Assessment A	B	O	N
			Glaucoma	1	0.19 %	0	0	0	1
			Hyphaemia	2	0.38 %	0	0	2	0
			Impaired vision	5	0.96 %	0	1	3	1
			Pupil disorder	2	0.38 %	0	0	2	0
		Hepato-biliary disorders		**206**	**39.39 %**	**3**	**122**	**62**	**18**
			Cholangiohepatitis	3	0.57 %	0	0	3	0
			Gall bladder & bile duct disorder NOS	2	0.38 %	0	2	0	0
			Hepatic failure	4	0.76 %	0	2	1	1
			Hepatic fibrosis	1	0.19 %	0	1	0	0
			Hepatic necrosis	3	0.57 %	0	2	0	1
			Hepatic neoplasm	3	0.57 %	0	2	1	0
			Hepatitis	4	0.76 %	0	4	0	0
			Hepatomegaly	11	2.10 %	0	4	5	2
			Hepatopathy	175	33.46 %	3	105	52	14
		Immune system disorders		**13**	**2.49 %**	**0**	**9**	**3**	**1**
			Allergic oedema	4	0.76 %	0	2	1	1
			Autoimmune disorder NOS	3	0.57 %	0	1	2	0
			Immune mediated haemolytic anaemia	3	0.57 %	0	3	0	0
			Other immune system disorder NOS	1	0.19 %	0	1	0	0
			Urticaria	2	0.38 %	0	2	0	0
		Metabolism and nutrition disorders		**25**	**4.78 %**	**0**	**11**	**10**	**4**
			Hypercalcaemic condition	1	0.19 %	0	1	0	0
			Hyperglycaemia	11	2.10 %	0	4	5	2
			Hypocalcaemic condition	3	0.57 %	0	1	1	1
			Hypoglycaemia	10	1.91 %	0	5	4	1
		Musculoskeletal disorders		**24**	**4.59 %**	**0**	**8**	**15**	**1**
			Arthritis	2	0.38 %	0	0	2	0
			Joint pain NOS	1	0.19 %	0	0	1	0
			Lameness	8	1.53 %	0	4	4	0
			Musculoskeletal disorder NOS	11	2.10 %	0	3	7	1
			Myopathy	1	0.19 %	0	1	0	0
			Myositis	1	0.19 %	0	0	1	0
		Neurological disorders		**146**	**27.92 %**	**0**	**49**	**64**	**32**
			Abnormal posture NOS	1	0.19 %	0	1	0	0
			Anisocoria	1	0.19 %	0	0	0	1
			Ataxia	49	9.37 %	0	14	25	10
			Central nervous system disorder NOS	8	1.53 %	0	2	5	1

Fig. 12.8 *Continued*

Species	Product	System	Sign	Reports	Reports/Cases	Causality Assessment A	B	O	N
			Coma	1	0.19 %	0	0	1	0
			Convulsion	32	6.12 %	0	16	8	8
			Hyperaesthesia	2	0.38 %	0	0	0	2
			Impaired consciousness	1	0.19 %	0	1	0	0
			Loss of consciousness	5	0.96 %	0	2	0	3
			Miosis	1	0.19 %	0	1	0	0
			Muscle tremor	18	3.44 %	0	5	8	4
			Mydriasis	4	0.76 %	0	1	3	0
			Myoclonus	2	0.38 %	0	0	2	0
			Nystagmus	5	0.96 %	0	2	2	1
			Paresis	12	2.29 %	0	3	7	2
			Proprioception abnormality	3	0.57 %	0	0	3	0
			Sedation	1	0.19 %	0	1	0	0
		No signs		**2**	**0.38 %**	**0**	**1**	**1**	**0**
			No sign	2	0.38 %	0	1	1	0
		Renal and urinary disorders		**170**	**32.50 %**	**7**	**81**	**55**	**27**
			Cystitis	5	0.96 %	0	4	0	1
			Oliguria	1	0.19 %	0	0	1	0
			Polyuria	17	3.25 %	0	6	5	6
			Renal disorder NOS	69	13.19 %	4	40	21	4
			Renal failure	11	2.10 %	3	7	1	0
			Renal insufficiency	1	0.19 %	0	1	0	0
			Stranguria	2	0.38 %	0	0	1	1
			Urinary bladder disorder NOS	4	0.76 %	0	1	2	1
			Urinary incontinence	12	2.29 %	0	1	8	3
			Urinary retention	2	0.38 %	0	1	0	1
			Urinary tract disorder NOS	17	3.25 %	0	7	8	2
			Urinary tract neoplasm	2	0.38 %	0	0	0	2
			Urine abnormalities	26	4.97 %	0	13	8	5
			Urolithiasis	1	0.19 %	0	0	0	1
		Reproductive system disorders		**3**	**0.57 %**	**0**	**0**	**1**	**2**
			Penile disorder NOS	1	0.19 %	0	0	0	1
			Preputial haemorrhage	1	0.19 %	0	0	0	1
			Scrotal disorder NOS	1	0.19 %	0	0	1	0
		Respiratory tract disorders		**74**	**14.15 %**	**0**	**25**	**32**	**16**
			Apnoea	2	0.38 %	0	0	1	1
			Bradypnoea	1	0.19 %	0	0	0	1

Fig. 12.8 *Continued*

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Species	Product	System	Sign	Reports	Reports/Cases	Causality Assessment A	B	O	N
			Cough	7	1.34 %	0	2	4	1
			Dyspnoea	10	1.91 %	0	4	4	2
			Epistaxis	5	0.96 %	0	3	2	0
			Hydrothorax	1	0.19 %	0	1	0	0
			Pleuritis	3	0.57 %	0	1	1	1
			Pneumonia	2	0.38 %	0	2	0	0
			Pulmonary disorder NOS	2	0.38 %	0	1	1	0
			Pulmonary haemorrhage	1	0.19 %	0	0	1	0
			Rale	3	0.57 %	0	1	2	0
			Respiratory tract disorder NOS	2	0.38 %	0	1	1	0
			Rhinitis	5	0.96 %	0	2	2	1
			Sneezing	1	0.19 %	0	0	0	1
			Tachypnoea	29	5.54 %	0	7	13	8
		Skin and appendages disorders		**78**	**14.91 %**	**0**	**20**	**42**	**14**
			Alopecia general	6	1.15 %	0	1	5	0
			Alopecia local	1	0.19 %	0	0	1	0
			Claw / hoof / nail disorder NOS	1	0.19 %	0	0	1	0
			Dermatitis	11	2.10 %	0	3	4	4
			Dermatosis NOS	2	0.38 %	0	0	2	0
			Desquamation	1	0.19 %	0	0	0	0
			Erythema	5	0.96 %	0	2	2	1
			Granuloma	1	0.19 %	0	0	1	0
			Malodour	1	0.19 %	0	0	0	1
			Pigmentation disorder	2	0.38 %	0	0	2	0
			Pruritus	11	2.10 %	0	6	3	2
			Pyoderma	7	1.34 %	0	3	3	1
			Seroma	2	0.38 %	0	1	1	0
			Skin abscess NOS	1	0.19 %	0	0	1	0
			Skin and/or appendage neoplasm NOS	2	0.38 %	0	0	2	0
			Skin haematoma	7	1.34 %	0	1	5	1
			Skin lesion NOS	6	1.15 %	0	3	2	1
			Skin necrosis	2	0.38 %	0	0	0	1
			Skin oedema	3	0.57 %	0	0	2	1
			Skin petechiation	5	0.96 %	0	0	4	1
			Skin vascular disorder NOS	1	0.19 %	0	0	1	0
		Systemic disorders		**749**	**143.21 %**	**10**	**340**	**279**	**115**
			Abnormal test result	125	23.90 %	5	53	46	20

Fig. 12.8 *Continued*

Species	Product	System	Sign	Reports	Reports/Cases	Causality Assessment A	B	O	N
			Adipsia	5	0.96 %	0	1	4	0
			Anorexia	173	33.08 %	2	97	53	20
			Collapse NOS	3	0.57 %	0	0	2	1
			Congested mucous membrane	1	0.19 %	0	0	0	1
			Cyanosis	2	0.38 %	0	0	0	2
			Death	93	17.78 %	0	23	44	26
			Dehydration	24	4.59 %	0	9	13	2
			General pain	8	1.53 %	0	2	3	2
			Glazed eye	1	0.19 %	0	0	0	1
			Hyperthermia	1	0.19 %	0	0	1	0
			Hypothermia	3	0.57 %	0	1	1	1
			Jaundice	33	6.31 %	1	23	7	2
			Lethargy	143	27.34 %	2	74	47	20
			Localised pain NOS	1	0.19 %	0	1	0	0
			Loss of condition	51	9.75 %	0	23	23	5
			Moribund	1	0.19 %	0	1	0	0
			Mucosa petechiation	1	0.19 %	0	1	0	0
			Neoplasia NOS	2	0.38 %	0	0	1	0
			Oedema NOS	8	1.53 %	0	1	5	2
			Pale mucous membrane	11	2.10 %	0	4	6	1
			Polydipsia	34	6.50 %	0	16	11	7
			Pyrexia	22	4.21 %	0	7	12	2
			Systemic disorder NOS	3	0.57 %	0	3	0	0
		Uncoded signs		**2**	**0.38 %**	**0**	**0**	**2**	**0**
			Uncoded sign	2	0.38 %	0	0	2	0
	Total Product X Canine Cases			**523**					

Total Number of Cases
Product X 523 Cases

VIEW RAW DATA

Report Run for Products: **Product X**

Fig. 12.8 *Continued*

PV SYSTEM

AR cumulative

Animal Health Company

Species	Product	System	Sign	Report Period 1 01-Jan-04 to 31-Dec-04		Report Period 2 01-Jan-05 to 31-Dec-05		Report Period 3 01-Jan-06 to 31-Dec-06		Report Period 4 01-Jan-07 to 31-Dec-07	
Canine											
	PRODUCT B										
		Application site disorders		**2**	**4.44 %**	**1**	**2.13 %**	**9**	**24.32 %**	**9**	**31.03 %**
			Application site blister	0	0.00 %	0	0.00 %	1	2.70 %	0	0.00 %
			Application site erythema	0	0.00 %	0	0.00 %	2	5.41 %	0	0.00 %
			Application site hair change	0	0.00 %	0	0.00 %	4	10.81 %	5	17.24 %
			Application site lesion	0	0.00 %	0	0.00 %	0	0.00 %	3	10.34 %
			Application site oedema	0	0.00 %	0	0.00 %	0	0.00 %	1	3.45 %
			Application site pain	2	4.44 %	1	2.13 %	1	2.70 %	0	0.00 %
			Application site pruritus	0	0.00 %	0	0.00 %	1	2.70 %	0	0.00 %
		Behavioural disorders		**4**	**8.89 %**	**1**	**2.13 %**	**3**	**8.11 %**	**3**	**10.34 %**
			Behavioural disorder NOS	2	4.44 %	1	2.13 %	0	0.00 %	2	6.90 %
			Disorientation	1	2.22 %	0	0.00 %	0	0.00 %	1	3.45 %
			Hyperactivity	0	0.00 %	0	0.00 %	2	5.41 %	0	0.00 %
			Vocalisation	1	2.22 %	0	0.00 %	1	2.70 %	0	0.00 %
		Blood and lymphatic system disorders		**3**	**6.67 %**	**7**	**14.89 %**	**4**	**10.81 %**	**0**	**0.00 %**
			Anaemia NOS	2	4.44 %	3	6.38 %	0	0.00 %	0	0.00 %
			Blood and lymphatic system disorder NOS	0	0.00 %	2	4.26 %	0	0.00 %	0	0.00 %
			Haemorrhage NOS	0	0.00 %	0	0.00 %	1	2.70 %	0	0.00 %
			Leucocytosis	1	2.22 %	0	0.00 %	1	2.70 %	0	0.00 %
			Lymphadenopathy	0	0.00 %	1	2.13 %	0	0.00 %	0	0.00 %
			Lymphocytosis	0	0.00 %	0	0.00 %	1	2.70 %	0	0.00 %
			Neutrophilia	0	0.00 %	0	0.00 %	1	2.70 %	0	0.00 %
			Thrombocytopenia	0	0.00 %	1	2.13 %	0	0.00 %	0	0.00 %
		Cardio-vascular system disorders		**3**	**6.67 %**	**3**	**6.38 %**	**2**	**5.41 %**	**0**	**0.00 %**
			Bradycardia	1	2.22 %	1	2.13 %	0	0.00 %	0	0.00 %
			Cardiac disorder NOS	1	2.22 %	1	2.13 %	0	0.00 %	0	0.00 %
			Tachycardia	1	2.22 %	1	2.13 %	2	5.41 %	0	0.00 %
		Digestive tract disorders		**21**	**46.67 %**	**19**	**40.43 %**	**28**	**75.68 %**	**16**	**55.17 %**
			Abdominal pain	0	0.00 %	1	2.13 %	0	0.00 %	0	0.00 %
			Bloat	0	0.00 %	0	0.00 %	1	2.70 %	0	0.00 %
			Diarrhoea	3	6.67 %	4	8.51 %	5	13.51 %	2	6.90 %
			Digestive tract haemorrhage NOS	0	0.00 %	0	0.00 %	0	0.00 %	1	3.45 %
			Distension of abdomen	0	0.00 %	0	0.00 %	1	2.70 %	0	0.00 %
			Emesis	13	28.89 %	10	21.28 %	12	32.43 %	8	27.59 %
			Gastroenteritis	0	0.00 %	0	0.00 %	1	2.70 %	0	0.00 %
			Haematemesis	0	0.00 %	0	0.00 %	1	2.70 %	0	0.00 %
			Haemorrhagic diarrhoea	1	2.22 %	0	0.00 %	3	8.11 %	1	3.45 %

Fig. 12.9 Adverse reaction cumulative report.

Animal Health Company

Species	Product	System	Sign	Report Period 1 01-Jan-04 to 31-Dec-04		Report Period 2 01-Jan-05 to 31-Dec-05		Report Period 3 01-Jan-06 to 31-Dec-06		Report Period 4 01-Jan-07 to 31-Dec-07	
			Hypersalivation	3	6.67 %	3	6.38 %	2	5.41 %	3	10.34 %
			Involuntary defecation	0	0.00 %	0	0.00 %	1	2.70 %	0	0.00 %
			Melaena	0	0.00 %	1	2.13 %	0	0.00 %	0	0.00 %
			Pancreas disorder	0	0.00 %	0	0.00 %	0	0.00 %	1	3.45 %
			Retching	0	0.00 %	0	0.00 %	1	2.70 %	0	0.00 %
			Stomatitis	1	2.22 %	0	0.00 %	0	0.00 %	0	0.00 %
		Ear and labyrinth disorders		**0**	**0.00 %**	**0**	**0.00 %**	**1**	**2.70 %**	**0**	**0.00 %**
			Internal ear disorder	0	0.00 %	0	0.00 %	1	2.70 %	0	0.00 %
		Endocrine system disorders		**0**	**0.00 %**	**0**	**0.00 %**	**1**	**2.70 %**	**0**	**0.00 %**
			Hypothyroidism	0	0.00 %	0	0.00 %	1	2.70 %	0	0.00 %
		Eye disorders		**0**	**0.00 %**	**1**	**2.13 %**	**1**	**2.70 %**	**1**	**3.45 %**
			Conjunctivitis	0	0.00 %	0	0.00 %	1	2.70 %	0	0.00 %
			Eye disorder NOS	0	0.00 %	1	2.13 %	0	0.00 %	0	0.00 %
			Periorbital oedema	0	0.00 %	0	0.00 %	0	0.00 %	1	3.45 %
		Hepato-biliary disorders		**2**	**4.44 %**	**1**	**2.13 %**	**3**	**8.11 %**	**1**	**3.45 %**
			Hepatopathy	2	4.44 %	1	2.13 %	3	8.11 %	1	3.45 %
		Immune system disorders		**3**	**6.67 %**	**2**	**4.26 %**	**3**	**8.11 %**	**3**	**10.34 %**
			Allergic oedema	1	2.22 %	0	0.00 %	0	0.00 %	2	6.90 %
			Anaphylaxis	1	2.22 %	0	0.00 %	2	5.41 %	0	0.00 %
			Autoimmune disorder NOS	0	0.00 %	1	2.13 %	0	0.00 %	0	0.00 %
			Urticaria	1	2.22 %	1	2.13 %	1	2.70 %	1	3.45 %
		Metabolism and nutrition disorders		**0**	**0.00 %**	**1**	**2.13 %**	**2**	**5.41 %**	**0**	**0.00 %**
			Hypercalcaemic condition	0	0.00 %	1	2.13 %	0	0.00 %	0	0.00 %
			Hyperglycaemia	0	0.00 %	0	0.00 %	1	2.70 %	0	0.00 %
			Hypoglycaemia	0	0.00 %	0	0.00 %	1	2.70 %	0	0.00 %
		Musculoskeletal disorders		**2**	**4.44 %**	**3**	**6.38 %**	**1**	**2.70 %**	**0**	**0.00 %**
			Lameness	0	0.00 %	2	4.26 %	0	0.00 %	0	0.00 %
			Muscle pain	1	2.22 %	0	0.00 %	0	0.00 %	0	0.00 %
			Musculoskeletal disorder NOS	1	2.22 %	1	2.13 %	1	2.70 %	0	0.00 %
		Neurological disorders		**18**	**40.00 %**	**28**	**59.57 %**	**27**	**72.97 %**	**7**	**24.14 %**
			Ataxia	5	11.11 %	11	23.40 %	7	18.92 %	1	3.45 %
			Central nervous system disorder NOS	3	6.67 %	1	2.13 %	0	0.00 %	1	3.45 %
			Convulsion	1	2.22 %	5	10.64 %	11	29.73 %	3	10.34 %
			Epileptic seizure	3	6.67 %	3	6.38 %	0	0.00 %	0	0.00 %
			Impaired consciousness	0	0.00 %	0	0.00 %	2	5.41 %	0	0.00 %
			Loss of consciousness	0	0.00 %	0	0.00 %	2	5.41 %	0	0.00 %
			Miosis	0	0.00 %	0	0.00 %	1	2.70 %	0	0.00 %

Fig. 12.9 *Continued*

PV SYSTEM
AR cumulative

Animal Health Company

Species	Product	System	Sign	Report Period 1 01-Jan-04 to 31-Dec-04		Report Period 2 01-Jan-05 to 31-Dec-05		Report Period 3 01-Jan-06 to 31-Dec-06		Report Period 4 01-Jan-07 to 31-Dec-07	
			Muscle tremor	5	11.11 %	8	17.02 %	3	8.11 %	1	3.45 %
			Nystagmus	0	0.00 %	0	0.00 %	0	0.00 %	1	3.45 %
			Paralysis	1	2.22 %	0	0.00 %	0	0.00 %	0	0.00 %
			Paresis	0	0.00 %	0	0.00 %	1	2.70 %	0	0.00 %
		Renal and urinary disorders		**2**	**4.44 %**	**1**	**2.13 %**	**2**	**5.41 %**	**2**	**6.90 %**
			Renal disorder NOS	0	0.00 %	0	0.00 %	0	0.00 %	1	3.45 %
			Renal failure	1	2.22 %	0	0.00 %	1	2.70 %	0	0.00 %
			Urinary bladder disorder NOS	1	2.22 %	0	0.00 %	0	0.00 %	0	0.00 %
			Urinary incontinence	0	0.00 %	0	0.00 %	1	2.70 %	0	0.00 %
			Urine abnormalities	0	0.00 %	1	2.13 %	0	0.00 %	1	3.45 %
		Respiratory tract disorders		**3**	**6.67 %**	**6**	**12.77 %**	**6**	**16.22 %**	**1**	**3.45 %**
			Apnoea	0	0.00 %	0	0.00 %	1	2.70 %	0	0.00 %
			Cough	0	0.00 %	0	0.00 %	1	2.70 %	0	0.00 %
			Dyspnoea	0	0.00 %	2	4.26 %	0	0.00 %	1	3.45 %
			Foam in respiratory tract	0	0.00 %	0	0.00 %	1	2.70 %	0	0.00 %
			Pulmonary congestion	0	0.00 %	0	0.00 %	1	2.70 %	0	0.00 %
			Pulmonary oedema	0	0.00 %	1	2.13 %	0	0.00 %	0	0.00 %
			Rale	0	0.00 %	0	0.00 %	1	2.70 %	0	0.00 %
			Respiratory tract disorder NOS	1	2.22 %	0	0.00 %	0	0.00 %	0	0.00 %
			Rhinitis	0	0.00 %	0	0.00 %	1	2.70 %	0	0.00 %
			Sneezing	1	2.22 %	0	0.00 %	0	0.00 %	0	0.00 %
			Tachypnoea	1	2.22 %	3	6.38 %	0	0.00 %	0	0.00 %
		Skin and appendages disorders		**25**	**55.56 %**	**19**	**40.43 %**	**9**	**24.32 %**	**7**	**24.14 %**
			Alopecia local	8	17.78 %	4	8.51 %	1	2.70 %	0	0.00 %
			Burn	0	0.00 %	0	0.00 %	1	2.70 %	0	0.00 %
			Dermatitis	0	0.00 %	0	0.00 %	2	5.41 %	1	3.45 %
			Dermatosis NOS	4	8.89 %	2	4.26 %	0	0.00 %	0	0.00 %
			Desquamation	0	0.00 %	0	0.00 %	1	2.70 %	0	0.00 %
			Erythema	4	8.89 %	1	2.13 %	0	0.00 %	1	3.45 %
			Excoriation	0	0.00 %	1	2.13 %	0	0.00 %	0	0.00 %
			Pigmentation disorder	0	0.00 %	1	2.13 %	0	0.00 %	2	6.90 %
			Pruritus	6	13.33 %	7	14.89 %	2	5.41 %	2	6.90 %
			Pyoderma	1	2.22 %	2	4.26 %	0	0.00 %	1	3.45 %
			Seborrhoea	1	2.22 %	1	2.13 %	0	0.00 %	0	0.00 %
			Self trauma	1	2.22 %	0	0.00 %	0	0.00 %	0	0.00 %
			Skin lesion NOS	0	0.00 %	0	0.00 %	1	2.70 %	0	0.00 %
			Skin oedema	0	0.00 %	0	0.00 %	1	2.70 %	0	0.00 %
		Systemic disorders		**40**	**88.89 %**	**44**	**93.62 %**	**38**	**102.70 %**	**19**	**65.52 %**

Fig. 12.9 *Continued*

Animal Health Company

Species	Product	System	Sign	Report Period 1 01-Jan-04 to 31-Dec-04		Report Period 2 01-Jan-05 to 31-Dec-05		Report Period 3 01-Jan-06 to 31-Dec-06		Report Period 4 01-Jan-07 to 31-Dec-07	
			Abnormal test result	5	11.11 %	7	14.89 %	4	10.81 %	1	3.45 %
			Adipsia	0	0.00 %	0	0.00 %	1	2.70 %	0	0.00 %
			Anorexia	9	20.00 %	7	14.89 %	5	13.51 %	3	10.34 %
			Collapse NOS	2	4.44 %	0	0.00 %	0	0.00 %	0	0.00 %
			Death	2	4.44 %	4	8.51 %	6	16.22 %	1	3.45 %
			Dehydration	0	0.00 %	1	2.13 %	2	5.41 %	1	3.45 %
			General pain	0	0.00 %	1	2.13 %	0	0.00 %	1	3.45 %
			Hypothermia	1	2.22 %	2	4.26 %	0	0.00 %	1	3.45 %
			Jaundice	1	2.22 %	0	0.00 %	0	0.00 %	0	0.00 %
			Lethargy	12	26.67 %	19	40.43 %	15	40.54 %	7	24.14 %
			Localised pain NOS	0	0.00 %	0	0.00 %	0	0.00 %	1	3.45 %
			Loss of condition	1	2.22 %	0	0.00 %	0	0.00 %	0	0.00 %
			Moribund	1	2.22 %	0	0.00 %	0	0.00 %	0	0.00 %
			Oedema NOS	0	0.00 %	0	0.00 %	0	0.00 %	1	3.45 %
			Pale mucous membrane	1	2.22 %	1	2.13 %	1	2.70 %	0	0.00 %
			Pyrexia	2	4.44 %	0	0.00 %	3	8.11 %	2	6.90 %
			Systemic disorder NOS	3	6.67 %	2	4.26 %	1	2.70 %	0	0.00 %
		Unspecified		**1**	**2.22 %**	**0**	**0.00 %**	**0**	**0.00 %**	**0**	**0.00 %**
			Unspecified	1	2.22 %	0	0.00 %	0	0.00 %	0	0.00 %
	Total Product B Canine Cases			**45**		**47**		**37**		**29**	

Report run for: Product B; Only Canine cases are displayed

No VIEW RAW DATA is available for this type of report because of the multiple time periods used. Although the case numbers are less than 200 cases per time period, the report is still useful to determine frequency of total reports over time as well as the relative frequency of clinical signs and report rate over time.

Fig. 12.9 *Continued*

time periods for comparison of clinical signs and their respective report rates. Report rates are compared for consistency with label information and are useful for estimating frequency, if the number of cases is ≥200. A clinical sign (preferred term) occurring with a report rate of >5% is considered to be frequently occurring, a report rate of 2–5% is considered infrequent, and a report rate of <2% is considered a rare event. Unexpected clinical signs (not present on the label) that occur at a report rate ≥5% should be reviewed individually to determine whether or not additional actions are needed to determine if there is a true safety or efficacy signal. Additional assessment should take into account the severity or seriousness of the adverse reaction, the causality assessment, concomitant medications, underlying disease conditions and other confounding factors associated with the reason for use. Cumulative reports can be run for any consecutive time periods, months, quarters, years, etc. In comparing cumulative time periods, a three-fold increase in report rate between time periods should prompt a more in-depth case review to determine if further action should be taken.

Analysis of *Figures 12.8* and *12.9* Crystal reports can also be developed for lack of efficacy cases, using 'lack of efficacy reasons'. This can be displayed as report rates by causality (*Figure 12.10*) and cumulative report rates (*Figure 12.11*).

Examples of product defect report rates (*Figure 12.12*) and a product defect cumulative report (*Figure 12.13*) are also given. Other Crystal reports, including human exposure reports, serial and lot number reports, mortality reports and product quality reports for manufacturing sites, can be extremely useful for product reports and allow for the quick review of data. It is also essential to be able to export the data behind the report to Microsoft Excel, in order to view the case numbers and case details to review individual cases when necessary.

While Crystal reports are extremely useful for querying the database and displaying data in a uniform method, it is also necessary to be able to write queries and display data depending on the question that needs to be answered. More flexible querying methods allow for every data field within a group of cases to be queried. PV Works (Vet) contains a 'Build Query' function that allows the user to choose criteria for case selection. Once cases meeting these criteria are selected, the system allows for the selection of data fields that can be exported to Microsoft Excel for further data manipulation. This type of function is essential for trends associated with age, breed, duration of treatment, etc.

Likewise, the creation of a Business Objects (or similar software) Universe allows for the communication of PV Works with a data querying and report system. This also allows for the ability to query every data field in the database and answer complex ad hoc safety and efficacy questions. The product BusinessObjects™ XI is produced by *Business Objects*, a French enterprise software company, specialising in business intelligence (BI). It is now part of SAP AG (*Systemanalyse und Programmentwicklung*). The benefit of a system like BusinessObjects™ XI is user flexibility in querying the database and the ability to create useful reports that can be used over and over again and refreshed with updated data, and also the ability to format data in multiple report formats (see http://www.businessobjects.com/).

It is also possible to manipulate and query data using other programs including Statistical Analysis Software (SAS) (http://sas.com/technologies/analytics/statistics/stat/index.html) and Epi Info. Both of these options require the importation of data as a dataset from the pharmacovigilance database. SAS software and licences must be purchased and Epi Info is available at no charge from the Centers for Disease Control (CDC) website, where training materials are also available (Epi Info, 2007).

Data mining and signal detection

Several data mining and signal detection methods have been developed and used for many years in the human health pharmacovigilance groups.

PV SYSTEM

LOE Report Rate

For Period01-Jan-2007 through 13-Dec-2007

Animal Health Company

Species	Product	LOE Reason	Reports	Reports/Cases	A	B	O	N
Canine								
	Product C							
		Inflammation	21	52.5%	0	7	7	7
		Infection	18	45.00%	0	1	15	2
		Accidental Overdose	1	2.50%	0	0	1	0
	Total Product C Canine Species		**40**					
Total Canine Species			**40**					
Grand Total Cases			**40**					

	Cases	% of Total
PRODUCT C	40	100%
Grand Total	40	100%

View Raw Data

Product(s): PRODUCT C

This report is designed to display lack of efficacy cases by LOE reason. LOE reason can be free test entries or can be chosen from drop down lists that are product specific, so that LOE reasons are chosen from label indications by product and by market

Fig. 12.10 Lack of efficacy (LOE) report rate.

PV SYSTEMS
LOE cumulative

Animal Health Company

Species	Product	LOE Reason	Report Period 1 01-Jan-04 to 31-Dec-04		Report Period 2 01-Jan-05 to 31-Dec-05		Report Period 3 01-Jan-06 to 31-Dec-06		Report Period 4 01-Jan-07 to 31-Dec-07	
Canine										
	PRODUCT C									
		Ear mites	2	11.11 %	10	28.57 %	6	23.08 %	6	16.67 %
		Fleas	3	16.67 %	3	8.57 %	3	11.54 %	4	11.11 %
		Hookworms	4	22.22 %	4	11.43 %	1	3.85 %	5	13.89 %
		Intestinal parasites	0	0.00 %	1	2.86 %	0	3.85 %	0	0.00 %
		Other	1	5.56 %	0	0.00 %	3	0.00 %	0	0.00 %
		Roundworms	0	0.00 %	2	5.71 %	5	11.54 %	1	2.78 %
		Sarcoptic mange	0	0.00 %	2	5.71 %	2	19.23 %	3	8.33 %
		Ticks	0	0.00 %	0	0.00 %	1	7.69 %	2	5.56 %
		Whipworms	0	0.00 %	0	0.00 %	1	3.85 %	1	2.78 %
	Total PRODUCT C Cases		**18**		**35**		**26**		**36**	
Total Canine Species			**18**		**35**		**26**		**36**	
Feline										
	PRODUCT D									
		Demodex	1	0.84 %	0	0.00 %	0	0.00 %	0	0.00 %
		Ear mites	39	32.77 %	38	31.67 %	48	27.27 %	49	26.20 %
		Ear mites, fleas	0	0.00 %	0	0.00 %	0	0.00 %	1	0.53 %
		Fleas	44	36.97 %	28	23.33 %	55	31.25 %	60	32.09 %
		Hookworms	4	3.36 %	2	1.67 %	7	3.98 %	7	3.74 %
		Intestinal parasites	0	0.00 %	3	2.50 %	3	1.70 %	7	3.74 %
		Lice	0	0.00 %	0	0.00 %	7	3.98 %	2	1.07 %
		Nematodes	1	0.84 %	0	0.00 %	0	0.00 %	0	0.00 %
		Other	8	6.72 %	11	9.17 %	11	6.25 %	6	3.21 %
		Roundworms	19	15.97 %	22	18.33 %	24	13.64 %	24	12.83 %
		Sarcoptic mange	1	0.84 %	4	3.33 %	7	3.98 %	6	3.21 %
		Tapeworms	0	0.00 %	2	1.67 %	11	6.25 %	13	6.95 %
		Ticks	3	2.52 %	7	5.83 %	13	7.39 %	4	2.14 %
		Whipworms	0	0.00 %	2	1.67 %	0	0.00 %	0	0.00 %
	Total PRODUCT D Cases		**119**		**120**		**176**		**187**	
Total Feline Species			**119**		**120**		**176**		**187**	
Total Number of Cases	**PRODUCT C CANINE**		**18**		**35**		**26**		**36**	
	PRODUCT D FELINE		**119**		**120**		**176**		**187**	

Fig. 12.11 Lack of efficacy (LOE) cumulative report.

PV SYSTEMS
PD Report Rate
For Period 01-Jan-2007 through 13-Dec-2007

Animal Health Company

Product	Class Category	Classification	Reports	Reports/Cases	Causality Assessment A	B	O	N
Product D								
	Filling/Packaging		**14**	**116.67 %**	**0**	**0**	**14**	**0**
		Cap/Seal	4	33.33 %	0	0	4	0
		Labeling/Coding/Insert	2	16.67 %	0	0	2	0
		Missing Tablets	5	41.67 %	0	0	5	0
		Overfilled/Underfilled	3	25.00 %	0	0	3	0
	Product		**2**	**16.67 %**	**0**	**0**	**2**	**0**
		Broken/Crumbling/Soft	1	8.33 %	0	0	1	0
		Palatability	1	8.33 %	0	0	1	0
Total PRODUCT D Cases			**12**					
Grand Total Cases			**12**					

	Cases	% of Total
PRODUCT D	**12**	**100%**
Grand Total	**12**	**100%**

View Raw Data

Product(s): PRODUCT D

By Convention: All product defect cases are assessed with causality 'O'.

Fig. 12.12 Product defect (PD) report rate.

PV SYSTEMS
PD cumulative

Animal Health Company

Product	Class Category	Classification	Report Period 1 01-Jan-04 to 31-Dec-04		Report Period 2 01-Jan-05 to 31-Dec-05		Report Period 3 01-Jan-06 to 31-Dec-06		Report Period 4 01-Jan-07 to 31-Dec-07	
Product Z										
	Filling/Packaging		**430**	**105.91 %**	**283**	**105.99 %**	**189**	**105.59 %**	**107**	**102.88 %**
		Appearance	3	0.74 %	3	1.12 %	2	1.12 %	0	0.00 %
		Cap/Seal	0	0.00 %	3	1.12 %	2	1.12 %	2	1.92 %
		Component Perform/Miss	0	0.00 %	0	0.00 %	0	0.00 %	3	2.88 %
		Container - Damaged	47	11.58 %	24	8.99 %	0	0.00 %	0	0.00 %
		Container - Empty	1	0.25 %	2	0.75 %	5	2.79 %	1	0.96 %
		Container - Leaking	0	0.00 %	0	0.00 %	0	0.00 %	1	0.96 %
		Container - Missing	0	0.00 %	0	0.00 %	0	0.00 %	4	3.85 %
		Foreign Matter	0	0.00 %	1	0.37 %	0	0.00 %	0	0.00 %
		Labeling/Coding/Insert	0	0.00 %	0	0.00 %	1	0.56 %	3	2.88 %
		Mixed Product	0	0.00 %	0	0.00 %	0	0.00 %	2	1.92 %
		Overfilled/Underfilled	0	0.00 %	0	0.00 %	2	1.12 %	0	0.00 %

Note: Product Quality Complaints can be classified by packaging defects and product defects. It is important to record and report each of these types of complaints and monitor these complaints over time. This is particularly true if the manufacturing site changes or there is a change in the formulation, or other change in the product.
This can be a report that is produced by the manufacturing site that has access to the PV System or by the Pharmacovigilance group and provided to the manufacturing group.

			68	16.75 %	36	13.48 %	0	0.00 %	0	0.00 %
	Product		**11**	**2.71 %**	**10**	**3.75 %**	**11**	**6.15 %**	**5**	**4.81 %**
		Appearance/Color	4	0.99 %	1	0.37 %	2	1.12 %	1	0.96 %
		Contamination/Infestation	0	0.00 %	0	0.00 %	0	0.00 %	1	0.96 %
		Odor/Rancidity	4	0.99 %	7	2.62 %	5	2.79 %	2	1.92 %
		Performance	0	0.00 %	0	0.00 %	1	0.56 %	0	0.00 %
		Precipitate/Sediment	1	0.25 %	0	0.00 %	2	1.12 %	1	0.96 %
		Viscosity	0	0.00 %	1	0.37 %	1	0.56 %	0	0.00 %
										%
	Unspecified		**1**	**0.25 %**	**3**	**1.12 %**	**2**	**1.12 %**	**3**	**2.88 %**
		Unspecified	1	0.25 %	3	1.12 %	2	1.12 %	3	2.88 %
Total PRODUCT Z Cases			**406**		**267**		**179**		**104**	
Grand Total Cases			**406**		**267**		**179**		**104**	

The totals may not equal the number of reports, as only partial data may be displayed in this sample report.

Fig. 12.13 Product defect (PD) cumulative report – extract.

One main advantage of these groups is that they have access to large amounts of adverse event data outside of their own company database. The FDA-Center for Drug Evaluation and Research (CDER) provides quarterly data files from the Adverse Event Reporting System (AERS) (see http://www.fda.gov/cder/aers/default.htm). The files contain raw data extracted from the AERS database for quarterly time periods and are not cumulative. The files, which are available in ASCII or SGML formats, include:

- demographic and administrative information;
- drug information from the case reports;
- reaction information from the reports;
- patient outcome information from the reports;
- information on the source of the reports.

These data can be used as comparators to the company's own product data or a specific product. There is no such comparator available for animal health companies. The FDA-CVM does not provide adverse event data in a format that can be used in this way. If FDA-CVM would provide similar access to data files as does CDER, or if the PV Works community could agree to share blinded data with each other, then data mining and signal detection software could be of value.

One of the main signal detection techniques is the use of the proportional reporting ratio (PRR) (Moore *et al*., 2003). This is illustrated in *Table 12.2*, which shows a 2 × 2 table for reporting PRR.

PRRs can be used in automated signal detection and a PRR >3 and a chi-square >5 could represent a signal. However, it all depends on what database is used for the 'All Other Drugs'. Sometimes this technique will work using a single company's database, but it all depends on what products are in the database and what adverse event is being looked at.

Table 12.2 A 2 × 2 table for reporting proportional reporting ratio (PRR). PRR = (A/(A + C))/(B/(B + D)).

	Specific drug	*All other drugs*
Specific adverse event	A	B
All other adverse events	C	D

No matter what signal is detected using any automated technique, it should be followed up with in-depth single case review to verify the signal. There are numerous examples in the literature where a drug was withdrawn from the market using information obtained from PRR analysis that was incorrect based on confounding factors present within the comparison database (Moore *et al*., 2003).

Another data mining technique is known as Empirical Bayes Screening (EBS). This method ranks drug–event combinations by comparing an observed event with what would be expected if the drug and event were statistically independent (Hauben and Zhou, 2003).

Lincoln Technologies, part of Phase Forward, provides software for data mining and signal detection (http://www.phaseforward.com/products/safety/stratpharma/). Our company conducted a recent pilot project with Lincoln Technologies to test the applicability of this type of software for use with our adverse event database. The software was tested looking for known safety signals. Because we were using our own database as the denominator comparator for expected, many of these signals were masked by the predominance of one product or another in the adverse event database. EBGM (Empirical Bayesian Geometric Mean) >2 is considered to be a possible signal (Hauben *et al*., 2007).

Figure 12.14 illustrates the type of report output from this type of software. For this particular product none of the listed clinical signs was assessed as a possible signal because not only was the EBGM <2 for all clinical signs, but also the confidence interval bars were extremely large. In this case, some of the known signals for this product were missed.

The other thing to consider is that if this software will identify 'false positive' signals because of the database limitation, this could result in a resource drain on the department, requiring an

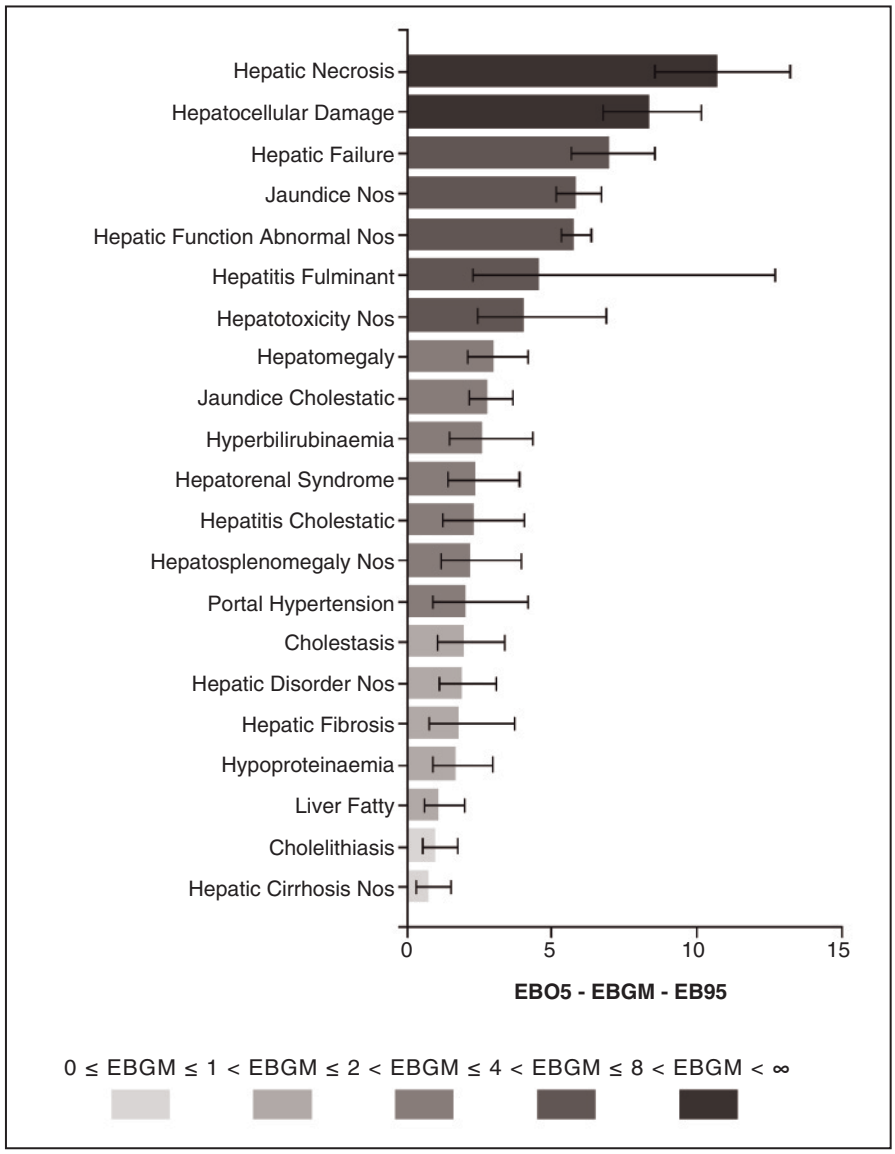

Fig. 12.14 EBGM signal detection data analysis.

investigation of each of these signals by individual, in-depth case review. This type of product trending software has potential once a larger animal health industry-wide database is available for comparative purposes.

Pharmacoepidemiological studies

Post-marketing epidemiologic studies are common in the human side of pharmacovigilance where case-control, cohort and registries are usual. On the veterinary side, pharmacoepidemiology is just coming to the awareness of most pharmacovigilance groups and company management is still reluctant to invest the resources in this type of investigation. It has been recognised by many that this is an area for future development (Chauvin *et al.*, 2002). Few veterinary databases exist that can be used for cohort studies.

The most notable veterinary practice database is that used by Banfield, The Pet Hospital. With

close to 600 animal hospitals located throughout the US, this database is an important source of clinical data and outcome information (Faunt *et al*., 2007). These data are available through DataSavant to researchers, and, in conjunction with Antech clinical diagnostic data, can be used to research the natural history of disease and monitor adverse events to particular products. Moore *et al*. (2005) used this data to determine the incidence rates and potential risk factors associated with adverse events following vaccine administration. Because the exact number of animals that received a particular vaccine was known and the adverse events were monitored for 3 days post vaccination, it was possible to create incidence rates using this data.

Other veterinary databases include the Veterinary Medical Database (VMDB) which includes the Equine Eye Registration Foundation (EERF) and the Canine Eye Registration Foundation (CERF). The VMDB contains patient encounter data from the US Veterinary Schools and currently contains almost 7 million records. Requests can be made for searches of this database (http://www.vmdb.org/vmdb.html).

The VetCancer Registry collects data about neoplasia in dogs and cats. Cases are submitted by veterinarians and must be diagnosed by histopathology. This site provides a search screen and is available to the public (http://www.vetcancerregistry.com/).

It is clear from a review of the databases that are available and a search of the pharmacoepidemiological studies done with animals to study the efficacy and safety of marketed products that this is an area where future attention should be placed. One of the easiest pharmacoepidemiological studies would be a registry. This would be particularly useful for cancer chemotherapeutic agents, where the majority of clinical cases would be handled in a specialty practice. The registry could be set up to follow all cases with a certain type of cancer and the treatment that was used or it could be set up to follow only those cases that were given the medication under study. Much useful information including incidence rate and outcome of adverse events could be monitored.

Regulatory outcome of pharmacovigilance

The outcome of pharmacovigilance varies between a confirmation that the product is performing in the market place according to expectations based on the pre-approval clinical studies or the uncovering of a safety or efficacy issue. Pharmacovigilance may detect a safety or efficacy issue that was not seen during the pre-approval clinical studies. When issues are observed the company may take action that includes further investigation into a particular safety or efficacy issue or it may lead to regulatory agency decisions. Animal health companies may voluntarily, or under the direction of a regulatory authority, initiate a variety of actions including:

- sending a safety alert (Dear Dr. Letter) to practitioners;
- changing product labels by adding warnings, contraindications or human safety information;
- conducting post-marketing research studies;
- recalling specific product lots;
- inspecting manufacturing facilities and records;
- withdrawing the veterinary medicinal product from the market.

(Bataller and Keller, 1999)

The CVM document *Adverse Reactions as a Basis for Regulatory Action* (Center for Veterinary Medicine, 2007) outlines the general review process of adverse reactions at CVM. The review process, in addition to assigning causality, includes a determination of the pattern of occurrence (is this an adverse reaction occurring in a widespread pattern versus a large number of reports collected from a single source), information collected from other sources including text books and the Merck Index, a review of the literature, and, in the case of serious problems, animal investigations carried

out by the Office of Research and, when warranted, consultations with experts in the field (Center for Veterinary Medicine, 2007).

Risk management and pharmacovigilance plans

The next step for pharmacovigilance groups – once they have achieved regulatory compliance, have an operational pharmacovigilance database that is efficient in recording and reporting regulatory submissions which is easy to query and manipulate data – is to consider processes for risk management planning. This is currently required by human pharmaceutical companies and can be found in the ICH E2E guidance document, FDA CDER and CBER (Center for Biologics Evaluation and Research) *Guidance for Industry: E2E Pharmacovigilance Planning and Guidance for Industry: Development and Use of Risk Minimisation Action Plans* (ICH, 2003; FDA-CDER and CBER, 2004, 2005).

Currently, risk management documents are divided into two major parts: the 'safety specification' and the 'pharmacovigilance plan'. The safety specification is a summary of the clinical trial data and the identified risks of a drug, the important potential risks and the major data gaps or missing information (ICH, 2003). A thorough evaluation of the currently known data within the safety specification allows for the development of a pharmacovigilance plan which contains actions to address the safety risks identified in the safety specification. If no potential safety risks are identified, then routine pharmacovigilance practices will be used to monitor the product after launch. In the case of potential safety issues or data gaps, enhanced pharmacovigilance practices may be outlined to address these concerns. These actions can include the collection of additional adverse event follow-up data including laboratory tests or other diagnostics, or it can include a proposal for pharmacoepidemiological studies to begin at the time of product launch.

It is clear that all stakeholders in pharmacovigilance – the regulatory agencies, the product manufacturers, the veterinarians and animal owners – have an interest in making sure that the products that are used on animals are safe and effective. Enhanced pharmacovigilance processes and cooperation between regulatory agencies and product manufacturers are ways that this can be achieved to the benefit of all.

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13 Practical veterinary pharmacovigilance

D. O'Rourke

General approach

Pharmacovigilance is the combined efforts of authorities, industry, the veterinary profession and end-users to evaluate safety and efficacy of veterinary medicines in practical use situations and to incorporate these findings in product availability and documentation in order to optimise animal health, welfare and public health.

There are limitations with the pre-authorisation clinical studies that are carried out:

1. *Short duration* – effects that develop with chronic use or those that have a long latency period are impossible to detect.
2. *Narrow population* – generally studies do not include special groups (e.g. young, old) to a large degree, and are not always representative of the population that may be exposed to a drug after approval.
3. *Narrow set of indications* – those for which efficacy is being studied which do not cover actual evolving use.
4. *Small size* – effects that occur rarely are very difficult to detect.

At least 30,000 animals need to be treated with a drug to discover (with a power of 0.95) at least one animal with an adverse reaction which has an incidence of 1 in 10,000 (*Table 13.1*). Therefore, these studies seldom detect or define the frequency of all important adverse reactions.

Furthermore, when a product is launched and used in practice we have:

- a larger number of animals (than in the trials);
- combination with other products;
- other environmental conditions;
- off-label use: dosage/time;
- age/condition: use in young or old;
- and sometimes . . . product failures.

So, in essence, the safety profile of a veterinary medicine evolves over its lifetime on the market.

Consequently, the aims and scope of pharmacovigilance are to monitor marketed veterinary medicines in order to ensure their safe use. The concepts of 'safety' and 'risk' are interrelated. Safety implies a *judgment* about the acceptability (or lack thereof) of a certain risk or set of risks. In essence, this is the concept of benefit : risk balance. Risk refers to (1) a *measure* of the chance the damage will occur to the animal's health, and (2) an appreciation of the severity of that damage. The role of the company pharmacovigilance department/team is to propose measures aimed

Table 13.1 Numbers of exposed animals required to detect true frequencies of adverse reactions.

Frequency of adverse reaction	*Statistical power**			
	95%	*90%*	*80%*	*63%*
1/100	300	231	161	100
1/500	1,500	1,152	805	500
1/1,000	3,000	2,303	1,610	1,000
1/5,000	15,000	11,513	8,048	5,000
1/10,000	30,000	23,026	16,095	10,000
1/50,000	150,000	115,130	80,472	50,000

*Statistical power is the probability of detecting an adverse reaction if it really occurs in the population under study (e.g. studying 11,513 treatments of which a product will allow, 9 out of 10 times, detection of an AR occurring in 1 out of 5,000 exposed animals).

at improving the safe use of the veterinary medicines concerned.

Objectives of pharmacovigilance

The objectives of pharmacovigilance are to:

- identify rare adverse reactions not detected during pre-licensure studies;
- monitor increases in known reactions;
- identify risk factors or pre-existing conditions that may promote reactions;
- refute false positive reactions.

However, there are limitations with pharmacovigilance:

- variability in reporting standards;
- reporter bias;
- significant under-reporting of reactions.

Variability in reporting standards

Veterinary surgeons must recognise and realise their key role in noticing adverse reactions. There must be a willingness to report, spend the time required to collate an accurate history and submit the report. Far too often they do not give sufficient time to ensure all the relevant information is collected.

Reporter bias

Outside issues can influence the views of the reporter. The length of time the product has been on the market can influence the reporting rate. An increased reporting rate is normally seen in the first 2 years after a product is launched, an illustration of the Weber curve or Weber effect (Amery, 1994; see Chapter 28).

A temporal reporting bias can often occur where the reporter only considers that reactions within 4 weeks of treatment are related to the product. This can result in the long-term effects of products being missed, e.g. liver problems in dogs related to non-steroidal anti-inflammatory drug (NSAID) therapy and fibrosarcoma in cats related to FeLV vaccination.

The reporting environment, e.g. news coverage, can influence the reporter. When NSAID tablets were launched in the US stories of possible adverse reactions appeared in the key newspapers as well as on Fox Prime Time News. Following this, the reporting rate of reactions significantly increased.

Finally, there can be individual bias where the veterinary surgeon or owner is convinced, regardless of available evidence, that the veterinary medicinal product is responsible for the reaction seen.

Significant under-reporting of reactions

A problem with spontaneous reporting on the human side is that less than 10% of all serious and only 2–4% of non-serious adverse reactions are reported.

In 2006 the Veterinary Medicines Directorate (VMD) received 2,384 suspected adverse reaction (SAR) reports, of which 1,084 (45%) were reports of authorised veterinary medicines used according to manufacturers' instructions (Dyer *et al*., 2007). There are just over 3,900 practices in the UK so this equates to about 1 in 3 practices reporting 1 SAR report per year. So in essence there is significant under-reporting. How do we compare to our medical colleagues? In 2005/2006 the Medicines and Healthcare products Regulatory Agency (MHRA), the authority in the UK that regulates human pharmaceutical products and medical devices, received 22,480 SAR reports, of which 68% were serious reactions and 4% were fatal (MHRA, 2007). With around 10,350 medical practices in the UK this equates to around 2 SAR reports per practice per year.

Analysis of data

Causality

After a case report is received it must be assessed to ascertain so far as is reasonably practicable whether the reaction noted is related to the product or not. There are a number of factors to take into account when assessing a case history:

- associative connection in time – including dechallenge and rechallenge following repeated administration (in clinical history) – or anatomic sites;
- pharmacological explanation, blood levels, previous knowledge of the drug;
- presence of characteristic clinical/pathological phenomena;
- exclusion of other causes;
- completeness and reliability of data;
- quantitative measurement of the degree of contribution of a drug to the development of the reaction (dose–effect relationship).

One of the key methods that attempt to assess the degree of certainty that the SAR is product related is causality assessment. Different methods for causality assessment are available (Gray, 1997; Woodward, 2005):

- informal guides such as the 'ABON system' elaborated by the EU's Committee for Medicinal Products for Veterinary Use (CVMP) Pharmacovigilance Working Party (PhVWP);
- structured algorithms and decision trees of various kinds such as the algorithm used by the US Center for Veterinary Medicines (CVM-FDA);
- Bayesian probability;
- expert system.

In veterinary pharmacovigilance the ABON system is used by the majority of companies. According to this coding system, four categories of causality can be made:

- category 'A': probable;
- category 'B': possible;
- category 'O': unclassified (cases where insufficient information was available to draw any conclusion);
- category 'N': unlikely to be related to the veterinary medicinal product.

For inclusion in category 'A' (probable), *all* the following minimum criteria should be complied with:

- There should be a reasonable association in time between the administration of the veterinary medicinal product and onset and duration of the reported adverse reaction.
- The description of the clinical event should be consistent with, or at least plausible, given the known pharmacology and toxicology of the veterinary medicinal product.
- There should be no other equally plausible explanation(s) of the case reported. (If such are suggested – Are they validated? What is their degree of certainty?) In particular, concurrent

use of other products, possible interactions or intercurrent disease should be taken into account in the causality assessment.

Where any of the above criteria cannot be satisfied (due to conflicting data or lack of information) then such reports can only be classified as 'B' (possible), 'N' (unlikely) or 'O' (unclassifiable/unassessable).

For 'B' (possible), the administration of the veterinary medicinal product is another possible and plausible cause for the reported event where the available data do not meet the criteria for inclusion in 'A'.

For 'N' (unlikely), sufficient information exists to establish beyond reasonable doubt that the veterinary medicinal product was not likely to be the cause of the adverse reaction.

'O' (unclassifiable/unassessable) is applied to all cases where reliable data concerning a SAR are unavailable or insufficient to make an assessment of causality.

In the majority of situations the breakdown of causality codes will be:

A – 5%
N – 5%
B and O – 90%

Expectedness

Another key requirement is to assess whether the SAR was expected. For human medicines, a company core safety information (CCSI) exists which lists the expected reactions that have been recorded worldwide. No such document exists on the veterinary side so expectedness is based on what appears in the product literature, labelling or summary of product characteristics (SPC). As the majority of product licences are national the list of expected reactions is national and not global. Consequently, we end up in a dilemma when assessing the expectedness of an SAR as it may be listed in the label in the US but not in the label or SPC in France. Therefore, if the reaction is noted in the US it is deemed as expected, yet as it is not labelled or on the SPC in France it is deemed as unexpected. This leads to problems in the analysis of worldwide cases for a particular product as well as the reporting of third country reports (see section 'Reporting to regulatory authorities').

Pharmacovigilance database

The majority of companies use some form of computerised database to analyse the spontaneous reports (cases) they receive. Use of a database has the following strengths:

- It is a fast means of tracking signals.
- It is less expensive than other systems.
- It is comprehensive in many respects as it covers:
  - a large number of animals: in principle the whole population exposed to the product;
  - all products;
  - any type of ADR;
  - all veterinarians.
- There is no limit to the duration of the investigational effort and it starts as soon as the first dose of a new product has been administered.
- It does not interfere with the prescribing habits of other aspects of day-to-day practice.

However, there are weaknesses of a database which should be noted and taken into account:

- It generates only signals, i.e. suspicious or causal connection.
- It is incapable of producing a reliable estimate of the incidence of an adverse effect.
- The amount of clinical information covered by the individual reports is usually too limited in exactness and detail to permit a thorough evaluation of the case owing to:
  - the poor quality of collected information and the usual lack of follow-up data;
  - the reported information being misleading.
- There is always a reporting bias if only because veterinarians are known to more easily report those ADRs that they consider as probably caused by the product. This bias is further enhanced if public awareness of a reaction

increases – this is known as the 'bandwagon' phenomenon.

A database allows data mining (see Chapter 28) to be carried out which may assist in identifying possible signals. However, additional review and scientific investigations will always be necessary to validate the signal and establish or rule out a causal relationship between a product and SAR. One key point to remember is that electronic pharmacovigilance systems assist but do not replace the knowledge of safety or veterinary reviewers.

Signal detection

A spontaneous SAR report database is not a reliable source for determining the incidence of a certain side effect. Moreover, individual ADR reports are rarely suggestive enough to generate a strong signal on their own. However, it is very useful in detecting signals pointing to a potential side effect and can also be helpful, if cautiously used, in delineating a population at risk. One must determine 'What is my aim?' and be aware of the constant danger of over-interpreting this type of information, which in essence consists of anecdotes.

A signal is reported information on a possible causal relationship between an adverse reaction and a drug, the relationship being unknown or incompletely documented. It consists of a hypothesis together with data and arguments; arguments in favour and against the hypothesis. These relate to numbers of cases, statistics, clinical medicine, pharmacology (kinetics, actions, previous knowledge), toxicology and epidemiology, and may also refer to findings with an experimental character. One key point to note is that a signal is an evaluated association which is considered sufficiently important to investigate further. After the launch of a product onto the market, initial signals soon become apparent (signal generation) and then become more obvious (signal strengthening) and reach a point where they warrant evaluation and follow-up (*Figure 13.1*).

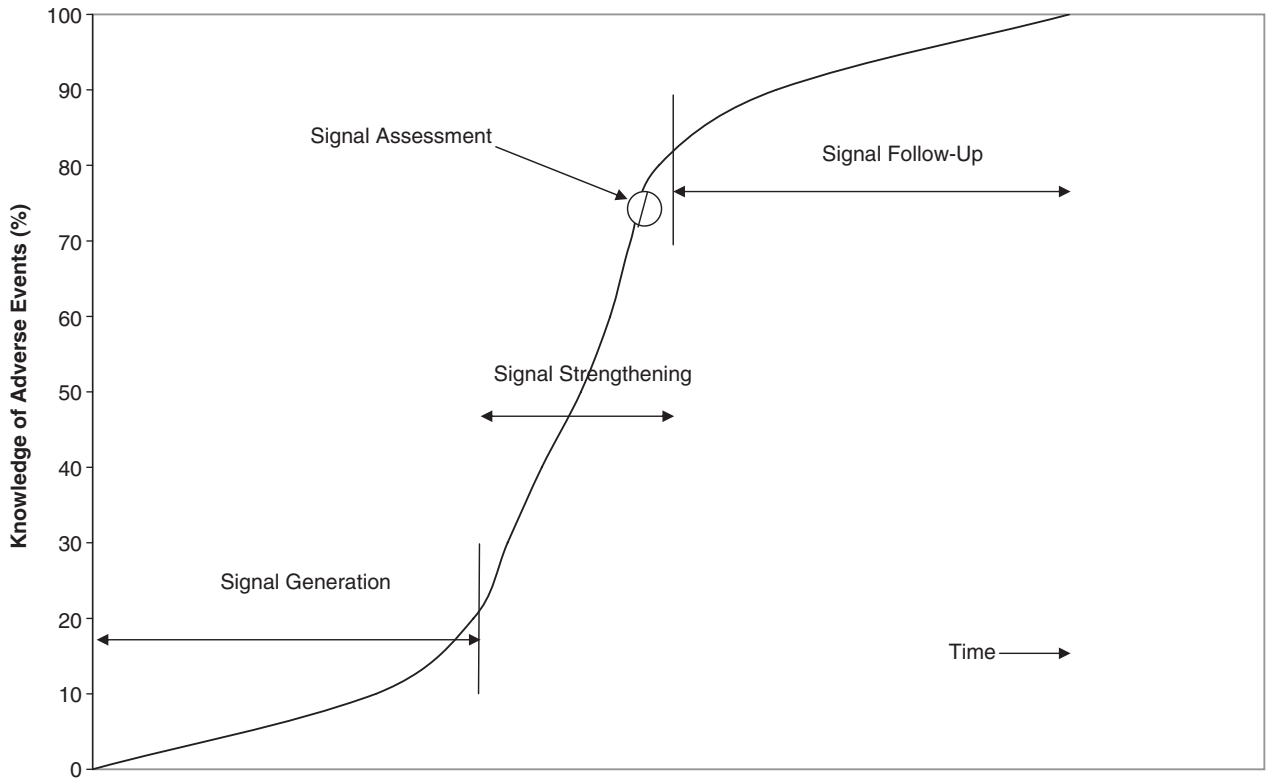

Fig. 13.1 The process of signal discovery, from earliest suspicion, to signal to explained and understood phenomenon.

A signal can be:

- a previously unrecognised safety issue;
- a change in severity;
- a change in frequency;
- identification of at-risk group.

Signal detection in pharmacovigilance comprises the process of:

- selection of a drug-related adverse association;
- preliminary assessment of the available evidence;
- follow-up of how the signal develops.

One key point to take into account is that there are no randomised controls in veterinary pharmacovigilance. So, for example, we do not know the incidence of death in a dog with liver disease. Consequently, if we calculate the incidence of death in dogs with liver disease that were given NSAID tablets we cannot compare this to the incidence of death in a dog with liver disease. If the incidence in these two groups were the same then it could be argued that the use of NSAID tablets did not lead to the death of the animal.

Signal generation methods generate more signals than true signals and, therefore, we need to have methods for ranking signals for further evaluation. Points to consider when ranking signals are:

- number of reports (consideration to estimated usage);
- quality of information;
- nature of the reaction (seriousness and severity);
- plausibility;
  - pharmacological
  - whether reported elsewhere;
  - occurs with drugs in class.

Frequency distribution

The frequency of SAR reports can be estimated using the following calculation:

$$Ft_2\,(\%) = [(N_{t1-2}) / N_{0-t}] \times 100$$

where Ft_2 (%) = the frequency over post-marketing interval t1 to t2, N_{t1-2} = total number of adverse drug reactions (ADRs) during period t1 to t2 and N_{0-t} = total reported ADR cases over entire data collection period.

The frequency distribution of SARs over time can provide an indication of the AR safety profile of drugs over the learning period and provides the rate of incidence of AR over time. It can be a powerful tool for providing a safety signal for newly marketed drugs.

Ajayi *et al*. (2000) carried out an analysis of 22 human drugs from time of approval using data obtained from the FDA Adverse Events Reporting System (AERS). Eight of these drugs were selected because they were removed from the market, while the other 14 drugs were randomly selected. Their results indicated that the SAR frequency distribution curves for the 22 drugs studied have two different characteristics. For drugs with more safety concerns or in which the SARs were considered to be clinically severe, higher SAR incidence rates were observed within the first 2 years of marketing. These high SAR incidence rates and the fact that the SARs were of a major public health concern led to their withdrawal from the market, or placement of restrictions on their use. On the other hand, drugs with fewer safety concerns tended to have a lower, evenly distributed SAR frequency distribution over time. For most of these drugs, the SAR profile reached a plateau after approximately 2–3 years of marketing. They appeared to maintain a relatively consistent SAR frequency distribution over the entire data collection period, which was expected to remain relatively comparable over the market life of the drug.

The authors concluded that these observations suggest that approximately 2–3 years of post-marketing experience is required to fully understand the safety profile of a new product. Lack of a high incidence rate of clinically significant SAR within this period usually suggests that the market life of the product will not be short

secondary to SAR with a subsequent in-depth understanding of the product.

Frequency of severe SAR cases

The relative frequency of severe SAR cases and those that led to deaths among the total number of reported SARs can provide an indication of the relative significance of the reported SARs (Ajayi *et al*., 2000). The analysis can also provide a signal for products with high safety concerns, such as to warrant their removal from the market or be placed on restricted use; for example, there can be products with relatively small numbers of total reported SAR cases. However, most of these are serious and result in deaths. Examples from human medicine studied in the work by Ajayi and colleagues include flosequinan, beractant, alfentanil and flecainide. Overall, these had very low numbers of adverse drug reactions, but the majority of these were serious or resulted in death. On the other hand, drugs such as albuterol, verapamil, triamcinolone and ipratropium have greater numbers of adverse reactions but relatively few serious ones or ones that resulted in death.

Frequency of VEDDRA signs

An analysis can be carried out on the frequency of VEDDRA (Veterinary Dictionary for Drug Regulatory Activities) signs in a series of case reports for a product. It is estimated that at least 200 case reports are required for this type of analysis to be valid. However, these 200 case reports could have up to 1,000 or more recordings of a VEDDRA sign and the resulting output can be difficult to make sense of. A more realistic analysis can be made by obtaining the frequency of VEDDRA SOCs (System Organ Class) for a product and comparing this with the frequency for other products used in the same species. One can then focus on those SOCs that have a higher number of SARs for the product under review.

Vaccines

The prophylactic use of vaccines, versus the therapeutic use of most drugs, imparts a different benefit : risk profile.

In the majority of cases a reaction occurs within hours of treatment, thereby leading to the suspicion of a link between treatment and the clinical signs (SAR) seen. However, one should keep in mind the possibility of a drug–drug (drug–vaccine) interaction which can lead to an adverse reaction some period after the initial treatment with the product.

The Dutch Authority (BBD) received 11 reports (170 cattle) of anaphylactic reaction to injection of tetracyclines and penicillins. The cattle had been inoculated previously with an inactivated vaccine. Following investigation it was found that the vaccine contained saponin, apparently contaminated with povidone, which had resulted in the animals becoming sensitised (Kamphuis, 1996).

Clark (1994) described a hypersensitivity reaction to the vaccine Torvac RSV that occurred when parenteral antibiotics were given to calves. On investigation it was found that the cause of the reaction was sensitisation to a compound erroneously present in identified batches of adjuvant which was similar to an excipient in some antibiotic formulations.

The VMD identified that sheep that have at any time previously been vaccinated with any brand of foot rot vaccine should not be injected with Cydectin 1% Injectable Solution (moxidectin) for Sheep.

Murphy and Arthur (2000) received reports of serious SARs in piglets following the administration of the *Mycoplasma hyopneumoniae* vaccine. The clinical signs reported ranged from drowsiness to convulsions and death. Subsequent investigation indicated that the vaccine had a component in the excipient that was also present

in other types of vaccines that the sow had been immunised with. As a result, the piglets had become sensitised via ingestion of colostrum from the dam.

These examples show that while an individual's experience may be limited to one or two cases, when collated with data from other sources, it may contribute considerably to the assessment of a potential safety hazard.

Reporting to regulatory authorities

Reports to authorities can be divided into two types – serious and non serious. Serious reports are deemed to be expedited and, depending on the legislation in force in the territory, are required to be submitted within a timeline to the local authority, usually 15 days, from receipt of the four criteria that are needed for a case report (Reporter, Animal, Product, Reaction). In order to meet the timeline for expedited reporting it is normal to set up procedures within a company to ensure that the report is on the database and available to the relevant pharmacovigilance assessor within 7 days of receipt of a serious case report.

Non-serious reports are submitted in a Periodic Safety Update Report (PSUR – EU) or Drug Experience Report (DER – CVM) at various time points, depending on the legislation, in the lifetime of the product. The report may also include a benefit : risk assessment of the product.

There is also a requirement in some regions/countries to submit third country reports, that is, reports of SARs from other regions/countries. As mentioned in the section on 'Expectedness' there are problems with the submission of these reports. For example, in the majority of cases a reaction that is noted on the US label (expected) is not noted on the French label (unexpected). So what is expected in the US is unexpected in France. A US case will be deemed as expected, as that is where it is reported. However, the requirement in the EU is to submit serious and unexpected cases. As it is impossible to make this call without reviewing all the US cases, the majority of companies are submitting all serious reactions from the US as third country reports. This is also good pharmacovigilance practice as it should only be the case owner (usually the person involved with recording and investigating the case) who makes key changes to the case record.

Electronic reporting

We are now in the era of information technology and regulatory authorities are gearing up to receiving reports electronically, thereby removing the need for transfer of paper reports. Before going live with electronic data transfer, standard data fields and lists of terms (species, breed, etc.) must be set up. In recent years considerable work in this area has been carried out.

The European Union has set up EudraVigilance as the schema for electronic transfer of SAR reports. A company has to forward the SAR report in the format required to the Eudra Vigilance gateway, whereupon, if accepted, it will be transferred to the relevant regulatory authority. Although EudraVigilance was due to go live on 1 January 2005 the majority of companies are still testing the gateway. Initially only serious SARS will be transferred via Eudra Vigilance. However, it is planned that in the future periodic safety update reports (PSURs) may also be transferred.

In the US, the CVM is also working towards electronic data transfer. However, they have chosen the ICSR Health Level 7 (HL 7) which harmonises the exchange of many types of health information between health care providers, competent authorities and market authorisation holders. Japan, the other partner in VICH, has not as yet decided which schema it will use for the electronic transfer of data.

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Further reading

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14 Preclinical safety testing and assessment of veterinary pharmaceuticals and pharmacovigilance

K.N. Woodward

Introduction

Medicinal products, and specifically veterinary medicinal products in this context, generally have to satisfy three major criteria before they are authorised, licensed or approved (depending on the terminology in place in the country or region concerned). These criteria are quality, efficacy and safety (Beechinor, 1993a, b; Kloos, 1993; Woodward, 2000), but from the viewpoint of pharmacovigilance it is largely the latter that concerns this chapter. Pharmacological and toxicological studies have traditionally been employed for predictive purposes in the safety evaluation of human and veterinary medicinal products (Perez, 1977; Morton, 1980). In fact, *safety* is a broad term which covers a number of areas including toxicological, pharmacological and microbiological safety and consumer, user and environmental safety assessment and risk evaluation.

These areas are often investigated in supplementary studies so that mechanistic aspects of their biological activities may be better understood (Ritchie, 1991; Woodward, 1991, 1992, 1996, 1997, 1998, 2000, 2004a, b Paige *et al*., 1997, 1998, 1999a; Friedlander *et al*., 1999; Dayan, 2000; Gad and Chengelis, 2001). The studies, regardless of whether they are for human or veterinary medicines, are conducted to enable predictions to be made for human health assessments, be these for human patients, exposed workers or for consumers, and sometimes to exclude effects that may have no relevance to human health. They may also have some predictive value for adverse drug events which might occur in treated animal patients (Woodward, 2005), and they should be reviewed in the context of the results of target animal safety studies.

Safety

Regulatory authorities need to be convinced that residues of veterinary drugs which may persist in edible tissues after slaughter, or that are excreted in milk or find their way into honey, are not going to elicit toxic responses in consumers who have eaten such produce. This topic is reviewed in more detail in Chapter 23 which deals with maximum residue limits (MRLs). Authorities and drug sponsors also need to be reassured that the drug and its veterinary

medicinal product or products will not pose unacceptable risks to users.

Over the last 30 years or so, authorities regulating a wide variety of chemical substances, from pesticides to human medicines, and industrial chemicals to biocides, have recognised a series of toxicological studies, or more accurately toxicity tests, that enable them to characterise the toxic properties of the substances which they aim to regulate, and often, but not always, to identify concentrations or doses that serve as safety limits (Diggle, 1999a–c). These might include occupational exposure limits or safe concentrations for drinking water. Veterinary drugs are no exception to this, and toxicologists have devised testing strategies to investigate the potential toxic properties of substances used in veterinary medicine, particularly from the viewpoint of consumer, user, animal and environmental safety (Farber, 1985; Woodward, 2000).

A variety of tests and testing regimes have therefore been developed to test chemicals, including veterinary drugs, to investigate their safety and many of these overlap. For example, results of toxicology tests may have relevance for target animal safety, while adverse effects in target animal studies may impact on the overall safety profile, and hence on consumer and user safety assessment (*Figure 14.1*).

Toxicity studies

The toxicity studies demanded by regulatory authorities for a range of chemical types, including medicines, veterinary medicines, pesticides, biocides and industrial chemicals, have a degree of similarity. They include studies designed to examine acute effects (single dose) and the effects of repeated exposure as well as those designed to examine specific end-points such as adverse effects on pregnancy, fertility and reproductive performance, neurotoxicity and the ability or otherwise of the substance to induce cancer, and be combined with careful clinical observations of the animals involved as well as rigorous investigations on organ systems (Dayan, 1986; Diggle, 1999a–c). Together, toxicity studies form an integrated chemical safety assessment approach (*Figure 14.2*). Further information on the range of tests required or available and toxicological interpretation can be found in specialist texts (Anderson and Conning, 1988; Woolley, 2003; Green, 2006; Jacobson-Kram and Keller, 2007).

Acute toxicity is generally not an important issue in the assessment of the safety of veterinary drug residues. This is because concentrations of residues of veterinary medicines in animal tissues or produce are unlikely to be sufficiently high to pose an acute toxic hazard and these studies

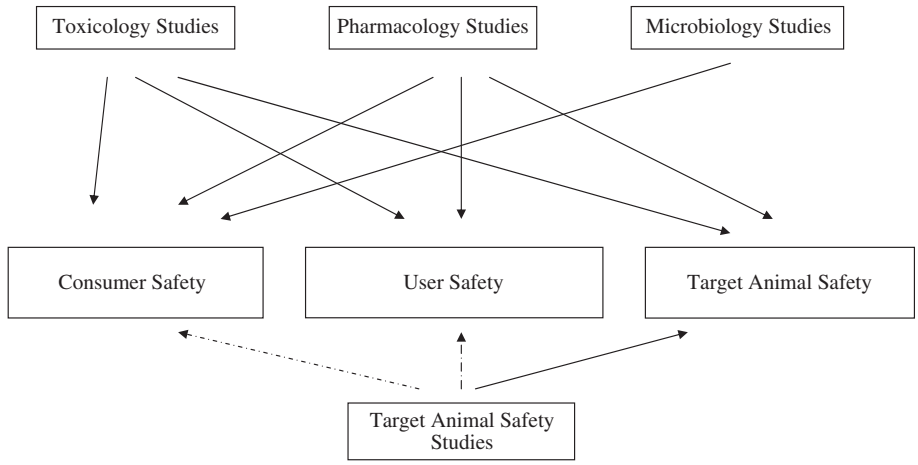

Fig. 14.1 Relationships in veterinary drug safety testing and assessment.

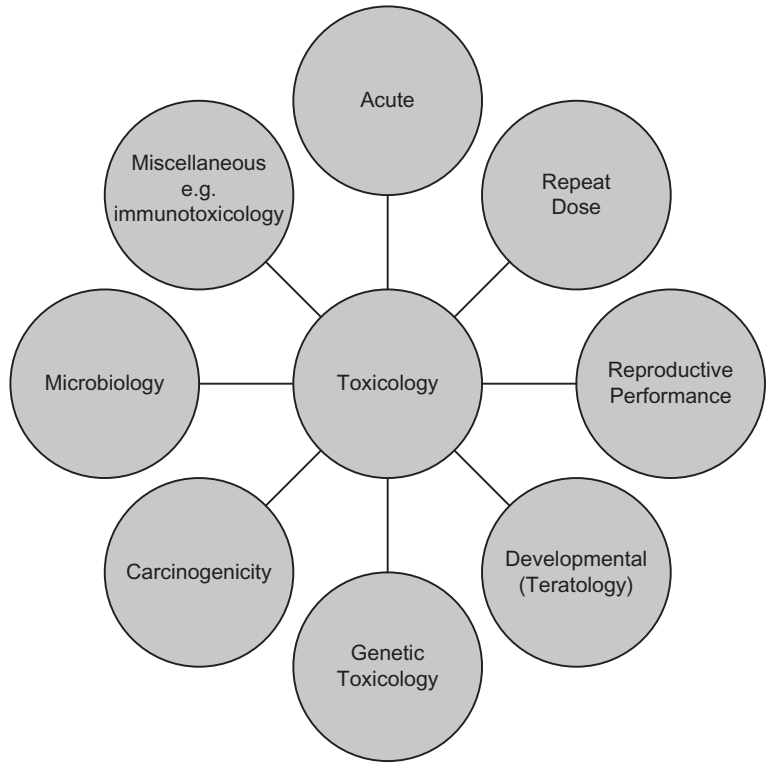

Fig. 14.2 Components of toxicity testing.

serve greater purpose in attempting to predict what might occur following accidental contamination with a veterinary medicine, for example in the occupational setting (Woodward and Atkinson, 1992; Woodward, 1996, 2000).

However, there are some exceptions. For example, concentrations of potent pharmacologically active drugs in animal tissues could conceivably elicit adverse reactions in human consumers. Indeed, this has occurred following the ingestion of meat containing residues of the β-agonist drug clenbuterol. Clenbuterol is a drug that is authorised in a number of countries for a variety of uses in food animals, including relaxation of the uterus in cattle prior to parturition. However, it has also been used illegally as a production enhancer. The drug acts as a repartitioning agent – lowering the amount of adipose tissue while increasing the muscle mass (Brambilla, 1992). In 1990, a total of 22 patients from 8 families suffered toxic effects, including headaches, tremors, dizziness and tachycardia after consuming veal liver later shown to contain relatively high concentrations of clenbuterol (Pulce *et al*., 1991). Similar episodes have occurred in Spain and Italy (Martinez-Navarro, 1990; Maistro *et al*., 1995; Salleras *et al*., 1995; Brambilla *et al*., 1997).

Properly conducted acute toxicity studies, with rigorous observation of the experimental animals, can provide indications of potential for toxicity in longer-term studies; for example, evidence of liver or kidney damage, and signs of neurotoxicity and other effects on physiological systems (Rhodes, 2000). Longer-term studies are designed to investigate the effects of repeat dosing. Such studies are generally of 28 (short-term repeat dose studies) or 90 days (subchronic studies) duration, and occasionally they may be of lifetime duration, namely 24 months in the rat and 18 months in the mouse as part of chronic toxicity tests (Ballantyne, 2000). It is in these studies that target organ toxicity is observed, either directly by histological examination of tissues or indirectly through clinical biochemistry and

examination and analysis of urine and faeces, for example.

More specialised studies are employed to investigate the potential of a drug to induce malformations in the offspring of animals exposed to the material during pregnancy. These are usually referred to as teratology studies and animals are given the drug at sensitive periods of organogenesis in the developing fetus to determine if a potential to produce birth defects exists or to examine whether the drug is generally toxic to the embryo or fetus. These studies are normally performed in a rodent and non-rodent (e.g. the rabbit) species, but it has been suggested that a tiered approach be taken for veterinary medicinal products (Hurrt *et al*., 2003). Similarly, some chemicals are known to affect sperm cells or otherwise have deleterious effects on reproductive performance, and these aspects too form part of toxicological screening.

A drug's potential to cause cancer is clearly of major concern and drugs may be tested in long-term carcinogenicity studies in rodents. However, the results of such studies are notoriously difficult to interpret because of species-specific tumours, non-specific effects, and the generation of tumours which may have no relevance to human risk assessment. The causes of cancer are manifold, but underlying the majority are genetic events involving damage to DNA, mutations and disruption of chromosomes. Chemicals that cause such genotoxic effects are immediately suspect from the point of view of their carcinogenic potential and a number of screening tests have been developed to investigate the effects, if any, of chemical substances. These involve studies using bacteria or mammalian cells, studies in experimental animals and other techniques to investigate effects on DNA and genetic material.

If positive results are obtained in such studies, then suspicion arises that the material could be a genotoxic carcinogen and this then leaves the investigator with three main choices. Initially, genotoxicity studies are usually conducted in a range of in vitro studies. If these provide negative results over a range of end-points (gene mutation, clastogenicity, DNA damage), then further data are unlikely to be required. However, if positive, in vivo studies may be necessary to demonstrate that the effect seen in vitro is an artefact or the result of ex vivo factors with no relevance for safety assessment, or to confirm that a real effect has been identified (Anonymous, 1989, 1991; Kirkland, 1990; Kirkland and Fox, 1993; Kirkland and Dean, 1994). Alternatively, the drug sponsor may decide to abandon the substance on the grounds that further development of a potentially carcinogenic material is likely to be a costly waste of time and money, as it is unlikely to gain regulatory approval. Finally, and as a last resort, the sponsor may decide to conduct animal carcinogenicity studies which may only serve to prove that the material is indeed a genotoxic carcinogen.

Even if the substance gives negative results in a carcinogenicity bioassay, the investigator, and therefore the company developing the product, has to attempt to explain why the material produced evidence of genotoxicity as this may still suggest a potential to affect germ-line cells and create hereditary mutations, or to otherwise have a deleterious effect on offspring.

Despite these considerations, it has been argued that on pharmacological grounds testing of veterinary drugs for carcinogenicity is unnecessary. First, because many veterinary drugs are similar to human drugs, and many of these have been shown to be carcinogenic in rodents but on mechanistic grounds are considered not to pose a human health risk; and second, because the concentrations of veterinary drugs in animal tissues mean that human exposure is likely to be very low (Galer and Monro, 1998a).

While these assertions might be true, it seems likely that on precautionary grounds, sponsors of veterinary drugs will continue to have to demonstrate a lack of genotoxic, and possible carcinogenic potential, before regulatory authorities will authorise their use in food animals, particularly in view of the difficulties involved in identifying thresholds for carcinogenicity (Purchase and Auton, 1995). Furthermore, it seems likely that in an increasingly open regulatory climate, with the

decisions of regulators open to increasing public scrutiny, and with the concerns of society reflected in decision making (Illing 1991, 1999, 2001), that testing of substances for genotoxic and where appropriate for carcinogenic potential will persist for the foreseeable future, while the presence of residues of carcinogenic drugs, unless they can be shown to be irrelevant to human risk assessment, will not be tolerated, contrary to earlier views that zero tolerance might not be a pragmatic solution (Somogyi, 1979), but other proposals, for example, that for a virtually safe level for residues (Farber and Guest, 1984), may yet warrant further consideration.

The need to conduct other studies is usually dependent on either the results from longer-term investigations, such as the 90-day study, or structural alerts in the molecule. Thus, if signs of adverse effects on the immune system are noted in the 90-day study, then investigations of the substance's immunotoxic potential may be considered necessary. If the drug is structurally related to substances known to be neurotoxic, then specific investigations into its neurotoxicity are likely to be deemed necessary.

The studies described above allow toxicologists to characterise the toxicological properties of substances used in veterinary medicinal products, to build up an overall picture of their toxicity profiles and to identify quantitative parameters based on the dosages used in the tests. They are similar to approaches taken for other groups of substances, including those used in food, as are the approaches to hazard identification and characterisation (Barlow *et al*., 2002; Dybing *et al*., 2002).

As toxicological data for older drugs may be available from a number of sources, including the open literature, the final toxicological profile constructed may well depend on a weight of evidence approach (Doull *et al*., 1996). Of the latter, the most important from a regulatory perspective is the no-observed effect level, or NOEL. The NOEL is identified for each study where toxic effects have been observed; for each study, it is the lowest dose at which toxic effects seen at higher doses did not occur, or, more precisely, where they were not seen, as it can never be excluded that subtle effects *did* occur but that they were beyond the observational capabilities of the test system. In fact the NOEL or no-effect level (NEL) as it was once called has been criticised because toxicity standardised toxicity tests do not investigate the full biological profile of a substance and specialised tests are rarely conducted; hence specific adverse effects may be missed (Zbinden, 1979).

It can be argued that the use of the term NOEL avoids this pitfall as it is clearly aimed at toxic effects noted in those studies that have been conducted, rather than toxic effects too subtle to observe, or those that might have been seen had other studies been employed. As mentioned earlier, it is now common practice to conduct specialised tests on the basis of either structure activity alerts or because of effects seen in the standard tests, so perhaps the criticism is partly assuaged, if not entirely removed. A suggestion that the NOEL could be refined by defining it as the dose that is statistically different from both the control group and the lowest observable (adverse) effect level (Calabrese and Baldwin, 1994) appears to have met with universal indifference, possibly because animal toxicity studies in general do not provide sufficient data to draw these distinctions with any confidence.

When it is not possible to identify a NOEL for a particular study, it is often necessary to repeat it using more carefully chosen and titrated doses so that a NOEL *can* be identified. The NOEL plays a crucial role in the safety assessment of other substances to which consumers are likely to be exposed, including food additives such as colorants and antioxidants, residues of pesticides and of course residues of veterinary drugs. Alternative approaches such as the identification of the threshold of toxicological concern (Delaney, 2007; Kroes *et al*., 2007) and tiered toxicity testing (Becker *et al*., 2007) have yet to be adopted, or indeed considered, in the veterinary regulatory area.

When conducting toxicological and other safety studies, regulatory authorities usually require that specific guidelines are followed. These may

be guidelines published by or on behalf of the authority concerned. For example, for veterinary medicines, the EU sets out its requirements for toxicity testing in Annex I to Directive 2001/82/EC as amended by Directive 2004/28/EC. However, this to a large extent only provides advice on what to do and what not to do. It suggests that guidelines developed for other EU regulations (e.g. chemicals) be followed or that sponsors refer to the guidelines published by the Organisation for Economic Co-operation and Development (*OECD Guidelines for Testing of Chemicals*, OECD, Paris, http://www.oecd.org/home/; dates vary according to the date of each guideline or revision of existing guidelines). The OECD produces a range of these recommendations covering physical–chemical properties, ecotoxicology, degradation and accumulation and health effects. The latter deal with toxicity testing and toxicokinetics. Most regulatory authorities require these safety studies, and other studies related to safety (physico-chemical properties, residues analysis) to be conducted according to the principles of Good Laboratory Practice, for which there is an OECD guideline available on which many national guidelines are based.

Increasingly, specific guidelines are being developed for testing of pharmaceuticals through the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH, http://www.vichsec.org/; see Chapter 2). There is also a VICH guideline on the general approach to testing, although this is largely aimed at food safety and residues. Many of these guidelines have been adapted from those produced by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), although some are specific for veterinary purposes.

A list of the VICH guidelines relevant to safety evaluation, and hence ultimately to pharmacovigilance, is given in *Table 14.1*. Further guidelines are available from the ICH (http://www.ich.org/), notably Guideline S7A which is useful in demonstrating pharmacological activity or, in the case of MRLs, and exclusion from the terms of the legislation, absence of pharmacological activity.

Table 14.1 VICH safety guidelines.

Number	*Guideline*
GL6	Ecotoxicity phase 1
GL22	Safety reproduction
GL23	Safety genotoxicity
GL28	Safety carcinogenicity
GL31	Safety: repeat-dose toxicity test
GL32	Safety: developmental toxicity test
GL36	Safety: microbiological ADI
GL37	Safety: repeat dose chronic toxicity
GL38	Ecotoxicity phase II
GL24	Pharmacovigilance
GL29	Pharmacovigilance PSU
GL30	Pharmacovigilance list of terms
GL35	ESTD
GL42	Pharmacovigilance: data elements

Elaboration of veterinary drug MRLs in the EU

Safety assessment

In the EU, veterinary medicinal products are subjected to a system of rigorous legislative requirements in order to demonstrate safety, quality and efficacy. The operation of this legislation is beyond the scope of this chapter and the interested reader is referred elsewhere (Woodward, 1997, 2000, and Chapter 2). However, before any veterinary medicinal product intended for use in food animals can be authorised it must first be entered into one of the Annexes of Council Regulation No. (EEC) 2377/90, the so-called MRL Regulation (see Chapter 23). The prime purpose of this legislation is to ensure that pharmacologically active substances, a definition that includes other constituents of the medicine in addition to the active ingredient or ingredients, are assessed for their toxic potential, and that

consumers of food of animal origin are adequately protected.

In fact, as we shall see, these assessments take into account factors other than toxicity. As noted with the example of clenbuterol, pharmacologic properties which may be desirable for the treated animal may not be at all desirable if they occur in the consumer who has eaten animal products. This sentiment applies not only to pharmacodynamic effects of drugs expressed in the animal (e.g. β-adrenergic effects, various hormonal effects, anaesthesia, analgesia), but it is also true of more indirect effects. For example, it is evidently desirable that the antimicrobial effects of antibiotics are seen in the treated animal, i.e. that the drug exerts its bacteriostatic or bactericidal effects on the pathogenic bacteria causing the disease, while it is not desirable that active residues of such drugs adversely affect the normal gastrointestinal flora of consumers eating meat containing antimicrobially active residues.

Finally, the presence of a particular drug in an edible product is not in itself problematic. What is critical is how much of the drug (and its metabolites) is present, and how long it persists. Veterinary drug residues may be composed of the original substance, the parent drug and, and often or, various metabolites. These are subject to various metabolic processes such as eventual conversion to non-toxic metabolic products including eventually water and carbon dioxide, and excretion in the urine, expired air or bile. In other words, they will eventually decrease in concentration as time passes, as a result of the animal's metabolism. This is known as residues depletion or depuration. So, the risks posed by residues of a veterinary drug depend not only on its toxic, pharmacological and microbiological activities, and those of its metabolites, but also on its rate of disappearance from the animal.

It is obvious from this that another factor therefore is the ability to measure the concentration of the drug and its metabolites, which in turn is dependent on having an adequate analytical method. All of these factors are important in the elaboration of MRLs.

Studies in target animals

As with humans, some of the adverse effects of medicines only become evident when the products are tested in the target species. Similarly, adverse effects noted in experimental laboratory animals may not be seen when tested in target animals. The EU has produced general guidance for target animal safety testing (European Commission, 1999a). These studies are intended to identify adverse effects arising from pharmacodynamic and toxicological origins. They are intended to be conducted using the likely commercial formulation and the method and route of administration. The dose that should be used is the commercial one and multiples of this, so that a safety margin can be identified. In addition to examining systemic effects, the studies are also aimed at identifying local effects such as injection site or skin reactions. There is a specific guideline on the local tolerance of intramammary products intended for use in cattle (European Commission, 1999b).

As veterinary medicinal products are also subject to clinical trials to characterise efficacy, adverse effects may also become evident from these studies.

Toxicity of veterinary drugs

Much of the toxicity data generated on veterinary drugs remain confidential by sponsor companies and is not generally available. Some of the data have become available in summarised form through the efforts of JECFA and through the EMEA by way of MRL summaries. These and data published in the scientific literature can be used to review the toxicological properties of some common drugs used in veterinary medicine, to demonstrate the principles involved in assessing data, identifying NOEL values and calculating acceptable daily intakes (ADIs). To some extent, they also show how different expert bodies can arrive at different conclusions on the basis of the same data.

By far the largest group of drugs used in veterinary medicine are the antimicrobial substances (Black, 1984; Wingfield and Appelbe, 1984; Ziv, 1986; MacNeil and Ellis, 1995; Woodward and Shearer, 1995). So, most of the examples discussed below are chosen from that category.

β-Lactam antibiotics

Penicillins

The term penicillin was given to an antibacterial substance derived from a mould of the genus *Penicillium* by Sir Alexander Fleming in 1929 (Fleming, 1946). The drug soon found its way into human clinical use, and into veterinary medicine (Lovell, 1946). The most common members of the family used in veterinary practice are benzyl penicillin, amoxicillin, ampicillin and penicillin G, the latter with benethamine, benzathine or procaine as the commonly used counter ion (Bishop, 1998; Wright and Wilkowske, 1991).

Penicillin drugs have been widely used since those pioneering days in both human and veterinary medicine (Neu, 1977; Black, 1984; Wright and Wilkowske, 1991; Wright, 1999). They are generally non-toxic in animals and in humans during clinical use (Stewart, 1967, Wilkowske, 1977; Campbell and Cox, 1992; Gentry, 1992; Bush *et al*., 1995) and are relatively non-hepatotoxic (Hautekeete, 1995; George and Crawford, 1996). They are also of low potential for nephrotoxicity except after very high doses (Morin *et al*., 1984; Geller *et al*., 1986).

However, for benzyl penicillin, there are virtually no published results of conventional toxicology studies (JECFA, 1991a). Along with other penicillins, it is known to be neurotoxic after intravenous injection in humans and animals but only after very large doses (Lerner *et al*., 1967; Currie *et al*., 1971; Johnson, 1971; Weiss *et al*., 1974; Schliamser, 1988; Schliamser *et al*., 1988a–c, 1991). It appears not to be a problem after oral administration, the route that consumers will be exposed to residues of the drug. In fact, benzyl penicillin only appears to be acutely toxic in animals when given at high doses to rats fed high sugar diets (Boyd *et al*., 1966).

Amoxicillin has a low order of toxicity in animals (Jones and Hill, 1974), and this is also the case for temocillin (Cockburn *et al*., 1985). There was no evidence of carcinogenicity for ampicillin either in a conventional gavage carcinogenicity study of 2 years duration in rats and mice, or in a 26-week experimental system in Tg-rasH2 mice (National Toxicology Program (NTP), 1987; Dunnick *et al*., 1989; Adachi *et al*., 2002). Penicillin VK gave negative results in rodent bioassays (Dunnick *et al*., 1989) while ampicillin gave negative results in a range of genotoxicity studies (NTP, 1987).

Overall therefore, the toxicological profile with regard to consumer safety is very reassuring. However, and as the 1991 JECFA Monograph notes,

> '. . . (benzyl penicillin) may induce all possible clinical forms of allergic reactions depending on dose, route, frequency of exposure, genetic predisposition and other factors' (JECFA, 1991a).

Penicillins are low molecular weight substances and not by themselves immunogenic. In fact they are haptens, substances that bind irreversibly to tissue macromolecules such as proteins to produce immunogenic materials. In the case of penicillins, the hapten is a metabolite of penicillin, the β-lactam ring or, more precisely, the penicilloyl moiety arising from cleavage of the β-lactam ring (Rosenblum, 1968; Ahlstedt *et al*., 1980; Erffmeyer, 1981, 1986; Davis, 1984; Mitchell *et al*., 1990; Wright, 1999).

As a result, penicillins may elicit a variety of allergic reactions ranging from mild skin rashes to potentially fatal anaphylaxis (Idsoe *et al*., 1968). It has been estimated that penicillin may account for up to 75% of deaths due to anaphylaxis in the United States (Delage and Irey, 1972), and that the frequency of allergic reactions to penicillins ranges from 0.7 to 10% of patients treated (Van Ardsel, 1965; Idsoe *et al*., 1968; Anderson and Adkinson, 1987). Even within the skin reactions, these may be mild to severe and life-threatening;

penicillins can cause mild urticaria and erythema, rashes, erythema multiforme and toxic epidermal necrolysis (Johnson, 1971; Russel and Lessof, 1971; Fellner, 1976, 1986; Takahashi, 1976; Herman and Jick, 1979; Rosenthal *et al*., 1979; Alanis and Weinstein, 1983; Herold *et al*., 1983; Tagami *et al*., 1983; de Haan *et al*., 1986; Chopra *et al*., 1989; Hoffman *et al*., 1989; Puavilai and Timpatanapong, 1989; Staretz and DeBoom, 1990; Ward *et al*., 1990; Romano *et al*., 1993, 1997; Egawa, 1994; Vega, 1994; Arias *et al*., 1995; Adcock and Rodman, 1996; Jiminez *et al*., 1997; Minguez *et al*., 1998; Saenz de San Pedro Morera *et al*., 1999; Gastaminza *et al*., 2000).

Contact dermatitis and urticaria, either as a result of systemic sensitisation or repeated dermal exposure can also occur, for example, following ingestion of contaminated foods or due to occupational exposure to penicillin present as residues in milk or to penicillin itself (Erskine, 1958; Vickers *et al*., 1958; Kautz, 1959; Zimmerman, 1959; Borrie and Barret, 1961; Vickers, 1964; Stewart, 1967, 1969; Reisma and Arbesman, 1968; Minkin and Lynch, 1969; Wicher *et al*., 1969; Mauranges, 1972; Olson and Sanders, 1975; Cany, 1977; Girard, 1978; Lindemayr *et al*., 1981; Rudzki and Rebandel; 1985; Pigatto *et al*., 1986; Woodward, 1991; Lisi *et al*., 1997).

There has been a report of a patient who experienced an anaphylactic reaction after a steak dinner. The patient, known to be sensitised to penicillin, developed generalised pruritus, difficulty in swallowing and speaking, and dyspnoea within 20 minutes of eating. The meat was later found to contain penicillin or penicilloyl moieties (Schwartz and Sher, 1984). A similar event occurred after pork consumption (Tscheuschner, 1972). There has even been a report of anaphylaxis in a patient after the consumption of a soft drink (Wicher and Reisman, 1980). Although penicillin was detected in the drink, its origins were obscure. There are some limited animal models for penicillin hypersensitivity, including cutaneous anaphylaxis (Kristofferson and Ahlstedt, 1982; Kornbrust *et al*., 1989; Kubo *et al*., 1989; Hattori *et al*., 1997), but it is not generally possible at present to predict which patients will react, and in which way.

As others have noted, it is difficult to quantify the public health risks of penicillin residues in foods (Dewdney and Edwards, 1984). Several factors combine to make the risk of adverse reactions to penicillin residues in food very low, including the dose received, oral intake and the low density of antigenic determinants (Dewdney *et al*., 1991) and, indeed, the literature supports this view; allergic reactions to antibiotic residues are very rare (Dayan, 1993).

In the establishment of MRLs, the CVMP in the EU and JECFA have addressed this problem. In fact, the CVMP estimated that 10 International Units (IU; 1 IU benzyl penicillin = 0.6 μg) of penicillin were required to evoke an allergic response, presumably in sensitised individuals. However, it concluded that concentrations as low as 0.01 IU could inhibit dairy starter cultures, and hence aspects of food processing, and recommended that residues of penicillin in milk should not exceed 0.005 IU. In order to protect both the consumer and dairy processing, the MRLs for a range of penicillins were established at between 50 (benzyl penicillin, ampicillin and amoxicillin) ppb for tissues and 4 ppb for milk, and 300 (oxacillin, cloxacillin and dicloxacillin) ppb for tissues and 30 ppb for milk (CVMP, *Penicillins, Summary Report*). JECFA on the other hand, which only considers safety and not food processing, considered that intake of benzyl penicillin should be kept below 30 μg of the drug. In practice, this resulted in MRLs for benzyl penicillin that are the same as those established by the EU, namely 50 ppb for tissues and 4 ppb for milk (JECFA, 1990).

Cephalosporins

The cephalosporins are chemically related to the penicillins and both share the β-lactam ring structure. However, in place of the thiazolidine ring of the penicillins, cephalosporins possess the six-membered dihydrothiazine ring (Van Heyningen, 1967; Abraham, 1987; Gustaferro and Steckelberg, 1991; Klein and Cunha, 1995).

A number of cephalosporins, including cefalonium, cefalexin, cefuroxime, ceftiofur, cefquinome, cefoperazone, cefazolin, cefapirin and cefacetrile, are used in veterinary medicine in food animals.

Like the penicillins, cephalosporins have low mammalian toxicity in mammalian toxicity tests and indeed in humans (Welles *et al*., 1968; Griffith and Black, 1970; Speight *et al*., 1972; Birkhead *et al*., 1973; Kradolfer *et al*., 1974; Capel-Edwards *et al*., 1979; Tauchi *et al*., 1979a–g; Fekety, 1980; Yoneda *et al*., 1980; Gerber and Craig, 1981; Norrby and Alestig, 1981; Smith and LeFrock, 1983; Parker and Park, 1984; Meyers, 1985; Esposti *et al*., 1986; Spurling *et al*., 1986; Norrby, 1987; Gustaferro and Steckelberg, 1991; Thompson and Jacobs, 1993; Grassi, 1995; Klein and Cunha, 1995). Cefuroxime and ceftiofur have a low order of toxicity (JECFA 1996a, 2002a).

Cephalosporins, again like penicillins, can be neurotoxic but usually after direct application to the brain surface or after high doses, particularly to renally compromised patients (Norrby, 1987; Weiss *et al*., 1974; Fekety, 1990; Schliamser *et al*., 1991; Thompson and Jacobs, 1993). Hepatotoxicity is rare (Hautekeete, 1995; George and Crawford, 1996). However, cephalosporins can cause renal damage through hypersensitivity-induced interstitial nephritis or through direct toxicity on the renal tubules. Again, this is rare, especially with the third-generation compounds, and when nephrotoxicity does occur, it is generally at high doses (Sack *et al*., 1977; Barza, 1978; Preziosi, 1981; Norrby, 1987; Cojocel *et al*., 1988; Fekety, 1990; Tune *et al*., 1996; Yilmaz *et al*., 1999).

In general, there is no evidence of genotoxicity in the cephalosporins group, but one compound used in veterinary medicine, ceftiofur, produced positive results in an in vitro cytogenetic assay (Aaron *et al*., 1995a), suggesting that it might have clastogenic potential. However, further investigations revealed this to be reversible, casting doubt on whether or not this was a true genotoxic effect. Furthermore, the drug had a major effect on the cell cycle kinetics and the effects thus appeared to be due to a prolongation of the cell cycle (Aaron *et al*., 1995b). In vivo studies for clastogenicity gave negative results (Aaron *et al*., 1995c). Hence, it can be concluded that ceftiofur is not a clastogen.

Like the penicillins, cephalosporins can induce hypersensitivity reactions leading to skin rashes, urticaria, contact dermatitis and toxic epidermal necrolysis, but anaphylaxis is very rare as are severe skin reactions (Speight *et al*., 1972; Milligan and Douglas, 1986; Hogan and Rooney, 1987; Christ, 1991; Dave *et al*., 1991; Romano *et al*., 1992, 2000, 2001; Blanco, 1994; Jick and Derby, 1995; McCloskey and Massa, 1997; Kelmar and Li, 2001). Occupational dermatitis has been reported (Foti *et al*., 1997; Straube *et al*., 2000). There is some degree of cross reactivity between penicillins and cephalosporins, but the degree and mechanisms are not clear although the actual incidence appears low (Beam and Spooner, 1984; Igea *et al*., 1992; Audicana *et al*., 1994; Dhar and Kulkarni, 1994; Kelmar and Li, 2001).

One study suggested that up to 10% of those sensitised to penicillin might have serious adverse events due to cross reactivity if exposed to cephalosporins (Herbert *et al*., 2000), although this might not be true in respect of anaphylaxis (Goodman *et al*., 2001). In general, the adverse hypersensitivity reactions with cephalosporins appear to be less frequent and less severe than those seen with the penicillins.

The CVMP has set ADIs for the cephalosporins based on microbiological effects on the gut microflora. In all instances, these are lower than the toxicological ADIs. The MRL for cefalonium was based on effects on dairy starter cultures, the NOEL for this being significantly lower than both those for the toxicological and microbiological ADI values. JECFA used the microbiological end-points to establish ADIs for ceftiofur and cefuroxime (JECFA 1996b, 2002b).

Macrolide antibiotics

Macrolide antibiotics possess a macrocyclic lactone ring to which are attached one or more deoxy sugar residues. Erythromycin is the major macrolide antibiotic used in human medicine

(Kapusnik-Uner *et al*., 1996). In veterinary medicine, the major macrolides are spiramycin, tylosin and tilmicosin.

Spiramycin Spiramycin has low acute oral toxicity in mice, rats and dogs, although some evidence of hepatotoxicity was observed at very high doses. It also had low toxicity after repeated oral administration to rats and dogs (Boyd and Price-Jones, 1960). There was no evidence of teratogenic effects in mice and rabbits, although embryotoxicity, probably due to toxic effects on the pregnant females, did occur at higher doses. It was not genotoxic in a range of in vitro and in vivo genotoxicity studies and no evidence of carcinogenicity was seen in a 2-year study in rats (Dubost *et al*., 1956; Boyd, 1958; Boyd and Brown, 1958; Boyd *et al*., 1958; JECFA, 1991b).

There is a paucity of data following use in humans, but there are isolated reports referring to effects on gastric motility and an ulcerated oesophagus (Qin *et al*., 1987; Perreard and Klotz, 1989); macrolides are known to reduce gastric motility when given in high doses (Pilot and Qin, 1988; Kapusik-Uner *et al*., 1996). There are no data to suggest that spiramycin has significant toxicity in humans when used therapeutically and adverse effects are limited to occasional nausea, vomiting and allergic skin reactions. There has been a single report of allergic vasculitis following the use of spiramycin (Galland *et al*., 1987).

Following occupational exposure, there have been a few reports of dermatitis and bronchial asthma (Davies and Pepys, 1975; Paggiaro *et al*., 1979; Veien *et al*., 1980; Veien *et al*., 1983; Moscato *et al*., 1984), including reports of occupational asthma in pharmaceutical company workers exposed to spiramycin (Nava, 1976; Malo and Cartier, 1988). It is evident that the adverse event profile for spiramycin is significantly lower than that for the penicillins.

The most sensitive safety studies were those of effects on the gastrointestinal flora where both in vitro and in vivo methods had been employed, and JECFA established the ADI for spiramycin on this basis (JECFA, 1991c, 1995a). A similar approach was adopted in calculating the ADI by the CVMP.

Tylosin Like spiramycin, tylosin has very low mammalian toxicity after oral administration, is not carcinogenic or genotoxic and shows no evidence of adverse effects in reproduction or teratology studies (Aiso *et al*., 1966; Anderson *et al*., 1966; JECFA, 1991c). Like spiramycin, there have been occasional reports of contact dermatitis and asthma in those occupationally exposed (Veien *et al*., 1980; Jung, 1983; Verbov, 1983; Barbera and de la Cuadra, 1989; Gollins, 1989; Lee *et al*., 1989; Caraffini *et al*., 1994; Danese *et al*., 1994; Tuomi and Rasanen, 1995; Pirkis *et al*., 1997).

Again, like spiramycin, the most sensitive endpoint for the calculation of the ADI was microbiological rather than toxicological, and the EU established the ADI for tylosin on this basis. Almost certainly, the same approach would have been taken up by JECFA, but the initial report required further data (JECFA, 1991c) and it seems the MRL was not pursued.

Tilmicosin Tilmicosin is structurally closely related to tylosin. It appears to have higher acute toxicity than either spiramycin or tylosin after oral administration to mammalian species. However, this higher toxicity was only seen in fasted animals; when given to non-fasted animals, the toxicity was similar to that of spiramycin and tylosin.

Dogs given oral doses of tilmicosin for 3 months showed increased heart rates, and 50% of animals given 70 mg/kg body weight per day died (Main *et al*., 1996). The NOEL was identified as 6 mg/kg per day. In a 1-year study in dogs, heart rates were increased at oral doses of 12 or 36 mg/kg per day and cardiac enlargement occurred at the higher dose. The NOEL here was 4 mg/kg per day. There were no notable adverse effects in a reproductive study in rats, or in teratogenicity studies in rats and rabbits. There was no evidence of genotoxicity in a range of in vitro and

in vivo studies (Jordan *et al*., 1993; JECFA, 1996c; Altunok *et al*., 2002). No carcinogenicity study was conducted, and JECFA felt that this was not necessary in view of the results from genotoxicity studies, the lack of any indication that carcinogenicity might be an issue in other studies and the fact that the closely related macrolide tylosin was not carcinogenic.

Unlike the other macrolides, there have been no significant reports of occupational exposure accompanied by allergy. However, there have been several reports of adverse effects in workers who have accidentally suffered a needle stick injury on needle contaminated with the drug. The majority of these were minor local effects resulting from needle punctures (McGuigan, 1994). However, there have been reports of cardiac effects in workers who have accidentally injected themselves with significant quantities of the medicine. These have included chest pains, electrocardiographic abnormalities and intraventricular conduction delays (Crown and Smith, 1999; Von Essen *et al*., 2003). There has been a report of a death following accidental intravenous injection (Kuffner and Dart, 1996) and a fatality in an 18-year-old woman (reported in Von Essen *et al*., 2003). Similar toxicity has been noted with erythromycin, including torsades de pointes (Regan *et al*., 1969; Nattel *et al*., 1990; Farrar *et al*., 1993; Brandriss *et al*., 1994; Orban *et al*., 1995). Experimental studies in dogs show that a negative inotropic effect developed after intravenous administration of tilmicosin, with reductions in left ventricular systolic pressure and electrocardiographic abnormalities. These studies indicate that tilmicosin might pose an occupational risk when administered by injection, but the quantities required orally to exert cardiac effects are too great for residues to pose a risk.

JECFA chose the NOEL of 4 mg/kg per day from the 12-month study in dogs as the toxicological ADI (JECFA, 1998a). The drug had little microbiological effect on the gut microflora of rats in an in vivo study. Consequently, JECFA on this occasion used the NOEL from the toxicological studies and a safety factor of 100 to calculate the ADI. The CVMP took a different strategy. It chose a NOEL from a study in germ-free rats infected with human gut flora, treated with tilmicosin. This NOEL was lower than the toxicological NOEL and so the ADI was based on microbiological effects.

Aminoglycosides

The aminoglycosides share a common structure of amino sugars linked to an amino hexose (aminocyclitol – a derivative of cyclitol, hexahydroxycyclohexane) moiety, via glycosidic bonds; hence the use of the term aminoglycoside. The most common examples used in veterinary medicine are neomycin, streptomycin, gentamicin and dihydrostreptomycin, although kanamycin and aminosidine are also used. Spectinomycin is a related compound; it is an aminocyclitol without the amino sugar residues (Burrows, 1980; Houdeshell *et al*., 1982; Chambers and Sande, 1996).

The two major class-related adverse effects of the aminoglycosides are ototoxicity and nephrotoxicity, both of which have been reported in animals and humans.

Ototoxicity

Studies in cats with an oral dose of 300 mg/kg dihydrostreptomycin for 21 days or 100 mg/kg for 60 days showed loss of hair cells in the cochlea, and damage to the sensorimotor epithelium. Dogs given streptomycin 50 or 100 mg/kg for 20 days showed auditory impairment. Degeneration of the nerve cells of the central nuclei, primarily the vestibular and cochlear nuclei, was seen in guinea-pigs given 100–400 mg/kg per day streptomycin for 3–6 weeks.

Ototoxicity has also been noted in cats with gentamicin and neomycin in mammals, including primates, and this may be exaggerated by the co-administration of loop diuretics (Christensen *et al*., 1951; Riskaer *et al*., 1952, 1956; Hawkins and Lurie, 1953; McGee and Olszewski, 1962; Tsang and Chin, 1963; Erlanson and Lundgren,

1964; Waitz *et al*., 1971; Webster *et al*., 1971; Brummett, 1981a, b; Yakota *et al*., 1984; Hodges *et al*., 1985; Ernst *et al*., 1994; Leake *et al*., 1997). Hearing loss and auditory and vestibular damage has been reported in humans treated with aminoglycoside antibiotics, including gentamicin, neomycin and streptomycin (Waisbren and Spink, 1950; Lindsay *et al*., 1960; Halpern and Heller, 1961; Erlandson and Lundgren, 1964; Greenberg and Momary, 1965; Meyers, 1970; Gailiunas *et al*., 1978; Dayal *et al*., 1979; Lerner *et al*., 1986; Chambers and Sande, 1996; Guthrie, 2008). Ototoxicity has been reported in the children of mothers treated with streptomycin and dihydrostreptomycin during pregnancy (Erlanson and Lundgren, 1964; Robinson and Cambon, 1964; Varpela *et al*., 1969; Warkany, 1979; Snider *et al*., 1980; Davies, 1991; Matz, 1993).

Nephrotoxicity

The aminoglycoside antibiotics have been shown to be nephrotoxic in experimental animals including mice (Molitor *et al*., 1946; Nelson *et al*., 1951; Waitz *et al*., 1971; JECFA, 1995a). Nephrotoxicity is relatively common in patients treated with aminoglycosides (Powell and Hooker, 1956; Greenberg and Momary, 1965; Hewitt, 1974; Masur, *et al*., 1976; Noone *et al*., 1978; Pratt and Fekety, 1986; Chambers and Sande, 1996; Solgaard *et al*., 2000) and neonates may be particularly susceptible (Heimann, 1983; Khoory *et al*., 1996). The incidence is relatively high; in patients given aminoglycosides for more than a few days, around 10–25% will develop mild renal impairment, and those exposed for longer or to relatively high doses will ultimately develop cellular necrosis of the proximal tubules (Fillastre *et al*., 1989; Chambers and Sande, 1996).

Renal damage caused by aminoglycosides may be tubular or glomerular, the latter possibly arising from toxic effects on mesangial cells (Martínez-Salgado, 2007). Toxicity due to aminoglycosides may have a circadian element (Beauchamp and Labreque, 2007). The effects are frequently reversible.

Other adverse effects

The only other common adverse effect associated with the use of aminoglycosides is contact dermatitis and this has been reported following neomycin and gentamicin treatment, largely following dermal application (Baer and Ludwig, 1952; Epstein, 1956, 1965; Calnan and Sarkany, 1958; Epstein and Wenzel, 1962; Hannuksela *et al*., 1981; Ghadially and Ramsay, 1988; Bigby *et al*., 1989; Goh, 1989; Gette *et al*., 1992). Streptomycin has been associated with anaphylaxis (Tinkelman and Bock, 1984).

The CVMP established ADIs for aminosidine, dihydrostreptomycin and streptomycin on the basis of conventional toxicity as the toxicological ADI was below that of the microbiological ADI. For gentamicin, the lowest ADI was the microbiological ADI and this served as the basis for the MRLs. Only with neomycin was the toxicological ADI the lowest *and* based on ototoxicity. A similar qualitative approach was taken by JECFA, with the ADI values for streptomycin and dihydrostreptomycin, gentamicin and neomycin being established on the basis of general toxicology, microbiology and ototoxicity (JECFA, 1995a).

Unlike its close relatives in the aminoglycosides group, spectinomycin is not ototoxic nor is it nephrotoxic (Novak *et al*., 1974; Holloway, 1982; JECFA, 1994a). Both JECFA and the CVMP based the ADI values on microbiological effects.

Fluoroquinolones

The earliest quinolone antimicrobial drugs, the so-called first-generation quinolones, are represented by oxolinic and nalidixic acids. However, the second-generation drugs, the fluoroquinolones, are typified by ciprofloxacin and enrofloxacin (Mitscher *et al*., 1993). The major fluoroquinolones used in veterinary medicine are flumequine, enrofloxacin, sarafloxacin, danofloxacin, orbifloxacin, ibafloxacin and marbofloxacin (Greene and Budsberg, 1993; NOAH, 2007). The major fluoroquinolone used in food animals is enrofloxacin.

The major toxic effect of fluoroquinolones is on the articular cartilages, and several fluoroquinolones have been shown to have the ability to cause juvenile arthropathies in a number of species including rats, dogs and birds (Schluter, 1987; Crist *et al*., 1988; Burkhardt *et al*., 1990; Stahlmann, 1990; Patterson, 1991; Hayem and Carbon, 1995; Kashida and Kato, 1997; Stahlmann and Lode, 1999; Takizawa *et al*., 1999a, b; Stahlmann *et al*., 2000; Kappel *et al*., 2002; Nagai *et al*., 2002; Peters *et al*., 2002). Grepafloxacin seems to have low toxicity in this respect (Takizawa *et al*., 1999a; Leone *et al*., 2003). The mechanisms involved are not fully understood, but studies with ofloxacin suggest that chondrocyte apoptosis may be involved, possibly as a result of effects on the caspase-8-dependent mitochondrial pathway (Sheng *et al*., 2008). They are also associated with a low incidence of tendonitis in humans (van der Linden *et al*., 2001; Leone *et al*., 2003), and there have been no reports of arthritis or other major diseases of joints in paediatric populations exposed to fluoroquinolones (Camp *et al*., 1994; Jick, 1997; Warren, 1997), although arthralgias and minor changes in cartilage have been noted (Hooper and Wolfson, 1993; Gendrel and Moulin, 2001).

Another notable toxic effect is prolongation of the QT interval in human patients, and this appears to be a class effect. Such effects are only seen at therapeutic doses (Hooper and Wolfson, 1993; Leone *et al*., 2003).

In the EU, the ADI values calculated by the CVMP were based on microbiological effects for enrofloxacin, sarafloxacin, difloxacin and marbofloxacin, as these were substantially below the toxicological ADI values. However, for danofloxacin, the toxicological ADI was based on a NOEL for arthropathy in dogs. A safety factor of 100 was used in the calculation of the ADI as the evidence suggests that these effects are rare in humans.

Sulphadimidine (sulfamethazine)

Sulphadimidine is a sulphonamide antimicrobial drug that has been widely used in food animal veterinary medicine, often potentiated with trimethoprim (Spoo and Riviere, 2001). Administration of sulphadimidine to rats, but not to mice, for 90 days induced thyroid hyperplasia (Heath and Littlefield, 1984a, b). Administration to mice for up to 24 months resulted in follicular cell adenomas of the thyroid (Littlefield *et al*., 1989). In rats, adenocarcinomas of the thyroid developed after exposure for up to 24 months in a two-generation study (Littlefield *et al*., 1990). Thus the data suggest that sulphadimidine was carcinogenic in rats and possibly carcinogenic in mice.

However, sulphadimidine has been shown to be goitrogenic in rodents, resulting in constant stimulation of the thyroid by thyroid-stimulating hormone (TSH); humans are insensitive to this mechanism of thyroid-induced neoplasia (Fullerton *et al*., 1987; McClain, 1995; Hill *et al*., 1996, 1998; Poirier *et al*., 1999). Hence, the tumours noted in rodents have no relevance to human risk assessment and are not predictive for human safety assessment (Galer and Monro, 1998a; Poirier *et al*., 1999). Sulphonamides also induce hypersensitivity reactions (Neuman *et al*., 2007).

JECFA, taking a precautionary approach, established a NOEL based on thyroid changes in rats and pigs and calculated the ADI using a safety factor of 100. The CVMP took a similar view. The MRL value was established at 100 μg/kg as this accounted not only for the toxicological ADI but also for any potential allergic and microbiological effects (JECFA, 1994b).

Carbadox and olaquindox

Carbadox and olaquindox are quinoxaline-1,4-di-N-oxide derivatives with antimicrobial activity. They were used as growth promoting agents in pigs and were used in the prevention and treatment of swine dysentery (Kornegay *et al*., 1968; Holder and Sinclair, 1972; Rainier *et al*., 1973; Bronsch *et al*., 1976; Schneider *et al*., 1976; Nabuurs and van der Molen, 1989; Nabuurs *et al*., 1990). In the EU, carbadox and olaquindox were

registered as feed additives under Directive 70/524/EEC and were not authorised as veterinary medicines, and so were not subject to the requirements for the establishment of MRLs. However, both drugs have been assessed by JECFA.

The most relevant aspect of the toxicity of carbadox is its carcinogenic potential. Carbadox was investigated in several studies in rats and doses in excess of 1 mg/kg per day were associated with an increased incidence of benign and malignant liver tumours. Tumours were even noted in a very limited study of only 11 months duration and in a second study where rats were dosed by the intraperitoneal route prior to weaning for 8–20 days, and/or in the feed at 300 ppm, for 1 year (Sykora and Vortel, 1986; JECFA, 1991d). A range of in vitro and in vivo mutagenicity studies with a variety of end-points has provided positive results (Oud *et al*., 1979; Negishi *et al*., 1980; Ohta *et al*., 1980; Voogd *et al*., 1980; Beutin *et al*., 1981; Yoshimura *et al*., 1981; Cihak and Srb, 1983; Cihak and Vontorkova, 1983, 1985; Scheutwinkel-Reich and von der Hude, 1984; JECFA, 1991d; Chen *et al*., 2008). Hence, it is evident that carbadox is a genotoxic carcinogen, and this might be considered to have signalled its demise as a drug used in food animals.

However, the major metabolites of carbadox in the pig, methyl carbazate, quinoline-2-carboxylic acid and desoxycarbadox, gave negative results in carcinogenicity studies and in genotoxicity studies (Truhaut *et al*., 1981; JECFA, 1991d). Relay toxicity studies were also employed to demonstrate the safety of carbadox residues. Relay toxicity studies are investigations whereby food containing residues of a drug is administered to experimental animals rather than the parent drug itself. Thus, the drug is administered to a food animal such as a pig, and the tissues of that animal are then used as the proxy test substance in toxicity studies, including carcinogenicity studies (Truhaut and Ferrando, 1975, 1981; Craine, 1977; Gallo-Torres, 1977, 1990; Jaglan *et al*., 1977; Ferrando and Truhaut, 1982; Evrard and Maghuin-Rogister, 1987; Lu *et al*., 1987, 1988, 1990; Arnold, 1990; Boisseau, 1990; Frazier, 1990; Guest and Fitzpatick, 1990; Weiss, 1990; Yong, 1990; Galer and Munro, 1998b).

While this methodology is useful in demonstrating the lability of residues bound to macromolecules (Huber *et al*., 1980; Mitsumori, 1993; Stevens and Wallin, 1990; Wislocki and Lu, 1990; Klee *et al*., 1999; Maume *et al*., 2001), its usefulness in toxicity testing has been disputed. For example, it has been criticised because:

- the doses of residues that are likely to be received in this way are too low to elicit a toxicological response;
- in carcinogenicity studies the dose is far from the maximum tolerated dose usually employed;
- the metabolites present as residues are usually unknown

(Arnold, 1990; Boisseau, 1990; Guest and Fitzpatrick, 1990).

Nevertheless, it can be argued for carcinogens at least that the doses likely to occur are somewhere towards the lower slope of a dose response curve, and that in the case of drugs like carbadox, the metabolites in the rat are reasonably well characterised qualitatively and quantitatively. Hence, relay carcinogenicity studies have some validity as part of a suite of toxicity studies and so they could complement standard studies, but not replace them (Arnold, 1990; Boisseau, 1990). The relay carcinogenicity studies conducted with carbadox were of 2 years duration in rats and 7.5 years duration in dogs. There was no evidence of an increased incidence of tumours (Ferrando *et al*., 1975, 1977, 1978).

Taken together with metabolism studies in various species including pigs, the data suggest that only carbadox itself is genotoxic and carcinogenic, and that its metabolites present as residues pose no risk to the consumer. As carbadox was a genotoxic carcinogen, JECFA was unable to identify a NOEL or establish an ADI, but it elaborated MRLs for the drug as it recognised that its residues did not pose a consumer risk (JECFA, 1990).

Olaquindox has similar genotoxic properties to carbadox (Voogd *et al*., 1980; Beutin *et al*., 1981;

Suter *et al*., 1978; Yoshimura *et al*., 1981; Cihak and Vontorkova, 1983; Scheutwinkel-Reich and von der Hude, 1984; Pokorna, 1986; Sram *et al*., 1986a–c; von der Hude *et al*., 1988; Nunoshiba and Nishioka, 1989). It has been tested in a number of carcinogenicity studies in rodents, some of them inadequate to assess carcinogenic potential. However, it has been investigated in two adequate carcinogenicity studies, one in rats and the other in mice, and there was no evidence of carcinogenic effects. Hence, it is a potent genotoxic agent but appears to lack carcinogenic activity. JECFA concluded that, like carbadox, it was unable to calculate an ADI because the drug was genotoxic, but provisionally concluded that the residues were acceptable (JECFA, 1991d).

Both carbadox and olaquindox were prohibited in the EU in 1998, not because of concerns over the safety of residues, but due to the hazards posed to those occupationally exposed to the substances (Anonymous, 1998).

Furazolidone and related compounds

Furazolidone is a member of the nitrofuran group of drugs which have been widely used as antimicrobials in veterinary medicine. A chemically related group, the nitroimidazoles, has also been used as antimicrobials and antiprotozoals; specifically, dimetridazole has been widely used in the treatment of histomoniasis in poultry (Brander *et al*., 1982; Papich and Riviere, 2001).

The nitrofurans and nitroimidazoles are genotoxic and some of them, including furazolidone and nitrofurazone, have been shown to be carcinogenic (National Toxicology Program – NTP, 1988; JECFA, 1993a, b). Such properties would ordinarily make them unacceptable for use in food animals unless, like carbadox, they could be shown to be converted to innocuous metabolites by the treated animals so that consumers were not exposed to potentially toxic residues. Unfortunately, some of the residues of these drugs were shown to be bound to macromolecules in vivo.

At first this might seem reassuring. If their residues are firmly bound to macromolecules, then this would suggest that they are safe. However, two questions arise from this observation:

- Are the residues 'lightly' bound so that toxic substances might easily be released?
- Are they firmly bound but with the potential for toxic substances to be released under severe conditions, e.g. when the macromolecules to which they are bound are digested in the human gastrointestinal tract?

To put both these questions more simply:

- How bioavailable are the bound residues? and
- How toxic/genotoxic/carcinogenic are they if they are bioavailable?

Various schemes have been put forward to address these questions. One approach involves examining the effects of weak acids and alkalis, then strong acids and alkalis on the bound material to see what exactly is released under various conditions. A natural progression from this is then to look at the effects of digestive enzymes to determine what might then be released. In the case of genotoxic and carcinogenic drugs, the release of reactive moieties would be of significance and further tests, e.g. genotoxicity studies, on these would be justified (Jaglan *et al*., 1977; Lu *et al*., 1987, 1990; Frazier, 1990; Matula, 1990; McCalla, 1990; Weiss, 1990; Vroomen *et al*., 1990a, b; Yong, 1990; McCracken and Kennedy, 1997). Another strategy would be to conduct relay toxicity tests, as already described for carbadox.

Unfortunately for the nitroimidazoles and nitrofurans, and unlike carbadox, these approaches have proved inconclusive. Furazolidone and ronidazole (a nitroimidazole) produce a number of metabolites, and there is significant binding to macromolecules, particularly proteins (Lu *et al*., 1984, 1988; Wislocki *et al*., 1984; Miwa *et al*., 1986; Sved and Foster, 1990; Vroomen *et al*., 1990 a, b; Wislocki and Lu, 1990; Hoogenboom, 1991; Alvaro *et al*., 1992; Hoogenboom *et al*., 1992, 1994; De Angelis *et al*., 1999). Some evidence suggests that bound furazolidone residues are degraded to non-toxic metabolites (Klee *et al*., 1999), but in general, for both classes of drugs,

there are insufficient data to state with certainty that the nitrofurans and nitroimidazoles do not pose a genotoxic or carcinogenic threat to the consumer, by way of their metabolites or from the release of bound residues from tissue macromolecules after consumption of food of animal origin.

Consequently, the CVMP recommended the entry of nitrofurans (including furazolidone) and the nitroimidazoles ronidazole, dimetridazole and metronidazole into Annex IV of Regulation (EEC) No. 2377/90, and this was adopted by the European Commission in 1977. As a result, these drugs may no longer be administered to food-producing animals in the EU.

Chloramphenicol

Chloramphenicol is a relatively simple antibiotic substance produced by *Streptomyces venezulae*. It is now made synthetically. It was first used for the treatment of epidemic typhus in South America, and scrub typhus in Asia in the late 1940s, and it produced dramatic therapeutic results. However, chloramphenicol was found to produce blood dyscrasias in humans. In fact it produces two distinct types of myelotoxicity. The least serious of these is a reversible bone marrow suppression due to mitochondrial damage which produces a mild anaemia (Keiser and Buchegger, 1973; Nijhof and Kroon, 1974; Chaplin, 1986; Holt *et al*., 1993). The more serious effect is bone marrow aplasia or aplastic anaemia with pancytopenia and acellular bone marrow. In fact aplastic anaemia has been estimated to occur in 1 in 500 to 1 in 100,000 cases treated with chloramphenicol, and it is often fatal (Sharp, 1963; Wallerstein *et al*., 1969; Polak *et al*., 1972; Keiser and Buchegger, 1973; Hausman and Skrandies, 1974; Modan *et al*., 1975; Al-Moudhiry, 1978; Benestad, 1979; Bottiger, 1979; Perez *et al*., 1981; Bamelou and Najean, 1983; Venning, 1983; Widayat *et al*., 1983; Aksoy *et al*., 1984; Najean and Baumelou, 1984).

These effects are not limited to adults and aplastic anaemia has been reported in children treated with the drug (Leiken *et al*., 1961; Awaad *et al*., 1975; Young *et al*., 1979; Lepow, 1986; White *et al*., 1986). There is no firm correlation with dose administered and the development of aplastic anaemia, although total doses are often high and of the order of 4–80 g (Hodgkinson, 1971; Hellriegel and Cross, 1974). However, cases of aplastic anaemia have been reported after topical administration (where the systemic dose may have been low), and after the application of ophthalmic drops (where the dose would have been low) (Rosenthal and Blackman, 1965; Carpenter, 1975; Abrams *et al*., 1980; Fraunfelder and Bagby, 1982; Plaut and Best, 1982; Issaragrisil and Piankijagum, 1985; Korting and Kifle, 1985), although the magnitude of the risk associated with the use of eye drops or topical application is probably very small (Lancaster *et al*., 1998; Walker *et al*., 1998). There has been one report of aplastic anaemia in a shepherd occupationally exposed to an aerosol spray containing the drug, for the treatment of infections in sheep (Del Giacco *et al*., 1981).

The mechanism of induction of aplastic anaemia is not fully understood. However, because of the lack of correlation with dose or duration of treatment, its seemingly random occurrence in treated populations, and its occurrence in identical twins, it possibly has a genetic background (Yunis and Bloomberg, 1964; Yunis, 1984, 1989). Although it has been possible to develop animal models of reversible bone marrow depression (Holt *et al*., 1997, 1998; Turton *et al*., 1999, 2000, 2002a, b; Festing *et al*., 2001), the same success has not been achieved with aplastic anaemia. It has been proposed that chloramphenicol is toxic due to the activation of the *p*-nitro group to give a toxic nitroso compound in susceptible individuals (Yunis and Bloomberg, 1964; Yunis, 1984, 1989) and in vitro the nitroso compound does appear to be more toxic than chloramphenicol or thiamphenicol. However, at the present time the mechanism of chloramphenicol-induced aplastic anaemia remains obscure (Malkin *et al*., 1990; Holt *et al*., 1993).

Not only is chloramphenicol-induced aplastic anaemia often fatal in its own right, but also it

can be the precursor to leukaemia (Krakoff *et al*., 1955; Yunis and Bloomberg, 1964; Scott *et al*., 1965). Leukaemia has been reported to follow chloramphenicol-associated aplastic anaemia in some patients who recover (Brauer and Dameshek, 1967; Fraumeni, 1967; Humphries, 1968; Seaman, 1969; Gadner *et al*., 1973; Meyer and Boxer, 1973; Forni and Vigliani, 1974; Meyler *et al*., 1974; Awaad *et al*., 1975; Schmitt-Graff, 1981; Scheres *et al*., 1985; Kapusnik-Uner *et al*., 1996).

JECFA considered chloramphenicol in 1987 and stated that as it could not identify a NOEL for aplastic anaemia, then it could not calculate an ADI (JECFA, 1988). It considered the drug again in 1994. There were no new data to address the NOEL for aplastic anaemia, and several genotoxicity studies carried out since JECFA's last review in 1987, using different end points, gave positive results. Positive results were noted with three mammalian metabolites of chloramphenicol. Consequently, JECFA remained unable to identify a NOEL, particularly now that the drug and some of its metabolites had been identified as being genotoxic (JECFA, 1994c; Robbana-Barnat *et al*., 1997). Hence, an ADI could not be calculated, and MRLs were not elaborated.

The same conclusions were reached by the CVMP. In fact, the CVMP went further and concluded that it could not calculate an ADI not only because of the lack of a threshold for aplastic anaemia and the genotoxicity of the drug, but also because there was no adequate carcinogenicity study, a NOEL could not be identified for fetotoxicity and there was no adequate reproductive study. In addition, there were a number of omissions from the residues file and the CVMP recommended its inclusion in Annex IV of Regulation No. (EEC) 2377/90, thus prohibiting its use in food animals in the EU.

The related drug thiamphenicol lacks the *p*-nitro group of chloramphenicol and in its place it has a methylsulphonyl group. Hence, based on the premise of Yunis (Yunis, 1988) it is less likely to be myelotoxic than chloramphenicol. This certainly seems to be the case. Although it can induce the reversible bone marrow suppression seen with chloramphenicol, it appears to have less of a preponderance to induce aplastic anaemia (Keiser and Buchegger, 1973; Kaltwasser *et al*., 1974; Frohli *et al*., 1984; Yunis, 1984, 1988, 1989; Ando *et al*., 1997; Turton *et al*., 2000, 2002a, b) and any bone marrow aplasia is rare (Gluckman *et al*., 1971; De Renzo *et al*., 1981). Unlike chloramphenicol, there is an adequate carcinogenicity study available and this gave negative results (Kitamura *et al*., 1997) and it does not induce DNA damage (Skolimowski *et al*., 1983).

JECFA was able to identify a toxicological ADI for thiamphenicol, but the ADI for microbiological effects on the gut flora was lower, and this was used as the basis of the MRL (JECFA, 2000). The CVMP also took a similar view and the MRL was eventually elaborated on the basis of a microbiological ADI.

Ivermectin and related compounds

Ivermectin belongs to a group of substances known as the avermectins. These are compounds that have as their central structure a macrocyclic lactone ring that adjoins a spiroketal structure. Since ivermectin was first introduced, a series of related compounds has become available including abamectin, doramectin, emamectin and eprinomectin for use in veterinary medicine. A related compound, moxidectin, a milbemycin (an avermectin macrocycle lacking a bisoleandrosyloxy substituent at the C-13 position) has also been introduced. They are used as endectocides in sheep and cattle, except for emamectin which is used as a parasiticide in farmed salmon. Many also have applications in companion animal medicine (Sutherland and Campbell, 1990; Shoop *et al*., 1995; McKellar and Benchaoui, 1996; Williams, 1997b).

Ivermectin has moderate acute toxicity when given orally to experimental animals. No major adverse effects were noted in subchronic studies, and there was no evidence that ivermectin was genotoxic or teratogenic at doses that were not toxic to the maternal animals; at doses that were maternally toxic, cleft palates in mice and clubbed fore-paws in rabbits were noted (Campbell and

Benz, 1984; Lankas *et al*., 1989; JECFA, 1991b; Burkhart, 2000) and in veterinary medicine, toxicosis usually arises from overdosage, particularly when small animals are treated with large-animal formulations (Roder and Stair, 1998).

Ivermectin has been safely used in humans for the treatment of onchocerciasis, filariasis due to *Wucheria bancrofti*, loiasis and strongyloidiasis (Aziz *et al*., 1982; Diallo *et al*., 1984; Lariviere *et al*., 1985; Kumaraswami *et al*., 1988; Richard-Lenoble, *et al*., 1988; Naquira *et al*., 1989; Cartel *et al*., 1992). Adverse reactions in humans to ivermectin are usually rare and generally mild (Burnham, 1993; Chippaux *et al*., 1993; Guzzo *et al*., 2002). Deaths have been reported after patients in a nursing home were treated for scabies (Barkwell and Shields, 1997), but these findings were not duplicated in a later study (Alexander *et al*., 1998), and the patients had been treated with other potentially toxic drugs including lindane, crotamiton and psychoactive drugs (Burkhart *et al*., 1997). The major adverse reaction to ivermectin in humans appears to be the Mazzoti reaction, caused by an immune response to dead parasites, possibly through the activation of neutrophilic granules (Ackerman *et al*., 1990; Njoo *et al*., 1993).

Ivermectin is neurotoxic and its mode of action appears to be through binding of the drug to glutamate-gated chloride channels, leading to increased chloride ion permeability and eventually to hyperpolarisation of nerve and muscle cells. It may also interfere with γ-aminobutyric acid (GABA)-mediated transmission of nerve impulses and the overall consequence is paralysis and death of the parasite, the therapeutic aim of the drug (Schaeffer and Haines, 1989; Martin, 1996; Dawson *et al*., 2000).

It is not generally neurotoxic in mammals as the blood-brain barrier protects the central nervous system. However, ivermectin has been studied in toxicity tests using the CF_1 mouse. This mouse strain is deficient in P-glycoprotein, a protein that is a constituent of cell membranes that determines their permeability (Didier and Loor, 1995; Schinkel *et al*., 1996; Sharom, 1997; Laffont *et al*., 2002). Hence, the CF_1 mouse, and neonatal animals, which are also deficient in P-glycoprotein, are more sensitive to the toxic effects of ivermectin, including its neurotoxic effects (Lankas and Gordon, 1989; Schinkel *et al*., 1994; Skopets *et al*., 1996; Lankas *et al*., 1997, 1998; Umbenhauer *et al*., 1997; Kwei *et al*., 1999; Marques-Santos *et al*., 1999).

This extra sensitivity is seen in acute toxicity, subchronic toxicity, reproductive toxicity and teratology studies with CF_1 mice, and in teratology studies, it results in NOELs that are 5–10 times lower than those noted with rats or rabbits (JECFA, 1993c). Some Collie dogs and Murray Grey cattle are also more sensitive to the toxic effects of ivermectin (Seaman *et al*., 1987; Fassler *et al*., 1991; Hopper *et al*., 2002). This may be due to decreased P-glycoprotein or to increased permeability due to other concomitant drugs (Hopper *et al*., 2002).

JECFA eventually established an ADI based on a NOEL from a reproductive study in CF_1 mice. It intended to use a very high safety factor in the calculation of the ADI, because of the implications of the neurotoxic and other effects seen in animals, for health assessment in humans. However, because the data in humans treated with ivermectin were reassuring and showed no evidence of neurotoxicity, the safety factor was reduced to 500. JECFA later reconsidered this opinion in the light of further reassuring data from use in humans, and data that showed that the drug produced developmental toxicity rather than being a frank teratogen. It also concluded that the CF_1 mouse was an extremely sensitive model. It continued to use the NOEL based on the data from the CF_1 mouse but reduced the safety factor to 100 (JECFA, 1993c). In evaluating other drugs in this class, doramectin, eprinomectin and moxidectin, JECFA based its decisions largely on neurotoxicity in dogs and safety factors of 200. However, as more evidence became available on the safety of ivermectin in humans, the size of the safety factor used in the calculation of the ADI has been reduced to 100 (for eprinomectin).

A similar approach has been adopted in the EU by the CVMP where extensive experience with

ivermectin contributed to decisions on related compounds, including doramectin, eprinomectin, emamectin and moxidectin. With the latter compound, the safety factor was reduced on a re-evaluation. The Joint FAO/WHO Meeting on Pesticide Residues (JMPR), a body that reviews pesticide residues in a similar manner to the way that JECFA deals with veterinary drugs, concluded that the CF_1 mouse was an inappropriate model for studying the toxicity of avermectins and instead used a NOEL from a reproductive study in rats to establish the ADI for abamectin. However, because it recognised that rats are extremely sensitive to the reproductive effects of avermectins it used a safety factor of only 50 in calculating the ADI (JMPR, 1998).

Griseofulvin

Griseofulvin is a fungal metabolite produced by *Penicillium griseofulvum* and *Penicillium patulum* strains. It is used in human medicine for the treatment of dermatomycoses in skin, hair and nails and until recently was used widely in veterinary medicine for the treatment of fungal infections, mainly against ringworm infections (Russel and Russel, 1992; Knasmuller *et al*., 1997).

Older studies showed it to have low acute and repeat dose toxicity in rodents and cats, and there were apparently no effects on reproduction in limited studies in rats (Sharpe and Tomich, 1960). However, studies in mice demonstrated that griseofulvin was hepatocarcinogenic in mice after oral dosing and resulted in thyroid tumours in rats (Rustia and Shubik, 1978). Dietary administration to mice resulted in hepatotoxicity, disruption of hepatic architecture and lesions that had the appearance of liver tumours (DeMatteis *et al*., 1966). Parenteral administration of griseofulvin to infant mice resulted in a high incidence of liver tumours (Epstein *et al*., 1967).

The mechanism of carcinogenicity is unclear (Williams, 1997a). The International Agency for Research on Cancer (IARC) concluded that griseofulvin was hepatocarcinogenic in mice and that there were inadequate data to assess the evidence for carcinogenicity in humans but that the substance was possibly carcinogenic to humans (IARC, 1974, 2002).

Griseofulvin has been tested in a number of assays for genotoxicity. In general, it has given negative results in tests for point mutations in bacterial systems, including the Ames test with strains of *Salmonella typhimurium*, and in a number of mammalian cell lines (Kuczuk *et al*., 1978, Leonard *et al*., 1979; Zimmerman *et al*., 1984; De Carli and Larizza, 1988; Zeiger *et al*., 1992). There is some limited evidence that griseofulvin is mutagenic in the mouse lymphoma TK^+/TK^- L5178Y assay (Sofuni *et al*., 1996). Results in the micronucleus test have generally been negative (Heddle *et al*., 1983; Kersten *et al*., 1999; Labay *et al*., 2001) although positive results were obtained in V79 cells and in a gut micronucleus test system (Kalweit *et al*., 1999; Vanhauwaert *et al*., 2001). Griseofulvin gave negative results in a test for DNA repair using rat and mouse hepatocytes (Mori *et al*., 1984) and in bacterial systems (Leifer *et al*., 1981).

However, in a number of studies for aneuploidy and other tests for chromosomal damage arising during mitosis and meiosis, clear positive results were seen (De Carli *et al*., 1973; Larizza *et al*., 1974; Grant, 1982; Curry *et al*., 1984; Waters *et al*., 1986; Marchetti *et al*., 1992, 1996; Tiveron *et al*., 1992; Mailhes *et al*., 1993; Kolachana and Smith, 1994; Inoue *et al*., 1995; Fahmy and Hassan, 1996; LeBoeuf *et al*., 1996; Parry *et al*., 1996; Bourner *et al*., 1998; Migliore *et al*., 1999; Qinghua *et al*., 1999).

The evidence demonstrates that griseofulvin is a potent aneugen in somatic cells and in germ cells. This may lead to loss of chromosomes and altered gene expression (Knasmuller *et al*., 1997). The results also show that griseofulvin is an antimitotic agent. The mechanism is unclear since it does not disrupt microtubules like some other spindle poisons, but it does appear to bind to tubulin or at least to microtubule-associated proteins (Grisham *et al*., 1973; Wehland *et al*., 1977; Ueno, 1985; De Carli and Larizza, 1988). Aneuploidy is regarded as an important change in the process of carcinogenesis (Oshimura and

Barrett, 1986) and this, taken with the results in animal studies, confirms griseofulvin's status as a carcinogen.

For such indirect carcinogens, it should be possible to determine a threshold dose or concentration (Parry *et al*., 1994; Kirsch-Volders *et al*., 2003), but the question arises as to which study to employ to determine this as the drug gives different responses depending on the test system chosen (Kirkland, 1998).

The problems for griseofulvin do not end there. The drug was found to produce teratogenic effects in rats when given oral doses of 250 mg/kg per day from days 6–15 after mating. No malformations were noted with 125 mg/kg per day (Klein and Beall, 1972). Similar results were noted in other studies in rats (Aujezdska *et al*., 1978; Steelman and Kocsis, 1978). An in vitro study with rat embryos also suggested teratogenic potential (Bechter and Schmid, 1987).

Therapeutic treatment of pregnant cats for ringworm resulted in malformations in the offspring including cleft palate, exencephaly, caudal displacement and hydrocephaly, along with multiple skeletal abnormalities including cranium bifidium, spina bifida and abnormal vertebrae. Cyclops and anophthalmia also occurred (Scott *et al*., 1975). Similar cases have been reported in cats treated therapeutically with griseofulvin (Gillick and Bulmer, 1972; Gruffydd-Jones and Wright, 1977; Turner, 1977). Cats appear to be more susceptible to the toxic effects of griseofulvin (Kunkle and Meyer, 1987), but it is not known if this species is also more susceptible to the teratogenic effects of the drug.

In humans, griseofulvin has produced CNS toxicity and disturbances of porphyrin metabolism. There are also numerous reports of dermatological effects, ranging from eczema to fatal toxic epidermal necrolysis (Savage, 1977; Thyagarajan *et al*., 1981; Shimoyama and Nonaka, 1987; Kojima *et al*., 1988; Taylor and Duffill, 1988; Boudghene-Stambouli and Merad-Boudia, 1989; Mion *et al*., 1989, 1990; Perfect *et al*., 1992; Mahboob and Haroon, 1998; Vassileva *et al*., 1998; Thami *et al*., 2001).

With this catalogue of adverse effects, it is perhaps not surprising that griseofulvin has not been supported by drug sponsors through either the EU or the JECFA MRL systems. The teratogenic effects are almost certainly due to the antimitotic effects described earlier, and hence it should be possible to determine thresholds for these too and eventually identify NOELs. However, faced with the research costs involved, and with no guarantee of success, it was perhaps always unlikely that companies would choose to defend the drug, particularly when newer antifungal drugs, with MRLs, are now available. Griseofulvin, without MRLs, is no longer permitted for use in food-producing animals in the EU.

Xylazine

Xylazine is a veterinary tranquillising agent. It is a thiazine derivative structurally closely related to clonidine and it acts by stimulation of α_2-receptors in the nervous system. Its effects include strong sedation and respiratory depression and it is used in both small and large animal veterinary medicines (Bongso, 1980; Bishop, 1998; Hoffmann *et al*., 2001). It can result in hypotension, coma and death in overdose in both animals and in humans (Arnbjerg, 1979; Carruthers *et al*., 1979; Gallanosa *et al*., 1981; Spoerke *et al*., 1986; Samanta *et al*., 1990; van Metre, 1992; Fyffe, 1994; Mittleman *et al*., 1998; Hoffmann *et al*., 2001).

JECFA concluded that one of the metabolites of xylazine might be genotoxic and carcinogenic and so was unable to calculate an ADI (JECFA, 1996d). However, the CVMP concluded that as the drug was given to small numbers of animals, and as these were unlikely to be sent for slaughter (as they had probably undergone surgery or other treatment), and in view of its extremely rapid metabolism and depletion of its residues, that no MRL was necessary, and that xylazine should be entered into Annex II of Council Regulation (EEC) No. 2377/90 (no MRL required to protect public health) for cattle and horses.

Azaperone

Azaperone is a tranquilliser used in both small and large animals, either alone or in combination with other drugs (Lees and Serrano, 1976; Serrano and Lees, 1976; Henrikson *et al*., 1995; Still *et al*., 1996; Radcliffe *et al*., 2000). However, it is also used as a sedative for pigs during transport prior to slaughter; this use can prevent economic losses which otherwise arise from stress and aggressiveness (Symoens and van den Brande, 1969; Symoens, 1970; Callear and van Gestel, 1973). Such uses have given rise to concern over the possible presence of pharmacologically active residues in the pigs and their meat products at slaughter.

JECFA concluded that pharmacological effects, rather than toxicological effects, were the most relevant from the point of view of safety assessment, and identified a NOEL based on neurobehavioral effects in dogs and a safety factor of 100 (JECFA, 1998b). The CVMP considered the dog to be a relatively insensitive model for the effects of azaperone and instead concluded that norepinephrine antagonism in a rat study was more suitable, presumably because it gave a much lower NOEL. It then calculated an ADI based on a 100-fold safety factor.

Carazolol

Carazolol is a β-receptor blocking agent used in obstetrics in pigs and in the treatment of tachycardia in this species (Kadir *et al*., 1990; Mejean *et al*., 1995; Bishop, 1996). High, prolonged doses in pigs can result in cardiac failure and this has been suggested as an experimental model for this condition in humans (Petzold *et al*., 1999).

The pharmacological effects of carazolol were considered by JECFA to be more relevant for consumer safety assessment than the toxicological effects, and the Committee established a temporary ADI of 0.1 μg/kg body weight using a NOEL based on inhibition of isoprenaline-induced tachycardia in rabbits and a safety factor of 200. Later, data from human patients became available. These were patients with chronic bronchitis or asthma and JECFA considered these to be an extremely sensitive group and here a NOEL of 0.5 μg/kg body weight was identified. A NOEL of 10 μg/kg body weight was identified for healthy subjects. JECFA concluded that a safety factor of 100 applied to the NOEL for healthy subjects and was in accord with the previous NOEL derived from the rabbit study, and it offered an extra safety margin of five-fold for patients with respiratory disease (JECFA, 1995b). An identical ADI, using similar reasoning, was calculated by the CVMP.

Summary reports, which give brief details of the data considered by the CVMP and its opinion, can be found on the EMEA's website under Veterinary Medicines, MRLs (http://www.emea.europa.eu/).

Conclusions

Veterinary medicines are subject to considerable assessment prior to marketing, and nowhere is this more critical than in the assessment of safety. At the national, multistate and global levels, toxicity and other preclinical data on veterinary medicines are examined in minute detail to determine whether or not they pose a risk to consumers, users or treated animals. The safety assessment is largely based on toxicity data, but other aspects are also considered, for example pharmacological activity and, for antimicrobial drugs, potential effects on the gastrointestinal flora. Studies are designed so that appropriate NOELs can be determined and toxicological, pharmacological and microbiological ADIs calculated. An exceptional amount of financial, intellectual, scientific and regulatory resources are employed so that consumers, users and animal owners will not be exposed to unacceptable risks.

From a limited examination of a small number of veterinary drugs, preclinical studies clearly have some predictive benefits for some of the adverse effects noted in target animal species, particularly for pharmacologically or toxicologi-

cally (type A) effects. This is similar to the situation with human medicines where there is often good predictability between animal toxicity studies and effects in humans, but where, nevertheless, there are limitations on the use of such data (Zbinden, 1990; Olson *et al*., 2000; Descotes, 2003) despite the level of advancement and sophistication seen in recent years in experimental toxicology.

Limitations may arise because of adverse effects seen post-marketing which were in populations not represented by the experimental studies (or even clinical trials), such as patients with renal failure, liver disease or other conditions that might affect the behaviour of the drug, or in other susceptible populations including the very young or the very old. For example, benoxaprofen was shown to have extended half-lives in elderly humans and those with renal impairment, and these kinetic variations may account for some of its adverse effects in this group – the group most likely to be treated with the drug (Chatfield and Green, 1978; Brogard *et al*., 1981; Aronoff *et al*., 1982; Hamdy *et al*., 1982; Kamal and Koch, 1982; James, 1985); these limitations are not restricted to human populations and they may also be seen in their animal counterparts.

Species differences undoubtedly also play a part. For example, the rat and dog are more susceptible to the gastrointestinal effects of non-steroidal anti-inflammatory drugs than guinea-pigs and some primates (Rainsford *et al*., 1984; Heywood, 1990).

Standard toxicological studies are less able to predict type B reactions – idiosyncratic, so that while animal toxicity studies were able to predict the gastrointestinal and haematological effects of NSAIDs, the neurotoxicity due to clioquinol, the liver damage due to halothane and possibly the dyskinesias resulting from phenothiazine in humans, they were not predictive of the anaphylaxis seen with alphaxalone, the photosensitivity and deaths due to benoxaprofen in the elderly, the retroperitoneal fibrosis arising from methysergide and the aplastic anaemia with chloramphenicol or phenylbutazone (Heywood, 1990; Dayan, 2000).

The examples reviewed above demonstrate many of the principles of safety assessment of veterinary drugs reviewed in the previous chapter. It is evident that a significant amount of subjective judgment is used in the evaluation of the scientific facts generated in safety studies, and that for some issues there is no clear scientific answer to a particular issue; instead the evaluation process is dependent on the views of both the individuals and scientific committees involved, and their own rules of procedure. Despite this, it is significant that the resulting evaluations are often remarkably similar and may, for example, differ only in the quantitative value ascribed to MRL values. In general, the overall processes involved lead to convergences of opinions and what is perhaps surprising is not that different evaluations occur occasionally, but that they tend to be very much in agreement.

In testing veterinary medicines, investigators have one major advantage over their counterparts in human medicine – the products being investigated for their safety for animal patients are usually tested, depending on local regulatory requirements, at multiples of up to five times the intended therapeutic dose in target animal safety studies, so that any toxicity specific to that animal, or specific to higher doses of the drug in that animal, will often be seen. Taken together with the results of laboratory animal toxicity studies and clinical trials in animals, a high degree of predictability for type A reactions can be realised. However, it must still be recognised that the population sizes used in animal clinical trials and target animal safety tests are usually too small to detect rare idiosyncratic type B reactions.

Overall, the results of laboratory animal studies, taken together with those from target animal safety studies, clinical trials and field safety evaluations should provide a significant degree of predictability for adverse effects in treated animal patients (Carakostas and Colaianne, 1996), which can be enhanced to a degree by a knowledge of any adverse effects seen in humans treated with the same or related drugs. This knowledge can

then be enhanced by data from spontaneous adverse reaction reporting schemes and, where appropriate, from post-marketing surveillance studies and pharmacoepidemiological techniques. The latter techniques will also be useful for investigating adverse reactions in susceptible subgroups not normally examined in laboratory species or target animal safety studies, and perhaps only investigated to a limited degree in clinical trials, including elderly animals, neonates and those with reduced hepatic or renal function, especially when the drug in question is aimed at one of those groups for example NSAIDs, frequently used in elderly dogs with muscular skeletal disorders, which may also have hepatic and renal impairment.

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15 Safety assessment of veterinary vaccines

M.J. Francis

Introduction

The majority of vaccines available today, whether for veterinary or human use, either rely on attenuation (weakening) techniques similar to those developed by Louis Pasteur over 100 years ago or are inactivated (killed) forms of the infectious agent.

Attenuated vaccines must be precisely controlled and characterised in order to provide the required level of protective immunity without causing significant disease symptoms within the animal. There is also a low risk that the attenuated antigen may revert to full virulence. As a result, careful back passage studies must be conducted within the host to ensure the stability of attenuation. Furthermore, in culturing the vaccine antigen it is possible that other infectious agents may be introduced which could themselves lead to undesired side effects when the vaccine is used in the field. In spite of these concerns, such vaccines provide the most direct means of eliciting a protective immune response within a host that will mimic the effect of a natural infection. Examples of such vaccines for veterinary use include canine parvovirus, canine distemper, feline rhinotracheitis, feline calicivirus, bovine viral diarrhoea, avian bursal disease and avian coccidiosis.

Inactivated vaccines must be totally innocuous and the large-scale processes used in the manufacture of a vaccine must be adequately validated. Problems with field outbreaks in the past have been attributed to incomplete inactivation. This problem should not, and would not, exist if more reliable inactivants, inactivation procedures and innocuity testing were used in the manufacturing process. Since the manufacture of such vaccine involves the culture of large amounts of the infectious agent, this can present a potential hazard to the personnel involved and the environment. Vaccines grown in eggs, tissue culture or simply culture medium may contain unwanted 'foreign' proteins which could affect the vaccine's immunological response or be potentially allergenic/reactogenic within the host. Inactivated vaccines do have certain limitations on their mode of presentation and as a consequence the nature of the immune response they will elicit. Often an excipient such as an adjuvant or immunostimulant is required in order to enhance the immunogenicity of such vaccines. Examples of these for veterinary use include bovine rotavirus, foot-and-mouth disease, equine influenza, swine

erysipelas, feline leukaemia virus, canine leptospira and porcine mycoplasma.

In cases where more traditional vaccination strategies prove to be sub-optimal or ineffective, alternative vaccines are explored. Such vaccines include split-product, subunit, recombinant protein, peptide, modified live and live vector approaches. The simplest and most basic form of subunit vaccine is one in which the infectious agent has simply been solubilised or broken up into its component parts. Other approaches would generally require recombinant DNA technology and genetic manipulation. These vaccines can be designed or tailored to meet specific requirements of safety and efficacy, which may not be possible using conventional approaches. Examples of such vaccines for veterinary use include equine influenza, feline leukaemia, clostridial toxoids, infectious pancreatic necrosis, rabies and *Escherichia coli*.

All new veterinary vaccines being developed for the open market must meet strict regulatory requirements of quality, safety and efficacy before they can be authorised for commercial use. Of these requirements the demonstration of product safety is arguably the most critical. This chapter provides a general guide to the safety assessment of veterinary vaccines. It is not designed to cover all the regulatory requirements or to be a substitute for official directives and guidelines, which must be closely adhered to for registration.

Regulatory requirements

The safety testing of vaccines is strictly governed by various regulatory requirements in different parts of the world:

- In the European Union (EU) the regulations are covered by European Council Directive 2001/82/EC and its associated amending directive 2004/28/EC.
- In the US the requirements are given in the Code of Federal Regulations 9 CFR.
- In Japan the governing regulations are laid out by the Ministry of Agriculture, Forestry and Fisheries (MAFF).
- In Australia they are given in the Australian Pesticides and Veterinary Medicines Authority (APVMA) Veterinary Manual of Requirements and Guidelines (Vet MORAG).

The safety testing of vaccines is currently being reviewed by the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH). This is a trilateral (EU–Japan–USA) programme aimed at harmonising technical requirements for veterinary product registration. Its full title is the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (see Chapter 2).

VICH was officially launched in April 1996 and it has set up a Target Animal Safety Expert Group comprising members from Japan, the EU, USA, Australia, New Zealand and Canada. This group published draft 17 of target animal safety guidelines for veterinary live and inactivated vaccines on the 5 June 2007. In addition to these overriding regulations there are a number of more specific guidelines. In particular the European Pharmacopoeia also has a monograph 5.2.6 on the Evaluation of Safety on Veterinary Vaccines as well as other specific monographs related to individual vaccines for different veterinary species. Generally, the data from safety tests on combined multivalent vaccines may be used to demonstrate the safety of vaccines containing fewer antigens and/or adjuvants provided that the components remain identical. For the purposes of this chapter the prime source of reference will be the European Medicines Agency (EMEA) and VICH Guidelines.

Quality requirements

Another important consideration in the safety testing of vaccines is the quality standard to

which the product should be tested. It is generally agreed that all safety testing carried out under laboratory conditions should be performed to Good Laboratory Practice (GLP) as laid out by the Organisation for Economic Cooperation and Development (OECD) within its 1997 revised document (ENV/MC/CHEM(98)17). This is a quality system concerned with organisational process and the conditions under which non-clinical health and environmental safety studies are planned, performed, monitored, recorded, archived and reported. If the safety testing is being carried out under field conditions then the work should be conducted to Good Clinical Practice (GCP) as defined by VICH in June 2000 – VICH GL9 (GCP). It is directed at individuals and organisations involved in the design, conduct, monitoring, recording, auditing, analysis and reporting of clinical studies in target species and is intended to ensure that such studies are conducted and documented to the required standard. Thus both GLP and GCP define clear international standards and procedures for the conduct of studies. These ensure the quality, reliability and consistency of the resultant safety data.

Single, repeat and overdose safety

In order to evaluate the safety of a new vaccine within the recipient host it should be administered at the recommended single dose or primary course and by each recommended route of administration to animals of each species and category in which it is intended to be used. In particular this should include animals of the minimum age that are likely to receive the vaccine. The animals should ideally be sero-negative at the time of vaccination, although this is more relevant for live attenuated vaccines. If sero-negative animals are not available then the use of low sero-positive animals should be justified. The vaccine itself should come from a 'pilot scale' manufactured batch that is fully representative of the final manufacturing process and formulated to contain a maximum antigen payload or potency.

In general a minimum of eight animals should be used per group, although this may vary significantly according to the requirements of specific monographs and for different species. The animals must be observed and closely examined for signs of local and systemic reactions. This should include post-mortem macroscopic examination and microscopic examination of injection sites. Other important criteria include pyrexia, general health and performance. The rectal temperatures should be recorded the day before vaccination or more frequently to establish a baseline, on the day of vaccination, 4 hours after vaccination and for the following 4 days or, if still elevated, until the level returns to normal.

Once again the frequency of these observations may be dictated by specific European Pharmacopoeia monographs or test guidelines. For example, the European Pharmacopoeia monograph for inactivated neonatal piglet colibacillosis vaccine (01/2008:0926) requires temperature monitoring at 2, 4 and 6 hours post vaccination. The study should continue for at least 14 days post vaccination and longer if any site reactions have not subsided.

For vaccines that require a single dose or primary course followed by a subsequent booster, a repeat dose study should be carried out. In this case the interval between administrations can be reduced to 14 days. Once again these tests should be performed in the most sensitive category of target animal using the recommended route of administration and animals should be observed for at least 14 days after the last administration. The measurement criteria are the same as those used for the single dose or primary course.

An overdose consisting of ten doses of live vaccine or two doses of an inactivated vaccine should also be administered to groups of animals in the most sensitive category of each target species and by each route of administration, unless otherwise justified. If a sufficiently high titre of live antigen cannot be dissolved in a single dose volume then a larger dose volume may be

used and the inoculum may be administered into multiple injection sites. The source of vaccine, potency, number of animals and measurements should be the same as those described for the single dose or primary course testing.

Reproductive safety

The safety of a vaccine within males, non-pregnant and pregnant females must be considered if it is to be used in breeding stock. This evaluation would generally involve a dedicated laboratory trial in combination with supplemental field data (see later). For this assessment a vaccine of standard dose/potency should be given to at least eight animals by all the recommended routes, unless a single route of greatest severity can be justified. Individual monographs may require larger numbers. For example, the monograph for neonatal piglet colibacillosis vaccine (01/2008:0926) requires no fewer than 10 pregnant sows that have not been vaccinated against colibacillosis.

The timing of the vaccination should cover each specific period of gestation required on the product data sheet and label and its effect in terms of fertility and reproductive performance should be monitored. In addition to this, animals should be observed for local and systemic reactions as laid out for single, repeat and overdose studies and the observations should cover parturition in order to assess the effect, if any, on the progeny, including teratogenic and abortifacient effects. Special consideration should be given to the study of laying hens where the efficacy and adverse effects on laying performance, egg size, hatchability and chick health must also be examined.

Effects on immunological function

In some cases it is possible that a vaccine may exert an adverse effect on the immune response of the recipient host. Where this is likely to occur or is suspected then appropriate tests must be performed to examine immunological functions. Further details of such tests may be specified in Pharmacopoeia Monographs. Often this involves the examination of any immunosuppressive effects at selected times post vaccination. When conducting such tests no difference should be observed between the vaccinated animals and unvaccinated controls.

Special requirements for live vaccines

In view of the potential risks associated with using live attenuated vaccines there are additional safety requirements designed to ensure that there is no risk to the animal itself, contact animals, the user and the environment.

Spread of the vaccine strain

Whilst the vaccinal line itself may be entirely safe within the recipient host animal, it is important that the shedding and potential spread to other animals is fully understood. For this to be achieved the vaccine should be administered by the recommended route that is most likely to lead to spread, which could, for example, be an intranasal mucosal route rather than a parenteral intramuscular route. The antigen used in this work should be the least attenuated and therefore have received the least number of in vitro passages from the Master Seed antigen. If possible the Master Seed itself should be used for this assessment. The investigation should also include a study of the potential spread to non-target species such as rodents and birds, since these could be susceptible and act as a means of disseminating the vaccinal strain more widely.

Dissemination within the host

In order to determine whether the live vaccine antigen has disseminated within the vaccinated

host it is recommended that samples of faeces, urine, milk, eggs, oral secretions, nasal secretions and other secretions should be tested for the presence of the vaccine antigen. Further studies may also be required to investigate the dissemination throughout the body of the host animal, with special attention being paid to normal sites of replication for the organism. This is particularly important in the case of live zoonotic organisms intended for use in food-producing animals.

Reversion to virulence

For a live attenuated vaccine, the potential for reversion to virulence must be studied using antigen from a passage level that is closest to the vaccine Master Seed and therefore least attenuated. This material needs to be administered by a recommended or intended route of vaccination that is most likely to lead to reversion to virulence; it needs to undergo five passages by this route in the target species. Where this is not possible due to the loss of replication through sequential passages then as many passages as possible should be carried out. If required, then in vitro culture may be carried out in between passages in order to restore the infectivity. Individual monographs may specify the number of animals that need to be used for each passage, but for companion animals generally two for each passage is adequate. The safety and virulence of the antigen after the final successful in vivo passage should be compared with that of the unpassaged material and no increase in virulence or adverse effects on safety should be observed.

Biological properties

For live antigens the characteristics on the parental antigens and the clinical symptoms of the disease should be described. In addition it may be necessary to study certain intrinsic biological properties of the vaccine strain, such as neurotropism.

Recombination or genetic reassortment

A theoretical analysis should be conducted into the likelihood of the recombination of genetic reassortment occurring between the vaccine antigen and field or other strains. This should result in a calculation on the probability of such an event occurring.

Residue studies

Residue studies are not normally required for vaccines. However, the possibility of residues remaining within foodstuffs in the case of food animals should be discussed where adjuvants and/or preservatives are used in the manufacture. If this is found to be of significance then the effects of such residues will need to be investigated. Furthermore, for live vaccines against zoonotic disease it will also be necessary to demonstrate that no residues containing live antigen remain at the vaccination site. In the EU, for the majority of adjuvants used within current vaccines they will have been placed in Annex II of the Regulation governing maximum residue limits (Regulation No. (EEC) 2377/90) (see Chapter 23) and thus no withdrawal period will be required. Where residue studies have been carried out, an acceptable withdrawal period will need to be proposed and justified.

Interactions

An analysis should be carried out into any potential interactions of a vaccine with other products used within the host animal.

Field safety trials

Under normal circumstances the results of controlled laboratory safety studies should be supplemented with data obtained from field studies. These field trials will require consent

from the licensing authorities within the country where the trials are to be conducted. To obtain such consent an official application must be made and comprehensive data should be provided on the vaccine's quality, its safety under laboratory conditions and a detailed study plan identifying the locations of the trials. All such work should be conducted according to Good Clinical Practice (GCP). The studies should include appropriate safety monitoring such as rectal temperature pre- and post-vaccination, performance and general well-being. They should also include observations on the size, persistence and frequency of any local reactions, e.g. at the site of injection. The numbers of animals involved may vary according to species, but minimum numbers may be specified within monographs and guidelines. For example, EU guidelines on companion animals recommend the use of at least 20 target animals on at least two premises. Trials should generally be conducted for a minimum of 14 days.

Ecotoxicity

The potential adverse or harmful effect that a vaccine may have on the environment in which it is intended to be used should be investigated. This is generally conducted in two phases.

The first phase is a preliminary investigation into the extent of environmental exposure to the vaccine, its active constituents and any relevant metabolites. This should include studies of the target species and the proposed method of using the vaccine, the mode of administration, the potential for excretion of the product and the disposal of waste/unused product.

Should this first phase analysis suggest the possibility of environmental exposure to the product or its constituents then a second phase study should be conducted. This will investigate the potential for ecotoxicity resulting from the exposure. The analysis should investigate the extent and duration of environmental exposure, and provide information on the pharmacological and/or toxicological properties of the vaccine. This may well involve specific studies on the impact of the vaccine on soil, water, air, aquatic systems and non-target organisms using defined protocols. The extent of this analysis will depend on the amount of pre-existing scientific data on the product and its components.

This environmental safety analysis should also take account of the disposal of waste material and unused product. In general such a disposal should be carried out by a licenced contractor in accordance with local requirements.

User safety

In addition to the safety of the vaccine within the host animal it is equally important to ensure that there is no risk to the user who administers the product (Woodward, 2008). This could be, for example, the veterinarian, the farmer or the animal owner. This assessment is unlikely to require specific studies and the risk can usually be assessed using information on the known properties of the product. The constituents of a vaccine would be generally expected to have a low inherent toxicity. The risk arising from the antigen will be largely dependent on whether it is live or killed. There should be no infection risk associated with a killed inactivated product. The risk arising from a live attenuated vaccine will depend on whether the antigen is zoonotic and known to be a human pathogen.

The presence of an adjuvant, in particular if it is oil based, does present a risk to the user if accidental self injection occurs. In general this risk is minimised by using only small volumes in a controlled delivery device such as a syringe and needle. However, multidose semi-automatic injectors significantly increase the risks associated with accidental self-injection. If self-injection does occur then users are advised to seek medical assistance immediately. The consequences of the failure to act quickly can be severe and potentially result in the loss of an affected finger. Advice is also provided on the product's data sheet advising a doctor that prompt surgical

attention is required and may necessitate early incision and irrigation of the injected area especially where there is involvement of the finger pulp or tendon. Care should also be taken to avoid contact with exposed areas of skin, although the potential for skin sensitisation and irritation is low.

Conclusions

As with all medicinal products, safety testing of veterinary vaccines is a critical part of pre-clinical testing that can reveal hazards to the treated animal, to the environment and to the user, and for vaccines containing zoonotic organisms to humans in general. Safety testing helps to identify potential hazards and, to a certain extent, associated risks. In turn, these can then be identified in the product literature so that veterinarians and other users can be made aware of potential adverse effects, and monitor these accordingly. As no preclinical testing schedule can ever be fully comprehensive, new and unexpected hazards will only be identified once the product is marketed and extensively used. At this point, veterinary pharmacovigilance plays a vital role in the development of the safety profile of vaccines and other biological products.

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16 Microbiological assessment of veterinary medicinal products and potential adverse effects

P. Silley

Introduction

There has been much debate and concern expressed in recent years in relation to potential microbiological hazards to human health arising from the veterinary use of antimicrobial compounds. One of the major concerns is that the use of antimicrobials in veterinary medicine may select for antimicrobial resistance among bacterial populations present in the target animal population. This bacterial population will likely include zoonotic pathogens, and consequently antimicrobial resistance may be selected. In itself this is of relatively minor significance; however, such resistant pathogens have the potential to infect susceptible persons through routes such as the consumption of improperly handled food products. Another concern is that antimicrobial resistance determinants present among the non-pathogenic commensal food-borne bacteria might be transferred to human pathogens. The consequence of both scenarios is that there is a potential for human illness to arise that has been caused by pathogens that are carrying antimicrobial resistance determinants and that no longer respond to antimicrobial therapy.

These concerns have given rise to additional regulatory guidance across the globe; this chapter will only consider how regulation from a European and US perspective is addressing this hazard. At the outset, however, it must be clearly stated that antimicrobial resistance should not be considered in the strictest sense as an adverse effect but rather as a natural consequence of use of these compounds. Whilst not considered in this chapter, attention must also be given to the benefit of antimicrobials in veterinary medicine, and for this the reader is referred to the review by Singer *et al*. (2007).

The plethora of new microbiology guidelines that have been introduced are despite any clear link between the use of antimicrobials in veterinary medicine and resistance development in human medicine being demonstrated (Phillips *et al*., 2004, 2007), although this has been contested by other workers. It is of course appropriate that all users of antimicrobial compounds examine the ways in which society uses these valuable resources. It is also crucial that we understand the context of use of veterinary medicinal products as a contributor to resistance development; it is merely one of a number of contributory factors and in my opinion not the most important in the context of impact upon public health. Indeed, as noted by Rehm and Weber (2007), the use of antimicrobials in

humans contributes greatly to the emergence of resistance, but veterinary, agricultural, aquacultural and industrial uses of antimicrobials also play a role.

In a keynote address to the National Foundation for Infectious Diseases 2006 Annual Conference on Antimicrobial Resistance, Robert C. Moellering, Jr, noted that the immense promise of antimicrobial chemotherapy, one of the major medical advances of the second half of the 20th century, has been dulled by the relentless development of resistance by the very microorganisms against which the therapy has been directed (Rehm and Weber, 2007). Moellering made the important point that there are no clinically important bacteria that have not developed some type of resistance to antibiotics, a situation anticipated by René Dubos more than 60 years ago (Moberg, 1996).

It is beyond refute that antibiotic resistance has now become a serious global problem and affects almost every bacterial species for which treatment with antibiotics is available. Olofsson and Cars (2007) made this point and emphasised that resistance to multiple antibiotics has developed among many common pathogens, such as staphylococci, pneumococci, *Pseudomonas* organisms and extended-spectrum β-lactamase (ESBL)-producing strains of *Enterobacteriaceae*.

The resistance problem is steadily increasing worldwide and as such the therapeutic options for the treatment of some infections are limited, especially in developing countries, where second- and third-line antibiotics are unavailable or unaffordable. World Health Organisation figures state that there are >11 million deaths annually[1] and treatment failure caused by antibiotic-resistant bacteria is a contributing factor, although the quantitative burden of antibiotic resistance is not certain.

Olofsson and Cars (2007) made the point that the development and spread of antibiotic-resistant bacteria is affected by several factors. Some of these are bacteria specific, such as mutation rate, transmission rate, biological fitness cost and the ability to compensate for such costs. Other, possibly more major factors in the emergence of resistance are the volume and quality of antibiotic use, including prescription when there is no clinical indication, over-the-counter sales or sales by drug vendors, inappropriate drug choice and suboptimal dosing (Ball *et al*., 2002; DeRyke *et al*., 2006). Dissemination of antibiotic resistance is also influenced by environmental factors in the community and hospitals. Direct or indirect person-to-person transmission is affected by population density and hospital structure and significantly increases in association with poor hygiene.

Regulatory systems

The European Medicines Agency (EMEA) is a decentralised body of the European Union based in London. Its main responsibility is the protection and promotion of public and animal health, through the evaluation and supervision of medicines for human and veterinary use. The EMEA coordinates the evaluation and supervision of medicinal products throughout the European Union (EU). The Agency brings together the scientific resources of the EU member states in a network of national competent authorities. It cooperates closely with international partners, reinforcing the EU contribution to global harmonisation.

The EMEA began its activities in 1995, when the European systems for authorising medicinal products were introduced. These provided for the centralised procedure and a mutual recognition procedure (to which has since been added the decentralised procedure). The EMEA has a role in each of these, but it is primarily involved in the centralised procedure. Where the centralised procedure is used, companies submit one single marketing authorisation application to the EMEA. For veterinary medicines a single evaluation is carried out through the Committee for Medicinal Products for Veterinary Use (CVMP).

[1] World Health Organisation (2003) *Shaping the Future*. http://www.who.int/whr/2003/en/whr03_en.pdf.

If the CVMP concludes that quality, safety and efficacy of the medicinal product is sufficiently proven, it adopts a positive opinion. This is sent to the Commission to be transformed into a single marketing authorisation valid for the whole of the EU.

Whilst the CVMP is responsible for publishing respective guidelines on the EMEA (see http://www.emea.eu.int), a welcome development in recent years has been VICH, a trilateral (EU–Japan–USA) programme aimed at harmonising technical requirements for veterinary product registration. Its full title is the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products. VICH was officially launched in April 1996.

The objectives of the VICH are:

- to provide a forum for a constructive dialogue between regulatory authorities and the veterinary medicinal products industry on the real and perceived differences in the technical requirements for product registration in the EU, Japan and the USA, with the expectation that such a process may serve as a catalyst for wider international harmonisation;
- to identify areas where modifications in technical requirements or greater mutual acceptance of research and development procedures could lead to a more economical use of human, animal and material resources, without compromising safety;
- to make recommendations on practical ways to achieve harmonisation in technical requirements affecting registration of veterinary products and to implement these recommendations in the three regions.

Once adopted, VICH recommendations replace corresponding regional requirements. Of the guidelines that will be discussed in this chapter, two are harmonised VICH guidelines. This initiative has clear benefits to sponsors in that a single data package should now satisfy the regulatory authorities in Europe, the USA and Japan. VICH is discussed in more detail elsewhere in this book (see Chapter 2).

Apart from the harmonised VICH guidelines, this chapter will also consider the regulatory framework in the USA where the US Food and Drug Administration (FDA) has responsibility for protecting the public health by assuring the safety, efficacy and security of veterinary drugs. The FDA is also responsible for advancing public health by helping to speed innovations that make medicines and foods more effective, safer and more affordable; and by helping members of the public obtain the accurate, science-based information they need to use medicines and foods to improve their health.

The FDA was formed in 1927 and was initially known as the Food, Drug and Insecticide Administration and employed its first veterinarian, Dr. Henry Moskey, to evaluate vitamins and minerals in light of their claimed nutritional and treatment uses. In 1953, a Veterinary Medical Branch was set up, and in 1965, the Bureau of Veterinary Medicine (BVM) was created, eventually becoming known as the Center for Veterinary Medicine (CVM). Today CVM is an internationally recognised public health organisation responsible for the evaluation, approval and surveillance of animal drugs, food additives, feed ingredients and marketed animal devices. CVM works to increase the availability and diversity of safe and effective products that relieve animal pain and suffering, sustain animal health and improve animal productivity without compromising public health.

Scope of the chapter

This chapter will address four guidelines with significant microbiology content: two of which are harmonised guidelines, two that solely relate to Europe and one that relates to the US yet takes into account the relevant harmonised guideline VICH 27:

1. CVMP/VICH/467/03-FINAL (Harmonised VICH Guideline 36): *Studies to Evaluate the Safety of Residues of Veterinary Drugs in*

Human Food: General Approach to Establish a Microbiological ADI.

2. CVMP/VICH/644/01-FINAL (Harmonised VICH Guideline 27): *Guidance on Pre-Approval Information for Registration of New Veterinary Medicinal Products for Food-Producing Animals with Respect to Antimicrobial Resistance*.
3. EMEA/CVMP/627/01-FINAL: *Guideline for the Demonstration of Efficacy for Veterinary Medicinal Products Containing Antimicrobial Substances*.
4. GUIDANCE FOR INDUSTRY #152: *Evaluating the Safety of Antimicrobial New Animal Drugs with Regard to Their Microbiological Effects on Bacteria of Human Health Concern*.

The intention of all the above guidance documents is to ensure that veterinary medicinal products are efficacious and safe, and implicit in this is the fact that adverse effects are minimised. In this context this chapter defines adverse effects as:

- a negative impact upon public health;
- ineffective products;
- a negative impact upon food industrial processes.

These respective effects will be discussed against the specific guidelines. The final section considers some general principles as to how such adverse effects might be mitigated by addressing the challenge of resistance development at source, and the role of the regulatory process in influencing this complex process.

It is often not appreciated that the requirements for a sponsor of a veterinary antimicrobial drug intended for use in food-producing animals are more onerous than those for companion animals or indeed for humans. This is primarily because of the issue of potential drug residues being ingested in the human diet. Indeed this is the rationale behind CVMP/VICH/467/03-FINAL (Harmonised VICH Guideline 36).

The rationale of CVMP/VICH/644/01-FINAL (Harmonised VICH Guideline 27) is to ensure that the potential for transfer of resistant organisms or resistance determinants from food animals to man through the food chain are minimised, whereas that of EMEA/CVMP/627/01-FINAL relates to demonstrating the efficacy of the antimicrobial whilst ensuring the dosage regimen minimises the risk of resistance developing in the target animal pathogens.

The FDA Guidance for Industry #152 is a risk assessment that addresses the safety issues of a new animal drug intended for use in food-producing animals with regard to human health. The FDA believes that human exposure through the ingestion of antimicrobial-resistant bacteria from animal-derived foods represents the most significant pathway for human exposure to bacteria that has emerged or been selected as a consequence of antimicrobial drug use in animals.

All the above guidelines are clearly detailed in full on the EMEA website (http://www.emea.eu.int/index/indexv1.htm) or the FDA Center for Veterinary Medicine website (http://www.fda.gov/cvm/Guidance/published.htm).

CVMP/VICH/467/03-FINAL (Harmonised VICH Guideline 36)

This Guideline is entitled *Studies to Evaluate the Safety of Residues of Veterinary Drugs in Human Food: General Approach to Establish a Microbiological ADI*. In Europe it is referred to as CVMP/VICH/467/03-FINAL and in the USA as Guidance for Industry #159.

Introduction

A variety of toxicological evaluations are performed to establish the safety of veterinary drug residues in human food. For drugs used in food-producing animals it is necessary to establish what is referred to as the acceptable daily intake (ADI), which is defined as an estimate of the amount of a substance, expressed on

a body weight basis that can be ingested daily over a lifetime without appreciable risk to human health. It may be necessary to determine a toxicological, pharmacological and microbiological ADI, depending on the type of drug under consideration and its pharmacodynamic activity.

In recent years there has been an increasing awareness of the potential impact of antimicrobial residues on the gastrointestinal flora. The impact of therapeutic antimicrobials on human gut flora is well established (Edlund and Nord, 1999, 2001; Sullivan *et al*., 2001; Nord *et al*., 2006a, b), but there is much conjecture as to the impact of residue levels of antimicrobials.

This is clearly a complex issue and has been handled in different ways by the regulators in different parts of the world. As a consequence and in an attempt to harmonise the different approaches, a Microbiological Task Force was set up by VICH and first met in July 2000 to consider the drafting of a harmonised guideline.

The Task Force recognised that the intestinal flora plays an important role in maintaining and protecting the health of individuals. It is well documented that the human colonic flora consists of at least 500 bacterial species (Suau *et al*., 1999; Hold *et al*., 2002; Eckburg *et al*., 2005). Essentially, the role of the colonic flora is confined to the fermentation of various substrates that escape digestion in the upper gastrointestinal tract. Saccharolytic fermentation of carbohydrates leads to production of short chain fatty acids that provide additional energy to the host, whereas proteolytic fermentation can give rise to toxic substances such as phenolic compounds, amines and ammonia (Vanhoutte *et al*., 2006).

This flora provides important functions to the host, playing an important role in maintaining health by preventing colonisation by pathogens, degrading dietary and in-situ-produced compounds, producing nutrients and shaping and maintaining the normal mucosal immunity (Hooper, 2004; Flint, 2006). It is also widely accepted that ingested antimicrobial drugs can potentially alter the ecology of the intestinal flora reaching the colon due to incomplete absorption or being absorbed, circulated and excreted via bile or secreted through the intestinal mucosa (Edlund and Nord, 1999; Sullivan *et al*., 2001).

Taking all these issues into account it was agreed that the microbiological endpoints of current public health concern that should be considered when establishing a microbiological ADI are the disruption of the colonisation barrier and a measure of the increase of the population(s) of resistant bacteria. For the purposes of the Guideline, resistance is defined as the increase of the population(s) of bacteria in the intestinal tract that is (are) insensitive to the test drug or other antimicrobial drugs. This effect may be due either to the acquisition of resistance by organisms that were previously sensitive or to a relative increase in the proportion of organisms that are already less sensitive to the drug.

Whilst the working party that was charged with drafting the Guideline had no problems reaching consensus with regard to the first endpoint (impact upon the colonisation barrier), there was much debate as to whether the scientific literature supported the view that antimicrobial residues were of public health significance in respect to development of resistance. The question asked was: 'What is the impact of residue levels of antimicrobials on human gut flora and is it possible to identify the impact of changing proportions of susceptible to non-susceptible isolates within an otherwise stable flora?'

The following section considers the issues that were debated by the Task Force at the time of drafting the Guideline, that is in the period 2000–2004, and as such any papers published after these dates would not have been considered.

The relative paucity of literature in this area is in part a function of the day-to-day fluctuations in resistant bacterial populations. Indeed Corpet (1992), in a human volunteer study, showed that the faecal excretion of resistant enterobacteria was not changed significantly in volunteers given ampicillin (1.5 mg/day) or oxytetracycline (2 mg/day), yet he pointed out that a possible

effect would be hidden in humans by the huge day-to-day fluctuations in the resistant bacterial populations.

An additional difficulty in addressing this issue can best be summarised by Hawkey (1986):

> '. . . the definition of the "normal" flora in man is difficult because it is now virtually impossible to find subjects who have not been exposed at some time to antibiotics or antibiotic resistant bacteria from other humans or their environment.'

Indeed as far back as 1969 a leading article in the *British Medical Journal* commented on the high level of carriage of antibiotic-resistant bacteria in apparently normal healthy members of the general population (Anonymous, 1969).

In this context it is important to consider the terms being used when considering issues of antimicrobial resistance. Davison *et al*. (2000) discussed definitions of antibiotic resistance and I am indebted to their excellent review for the thoughts presented in this section. Although, as pointed out by Davies (1994), some bacteria are known to have exhibited natural resistance prior to the introduction of antibiotics, the emergence of resistance in previously susceptible bacterial populations has been associated with antimicrobial use. What is abundantly clear is that published definitions of antibiotic resistance vary considerably.

Harrison and Lederberg (1998) defined antibiotic resistance as a property of bacteria that confers capacity to inactivate or exclude antibiotics, or a mechanism that blocks the inhibitory or killing effects of antibiotics. The HMSO *Report on Microbial Antibiotic Resistance in Relation to Food Safety* (HMSO, 1999) simply defined it as the ability of an organism to withstand an antibiotic. The CVMP, in an EMEA report, *Antibiotic Resistance in the European Union Associated with Therapeutic Use of Veterinary Medicines* (EMEA, 1999), defined microbiological resistance as either (1) where resistant organisms are those that possess any kind of resistance mechanism or resistance gene, or (2) where a bacterium is classified as susceptible or resistant depending on whether an infection with that bacterium responds to therapy or not.

Resistance has historically been described in terms of expressed phenotype or more recently genotype. This change, resulting from advances in molecular techniques, has meant that it is not always possible to compare data generated using different methodologies. The origin and type of resistance is also important, and so terms such as intrinsic and acquired resistance and single, multiple and cross-resistance have been introduced (Prescott and Baggott, 1993). Antibiotic resistance is usually defined in a clinical context as an indication of the likely outcome of therapy, rather than as an epidemiological attribute. Whilst there are no agreed definitions for antibiotic resistance in the context of an epidemiological study, Davison *et al*. (2000) have suggested that any such definition must consider the following criteria:

- Resistance must be regarded as a quantifiable (qualitative or quantitative) variable at the level of either the bacterial or host population and must be defined with respect to a reference population.
- The detection methodology must have known, quantified sensitivity, specificity, repeatability and reproducibility.
- The target bacterial and host population must be precisely defined.
- The sampling framework must be fully specified, indicating how the samples are selected from the bacterial or host populations or the environment, including the various levels of organisation within these populations or ecosystems and the number of units from which samples are selected.

Definitions of antibiotic resistance will vary depending on the purpose of study. The use of more precise definitions in the future will facilitate comparison between similar studies or indicate that such comparisons are invalid. The important differentiation between clinical resistance and epidemiological cut-off values has recently been more widely discussed (Bywater *et al*., 2006; Turnidge and Paterson, 2007).

Whilst the gastrointestinal tract provides for a relatively stable microbial population within any one individual, the population of *E. coli* may, for example, be stable at 10^8 cfu/g gut contents (where cfu is colony-forming units). It is, however, generally true to say that little attempt has been made to determine whether that population is consistent with regard to the proportions of strains that make up the population.

The heterogeneity of any population of a single bacterial species within the gastrointestinal tract is largely unknown. Indeed it is somewhat difficult to extract such information from the literature. Selection of samples from defined populations requires a framework to accurately estimate resistance in the target population and to enable statistical inferences to be made. This has been considered by Davison *et al*. (2000) in some detail, but suffice to say it is important that the number of samples required to make meaningful conclusions will depend on the purpose of the study, the target population size, the expected prevalence of resistance and the required power of the study. Despite this there are few studies that address these issues and as such only general comments can be inferred from much of the published literature with regard to changes in the balance of susceptibility profiles of individual populations.

It is difficult to find studies that have specifically examined the health impact of a gut flora that has changed with respect to the relative proportion of antibiotic-susceptible and -resistant strains of any one genus. The deficiency of most studies when trying to extract such information is that the data set is biased as it is looking at hospitalised, and therefore ill, patients. In such circumstances the data relate to the balance of flora in the sick patient with no reference data for that individual, pre-hospitalisation. There is thus no way of discerning whether a change in balance of flora has constituted a health risk. Furthermore most studies have simply isolated organisms from an infection and carried out susceptibility tests without making any attempt to compare the resulting susceptibility profile to that which was prevalent in the hospital at that time.

It is known that most of the anaerobic microorganisms that cause clinical infection originate from the normal oropharyngeal and gastrointestinal microflora, although there is little evidence that an imbalance in the normal flora alone is sufficient to give rise to infection (Tancrède, 1992).

Indeed there has been much controversy over the years as to whether pseudomembranous colitis arising from *Clostridium difficile* results from exogenous infection or simply from overgrowth of endogenous organisms in a susceptible host. Larson *et al*. (1980) first suggested that high mortality among clindamycin-treated hamsters was due to acquisition of *C. difficile* from the environment, and as such in the development of enterocecitis, clindamycin treatment and infection with *C. difficile* are separate events. It was postulated that there might be parallels between the hamster model and human pseudomembranous colitis where the initial event in human disease is the induction of susceptibility by antibiotics, surgery or chronic illness. Subsequent exposure to *C. difficile* would then determine the occurrence of pseudomembranous colitis. This hypothesis clearly demonstrates that it is not the perturbation of the gastrointestinal tract per se by antibiotics that causes subsequent disease, as there additionally needs to be exposure to the causative organism.

Indeed, Gorbach *et al*. (1988) reported on human volunteer studies in which cefoxitin, piperacillin, cefoperazone or aztreonam were administered intravenously to healthy volunteers in order to study changes in intestinal flora and acquisition of new strains. Seven of sixteen treated individuals were colonised with Gram-negative bacilli, but no correlation was observed between this colonisation and the suppression of either anaerobes or any other component of the faecal flora.

Marked strains of *E. coli* and *Pseudomonas aeruginosa* were administered by mouth to the volunteers and were found in the stools of both antibiotic-treated and control volunteers, demonstrating that the antibiotics had no apparent influence on the ability of these strains to colonise

the intestinal tract. The cefoxitin-treated subjects showed minimal derangement in their aerobic and microaerobic flora and were among the most heavily colonised, whereas the cefoperazone-treated subjects were subject to the greatest alterations in flora yet exhibited no discernible colonisation by the ingested organisms. Furthermore, subjects given cefoxitin or aztreonam, drugs that had the least effect on the anaerobic flora, experienced the greatest frequency of colonisation by Gram-negative bacilli.

Gorbach *et al*. (1988) concluded that colonisation resistance occurs in humans and is diminished by antibiotic administration but felt that their studies did not support the hypothesis that colonisation resistance is related to the anaerobic flora alone. They acknowledged that their findings were contrary to animal studies which had largely been carried out with mice (Van der Waaij *et al*., 1971; Freter and Abrams, 1972; Welling *et al*., 1980; Hentges, 1985).

What is also significant from this study and which is relevant to the topic under discussion is that there was no reported illness amongst the volunteer groups. Whilst the study was not designed to address this issue, the authors do make mention that healthy volunteers were studied rather than sick patients in order to minimise the variables and because of the ethics of feeding sick individuals potentially infective bacterial strains. That no volunteers were withdrawn from the study suggests that the changes in microbial flora, whether in terms of absolute numbers or changes in proportion of resistant strains, did not lead to morbidity.

Human studies

Enterococci are part of the normal intestinal flora. Prior to the identification of multiple-antibiotic-resistant strains in the 1970s, enterococci were considered to be relatively innocuous organisms. They are now considered as agents of nosocomial infection, with a frequency of isolation paralleling the accretion of antimicrobial resistance to most currently authorised antimicrobial products (Mundy *et al*., 2000). It is worth considering that some studies with enterococci (Lautenbach *et al*., 1999; Garbutt *et al*., 2000) have shown that vancomycin-resistant *Enterococcus faecium* infection did not independently increase mortality risk compared with patients who had vancomycin-susceptible *E. faecium* bacteraemia, when adjustments were made for severity of illness.

Other studies similarly support these findings in which a comparison of patients with vancomycin-resistant enterococci and vancomycin-susceptible enterococcal bacteraemias revealed no significant differences in mortality, especially after controlling for factors such as age and APACHE II score (Boyce *et al*., 1994; Wells *et al*., 1995; Tornieporth *et al*., 1996).

Such reports are not restricted to enterococci. Similar reports have been forwarded by Harbarth *et al*. (1998) with regard to a comparison of crude mortality among bacteraemic patients with methicillin-susceptible *Staphylococcus aureus* versus methicillin-resistant *S. aureus*.

If the study design is inadequate then it can be difficult to draw any real conclusions. Huycke *et al.* (1991) isolated *E. faecalis* strains over a 4-year period and examined them for a cytolytic toxin which conferred a haemolytic phenotype and gentamicin resistance. The carefully analysed data showed that neither gentamicin resistance nor the cytolytic toxin was prevalent within the species, yet there was an implication that these two factors together might have been working synergistically to cause disease. Unfortunately patient severity of illness was not assessed in this cohort to determine if there was an interaction between cytolysin production and severity of illness.

In a focus article regarding relationships between enterococcal virulence and antimicrobial resistance, Mundy *et al*. (2000) stated that in clinical infectious disease management, two assumptions are frequently made with respect to multidrug-resistant pathogens. The first assumption is that more antimicrobial drug resistance equates with greater virulence. The second is that attributable mortality is linked to the pathogens'

antimicrobial susceptibility profile rather than the availability and prompt initiation of suitable antimicrobial chemotherapy. To date, data in support of either position are lacking (Mundy *et al*., 2000), thus suggesting that antibiotic resistance and intrinsic virulence both contribute to disease, but in separate and complementary ways.

One of the few studies to categorically report the health outcome of a change in balance of antibiotic susceptibility in the faecal flora was an early study by Levy (1978) in which the emergence of antibiotic-resistant bacteria in the intestinal flora of farm inhabitants was determined subsequent to poultry being fed tetracycline and oxytetracycline supplemented diets. Tetracycline-resistant flora appeared in faecal stools of the farm workers within 3–5 months after introduction of the antibiotic supplemented feed, yet Levy (1978) was able to report that no sickness was associated from these resistant bacteria during the time of the study which was over at least a 15-month duration.

In an even earlier study Siegel *et al*. (1975) collected faecal samples from five different groups of people: farm workers involved in animals receiving antibiotic rations, people on the same farms but with minimal animal contact, people receiving antibiotics, untreated individuals residing with treated individuals and untreated people with direct or indirect exposure to antibacterials. Not surprisingly it was concluded that the enteric flora of human-beings in contact with farm animals or medicated people contain greater frequencies of resistant organisms than those of people unexposed to farm animals or treated individuals. No comment was made as to any health implications of this people-centered study, from which we might assume that no adverse health effects were observed during the period of the study.

Antibiotic resistance in faecal flora

There has been little published data on the natural frequency of antibiotic resistance genes in the normal non-pathogenic flora of ambulatory and hospitalised individuals. Ismaeel (1993) surveyed the faecal bacteria from 197 hospital patients, 58 laboratory workers, 66 urban dwellers and 19 rural dwellers for resistance to ampicillin, streptomycin, tetracycline, kanamycin and gentamicin. The study was seeking to determine the relative frequency of potential reservoirs of resistance genes in individuals with and without a history of taking antimicrobials in the 2 weeks prior to sampling. This time period was selected on the basis that other studies had shown that changes in faecal flora subjected to antibiotics were usually reversed 10–14 days after stopping the drug (Richmond, 1977; Levy, 1986).

The study concluded that there was a high prevalence of resistant bacteria in the gut flora of ambulatory and hospitalised individuals whether or not they were taking antimicrobials. As in most of these studies there was no reference to whether antimicrobial resistance was associated with illness. The ambulatory contributors in this study all answered a comprehensive questionnaire, although no mention was made of any association between increased prevalence of resistance and predisposition to illness.

Levy *et al*. (1988) reported on the high frequency of antimicrobial resistance in human faecal flora in a study of over 600 individuals from hospitals, from laboratories where antibiotics were used and from urban and rural communities. They found only a minimal difference in resistance to a range of drugs in faecal samples from the hospitalised individuals compared with the ambulatory groups. The fact that there were no significant differences between the groups does suggest that the balance of resistance isolates is not a factor that predisposes an individual to infection.

As has been stated previously, only a small number of studies have examined healthy ambulatory populations (Smith and Halls, 1966; Moorhouse, 1969; Linton *et al*., 1972). Linton *et al*. (1972) examined 309 children and adults from urban and rural areas of England and in all cases some level, albeit usually a low level, of organisms resistant to ampicillin, streptomycin,

tetracycline, kanamycin, chloramphenicol, nalidixic acid, nitrofurantoin or sulphafurazole were found. In reviewing these studies in context with his own work, Levy *et al*. (1988) acknowledged that there is present-day carriage of high numbers of resistant bacteria in the human gut flora even in the absence of concurrent or recent antibiotic consumption. It is intuitive to say that such general increases in the proportion of antibiotic-resistant bacteria in the gastrointestinal tract have not been mirrored by a concomitant increase in infectious disease.

Selective decontamination studies

It would be inappropriate not to consider studies relating to selective decontamination of the digestive tract (SDD) whereby the anaerobic bacteria are maintained and potentially pathogenic aerobic bacteria are eliminated from the oropharynx and gastrointestinal tract by means of enterally administered non-absorbable antibiotics. Bonten and Weinstein (1996) made clear that because of flaws in the design of these studies, including use of historical control groups and small numbers of patients, the results with regard to reducing patient mortality are largely inconclusive.

In a review of this subject Ebner *et al*. (2000) commented that most authors investigating the efficacy of SDD have not found antimicrobial resistance to be a major problem. Individuals subject to SDD are usually acutely ill and immuno-compromised and as such it is reassuring that changes in the balance of flora brought about by the treatment are not considered to predispose to but rather to lead to a reduction in infection. It was Daschner (1992) who pointed out the danger of resistance developing within the scope of SDD. Since that time many studies have investigated the impact of SDD on the incidence of nosocomial pneumonia and on mortality among intensive care patients. Whilst there are no major problems with resistance, the studies, as reviewed by Ebner *et al*. (2000), do show that SDD favours the emergence of bacterial resistance equally among Gram-positive and Gram-negative organisms as well as reducing morbidity and mortality in the intensive care unit.

Giuliano *et al*. (1989) monitored the modifications of oral and intestinal flora in ten allogenic bone marrow recipients who received randomly either norfloxacin or perfloxacin as a selective decontamination procedure. In all cases *Enterobacteriaceae* were eliminated, and in all but one perfloxacin-treated patient enterococci were eliminated in the intestine. The anaerobic flora was not affected although *Bacteroides* spp. were markedly reduced with perfloxacin treatment. In most patients the most striking observation was a marked increase in antibiotic-resistant staphylococcal counts. Whilst there was no emphasis on subsequent infection outcomes reported in the paper the authors did report that they observed a strain of *Staphylococcus epidermidis* causing bacteraemia with the same pattern of resistance of the strain present in the faeces of the patients. They further commented that all the documented infections occurred in the norfloxacin group, although the number of patients was too small to allow any clinical considerations to be made.

Intestinal flora and disease

Systemic prophylaxis with antibiotics has been shown to significantly reduce the rates of septic complications after abdominal surgery (Höjer and Wetterfors, 1978; Rietz *et al*., 1984). Studies such as those reported by Myrvold *et al*. (1989) in which patients were prophylactically treated with doxycycline can be considered to shed some light on the impact of altering the antibiotic susceptibility profile of the gastrointestinal flora. In this study there were significant increases in the incidence of doxycycline-resistant isolates at 3 days and 1 week post operation, although levels returned to normal after 4 weeks in 22 of the 24 patients. It can thus be construed that despite the increase in proportion of doxycycline-resistant isolates in the faeces, the regimen was

not predisposing these ill patients to additional infection.

Whilst not related to changes in the antibiotic resistance profile there are a number of reports that link disease with changes in the balance of intestinal flora. Eerola *et al*. (1994) suggested that the intestinal flora of patients with early rheumatoid arthritis is significantly different from that of controls primarily due to changes in the anaerobic part of the flora. Despite this, the authors had not been able to determine which intestinal bacteria had increased and which had decreased. Subsequent work from this group concluded that a vegan diet changes the faecal flora in rheumatoid arthritis patients, leading to an improvement in the condition.

Sugawara *et al*. (1989) followed changes in intestinal flora of 15 cirrhotic patients aged between 38 and 63. Cirrhotic patients often exhibit hyperammoniaemia and as such can be treated with antibiotics. In this study, long-term oral administration of latamoxef was evaluated. The authors also included oral administration of a multidrug-resistant *Bifidobacterium longum* to just one of the 15 patients presumably in an attempt to stabilise the gastrointestinal flora. The paper presents little details but does state that:

> 'The cirrhotic patient who received oral administration of multi-drug-resistant *Bifidobacterium longum* in combination with Latamoxef, did not have an increase in drug-resistant bacteria, such as *Clostridium*'.

The patient must, however, have exhibited an increase in the proportion of drug-resistant *Bifidobacterium longum* which obviously did not prejudice the health status of that individual as the authors concluded that administration of the organism enabled long-term administration of antibiotics. It may of course have been that any health impact was masked by the continued latamoxef administration.

Nord *et al*. (1984) reviewed the impact of antimicrobial agents on the gastrointestinal microflora and concluded that the risk of infection arises from overgrowth of potential pathogens in response to antibiotic treatment and not a change in the balance of resistant phenotypes of the flora. It must be noted that in these studies the changes in flora are arising as a result of therapeutic doses of antibiotic.

Giuliano *et al*. (1987) performed a study in healthy volunteers in which various broad-spectrum parenteral antibiotics were administered via the intravenous route resulting in an increase in resistant *Bacteroides* spp. The most dramatic changes were seen with cefoxitin and piperacillin to the extent that the resistant *Bacteroides* spp. became the dominant population. The report makes no comment other than that the volunteers were healthy and as such we must again conclude that there was no direct effect on the health status of the individual volunteers.

In a Japanese study, Hachimori (1976) compared the bacterial flora of healthy and acutely diarrhoeal infants and concluded that there was scarcely a significant difference in the antibiotic sensitivity of faecal *E. coli* and *Klebsiella* between the patients and the healthy infants. The percentage of *E. coli* and *Klebsiella* spp. isolates resistant to sulphonamide, streptomycin, chloramphenicol and tetracycline was higher, however, in the patients compared to the healthy infants, namely 38.5% *E. coli* and 28% *Klebsiella* spp. in the healthy infants compared with 48.4% and 42% in the patients.

In a review of the effect of antimicrobial therapy on bowel flora, Hooker and DiPiro (1988) considered the clinical implications of altering the flora. These consequences were detailed as diarrhoea and increased risk of infection in high-risk patients arising from disruption of the colonisation barrier. In what was a comprehensive review there was no mention of the clinical impact of a change in balance of the antimicrobial-susceptible gut population, presumably because there is no obvious evidence of this being a clinical issue.

Other human studies

There are only few detailed long-term studies reported in the literature; McBurney *et al*. (1999)

followed the enterobacterial populations of faecal samples collected from two human subjects during a 12-month period. Subject 1 showed marked variation in the total number of enterobacteria present in the faecal samples, yet a single *E. coli* strain persisted and predominated throughout the 12-month period. In subject 2 two strains of *E. coli* predominated at the beginning of the study.

The authors commented that whilst they could not prove a cause and effect, administration of a standard treatment regime of amoxicillin was followed by perturbation in the composition of the microflora. The perturbation was not initially obvious from examination of total enterobacterial numbers as they had always fluctuated widely in subject 2 but became apparent when the strain composition and antibiotic sensitivity of the enterobacterial population was determined. *E. coli* strains that exhibited multiple resistances predominated in the faecal samples for a period of about 13 weeks following administration of the antibiotic. The multiple drug-resistant strains were then no longer detectable but were replaced by a complex community containing ampicillin-resistant *Klebsiella* and antibiotic-susceptible *Enterobacter* and *Serratia* strains. At no point in this detailed study did the authors suggest that subject 2 suffered any health effects as a result of the changing balance of antibiotic susceptibility within the *Enterobacteriaceae*.

A detailed study on the impact of macrolides on changes in oral flora has been published by Sefton (1999). It was shown that increased numbers of resistant oral streptococci could be isolated from the mouth after administration of macrolides and other antimicrobial agents. It was considered that the increased proportion of strains with decreased susceptibility is likely to be as a result of resistant strains normally present in the mouth filling the vacuum left vacant by the inhibition of susceptible organisms, which may include potential pathogens in the disease state. Although Sefton found that resistant oral streptococci persisted for up to 3 months, she concluded that they were not considered to be clinically relevant. Indeed in the abstract to the paper Sefton stated, 'In our studies, no clinical problems resulted from the transient increase in macrolide-resistant streptococci'.

Animal studies

It is interesting to note that there are a number of reports in animals of changes in the balance of antimicrobial resistance being brought about by factors other than administration of antibiotics. Changes in antimicrobial resistance after transport and holding in swine have been reported (Langlois *et al*., 1984; Molitoris *et al*., 1987) as well as after movement of animals into and out of their pens (Hedges and Linton, 1988). There have also been reports describing increasing antimicrobial drug resistance of *E. coli* in the intestinal flora of swine after animals were exposed to stress (Dawson *et al*., 1984; Langlois *et al*., 1986). Moro *et al*. (1998, 2000) working with swine from a farm that had not used antimicrobials in feed for 10 years were able to show that heat stress for as little as 24 hours caused a significant increase in antimicrobial resistance to a number of antibiotics and that this high level of resistance was able to persist until slaughter.

There are two relevant points to be made from this study. First, that the balance between antibiotic-sensitive and -resistant isolates can be affected by parameters other than antibiotic exposure. Second, and probably of more significance to the question under consideration, is that despite the changes in resistance patterns there was no report of any resulting infection in the reported studies. It must be noted, however, that the numbers of swine used in the studies were low.

The correlation between intensive use of antimicrobial agents and development of resistant bacteria is well documented for pathogenic bacteria (Davies, 1994) whether they are human or animal pathogens. The impact of antibacterials on non-target indigenous bacteria has received less attention. In a relatively recent study Sunde *et al*. (1998) phenotypically screened 1,200 enterobacterial *E. coli* isolates from swine for antibiotic

resistance. The bacteria were isolated from ten herds of swine with different histories of exposure to therapeutic antimicrobials. The isolates were part of the normal intestinal flora of healthy individual animals.

The outcome from this large study was that multi-resistant bacteria and a broad spectrum of resistance genes exist in the normal flora of healthy swine. Of particular interest was the fact that the herds were selected on the basis of the level of antimicrobial agents used. The use of therapeutic antimicrobials was considered to be high for two herds, medium for one herd and low for the remaining seven herds. There were differences in the resulting resistance profiles, with streptomycin resistance being the highest followed by sulphonamide and then tetracycline resistance, although because of the non-random way in which the herds and isolates were chosen no general conclusions regarding the prevalence of antibiotic resistance could be made.

No statistical analysis of the results was carried out and thus the authors were unable to separate the effects of antibiotics from other confounding variables. However, it is clear for our purposes that despite the differences in resistance profiles between herds there were no reports of diseased or sick animals. The reverse was in fact true in that the authors commented on the fact that the study was with healthy animals.

Studies in animals by Orden *et al*. (1999) also suggest that the virulence factors and antibiotic resistance are not positively correlated. In fact it was suggested by these authors that possession of some potential enteric virulence factors among strains of *E coli* appeared to be associated with increased sensitivity to fluoroquinolones.

It can thus be concluded that changes in the balance of susceptibility will not in isolation predispose animals to disease.

The Task Force was therefore faced with the suggestion that there was no substantial body of evidence to link changes in the proportions of antibiotic-susceptible isolates in the human gut flora following antibiotic exposure, with a predisposition to disease. It must be stressed that the question being considered was not whether antimicrobial exposure arising from therapeutic dosage regimens resulted in development of resistance. Despite this lack of evidence at that time it was accepted that there was a public perception that antimicrobial residues were linked with resistance and public health issues and as such there was a need for regulation.

The current Guideline is described as an attempt to address the complexity of the human intestinal flora (Eckburg *et al*., 2005) and reduce uncertainty when determining microbiological ADIs. However, it is not the purpose of this chapter to review the ecology of the human gastrointestinal flora. The Guideline outlines a process for determining the need for a microbiological ADI and discusses test systems that take into account the complexity of the human intestinal flora. These test systems could be used for addressing the effects of antimicrobial drug residues on human intestinal flora for regulatory purposes. The Guideline makes clear that further research is needed to confirm the reliability and validity of all test systems and it does not recommend any one particular system for use in regulatory decision-making. Instead, it provides recommendations for a harmonised approach to establish a microbiological ADI and offers test options rather than specifying a testing regimen. For a review of the history of this subject the reader is referred to the paper of Cerniglia and Kotarski (2005).

Outline of the guideline

The five steps

The Guideline requires the determination of two distinct microbiological ADI values. The essence of this is summarised in the five steps outlined below. The data may be obtained experimentally, from the published literature, or from other sources.

- **Step 1**. Are residues of the drug, and (or) its metabolites, microbiologically active against representatives of the human intestinal flora?

  - Recommended data:
    Minimum Inhibitory Concentration (MIC) data from the following relevant genera of intestinal bacteria – *E. coli*, and species of *Bacteroides, Bifidobacterium, Clostridium, Enterococcus, Eubacterium* (*Collinsella*), *Fusobacterium, Lactobacillus, Peptostreptococcus/Peptococcus*.

It is recognised that the understanding of the relative importance of these microorganisms is incomplete and that the taxonomic status of these organisms can change. The selection of organisms therefore should take into account current scientific knowledge. If no information is available, it should be assumed that the compound and (or) its metabolites are microbiologically active.

- **Step 2**. Do residues enter the human colon?
  - Recommended data:
    Absorption, distribution, metabolism, excretion (ADME), bioavailability, or similar data may provide information on the percentage of the ingested residue that enters the colon.

If no information is available in humans, appropriate animal data should be used and again, if there is no available information, it must be assumed that 100% of the ingested residue enters the colon.

- **Step 3**. Do the residues entering the human colon remain microbiologically active?
  - Recommended data:
    Data demonstrating loss of microbiological activity from in vitro inactivation studies of the drug incubated with faeces or data from in vivo studies evaluating the drug's microbiological activity in faeces or the colon content of animals.

If the answer to any of the questions in steps 1, 2 or 3 is 'no', then the ADI will not be based on microbiological endpoints and the remaining steps need not be addressed.

- **Step 4**. Assess whether there is any scientific justification to eliminate the need for testing either one or both endpoints of concern. Take into account available information regarding colonisation barrier disruption and resistance emergence for the drug. If a decision cannot be made based on the available information, both endpoints need to be examined.
- **Step 5**. Determine the NOAECs/NOAELs for the endpoint(s) of concern as established in step 4. The most appropriate NOAEC/NOAEL is used to determine the microbiological ADI.

NOAEC refers to no observable adverse effect concentration and NOAEL to a no observable adverse effect level.

The studies referred to in the Guideline are complex and it is crucial that all the issues and potential pitfalls are understood before embarking on a series of studies. One of the positive aspects of this Guideline is that it does offer alternative approaches to addressing microbiological ADI determinations; however, it is my opinion that to fully exploit these opportunities a drug sponsor and the regulatory authorities must sit down together to discuss the most appropriate study approach for the respective active ingredient. One size does not fit all.

It is also important to point out that the approach to the microbiological ADI determinations for colonisation barrier effects and resistance development are fundamentally different. In the former it is possible to carry out simple MIC studies and/or alternative short-term in vitro approaches, whereas the latter requires complex long-term population studies which can be carried out in in vitro or in vivo test systems.

How the data are handled

Colonisation barrier

The Guideline is relatively new and only came into effect in Europe and the USA in May 2005; there are few antimicrobial compounds that have been fully evaluated for which a microbiological ADI has been agreed and for which the data are in the public domain. In an attempt to under-

Table 16.1 MIC_{50} of enrofloxacin against bacterial species of human intestinal origin at an inoculum level of 10^7 cfu/ml (from JECFA, 1997).

Genus	*n*	*MIC_{50} (μg/ml)*
Escherichia coli	10	0.031
Enterococcus spp.	10	1.0
Lactobacillus spp.	10	0.5
Bacteroides spp.	10	1.0
Bifidobacterium spp.	10	0.5
Fusobacterium spp.	10	0.125
Eubacterium spp.	10	0.25
Peptostreptococcus spp.	10	0.25
Clostridium spp.	10	0.5

stand how the microbiological data are handled, examples of typical data will be taken from old information that is in the public domain.

Step 1 requires the determination of MIC data against at least 10 strains of listed genera as described above. All strains must be sourced from the faecal microbiota of healthy non-medicated humans and the MIC determinations must be carried out using standardised procedures as described by organisations such as the Clinical Laboratory Standards Institute (CLSI) and in particular using the guideline for testing of anaerobes. Even this raises challenges as the described guideline is not necessarily appropriate for all the organisms of interest in ADI studies. Typical MIC data for enrofloxacin are shown in *Table 16.1* (JECFA, 1997).

In the absence of data to the contrary it is often assumed that all the ingested residue enters the human colon, and in accordance with step 3 of the Guideline studies need to be carried out to determine whether any residual antimicrobial activity remains after residue concentrations of antimicrobial compound have interacted with the human digesta. Currently there are no such data in the public domain, but data are currently under review by the various regulatory authorities for a range of classes of antimicrobials; in all cases the degree of inactivation of the active agent exceeds 80% and for many drugs that have been tested to date this value exceeds 95%. These data are sufficient to allow the calculation of the ADI_{micro} for colonisation resistance, in accordance with the calculation detailed in the Guideline.

The approach to this type of inactivation study has not been universally accepted by all regulatory authorities despite the protocols being worked out with and agreed by one of the major regulatory authorities. This seems to conflict somewhat with the objectives of VICH.

Calculations

The ADI with respect to disruption of the colonisation barrier is calculated according to the formula detailed in Guideline CVMP/VICH/467/03-FINAL:

$$\frac{\text{ADI}}{(\mu\text{g/kg bw})} = \frac{\text{MIC}_{\text{calc}} \times \text{mass of colonic contents}}{\text{Fraction oral dose available} \times \text{weight of human}}$$

The Guideline introduces the term MIC_{calc} and details that this value is derived from the lower 90% confidence limit for the mean MIC_{50} of the most relevant genera for which the drug is active.

The formula for the confidence limit is:

$$\text{lower 90\% CL} = \text{Mean MIC}_{50} - \frac{\text{Std Dev}}{\sqrt{n}} \times t_{0.10.\text{df}}$$

where:

- Mean MIC_{50} is the mean of the log transformed MIC_{50} values;
- Std Dev is the standard deviation of the log transformed MIC_{50} values;
- n is the number of MIC_{50} values used in the calculations
- $t_{0.10.df}$ is the 90th percentile from a central t-distribution, with df degrees of freedom, and df = $n - 1$.

Within the Guideline an example calculation is provided in which it advises that the MIC_{50} of the relevant genera are examined and the summary MIC_{50} values of those genera not inherently resistant to the test compound considered.

In this respect the data presented in *Table 16.1* suggest that all the tested genera should be considered as appropriate input data, as there is no evidence that any of the genera are intrinsically resistant. No guidance is provided as to what MIC values suggest intrinsic resistance, but within the example cited in the Guideline a value of 32 μg/ml is considered as sensitive. In this example we will consider a hypothetical drug referred to as 'Superkill' with MIC values against human gut flora typical of many drugs currently used in veterinary medicine. The following MIC_{50} values can be used to determine MIC_{calc}.

Bacteroides fragilis	4 μg/ml
Other *Bacteroides* spp.	4 μg/ml
Bifidobacterium spp.	0.25 μg/ml
Clostridium spp.	0.125 μg/ml
Enterococcus spp.	2 μg/ml
Escherichia coli	4 μg/ml
Eubacterium spp.	0.5 μg/ml
Fusobacterium spp.	0.5 μg/ml
Lactobacillus spp.	32 μg/ml
Peptostreptococcus spp.	2 μg/ml

From these input data the MIC_{calc} can be calculated to be 0.74 μg/ml for 'Superkill'. This value will subsequently be used to calculate the microbiological ADI with respect to disruption of the colonisation barrier.

It is accepted in regulatory circles that the mass of colon contents is agreed to be 220 g and the standard weight of a human is 60 kg. The fraction of oral dose available is described in the Guideline thus:

> '. . . the fraction of an oral dose available for colonic microorganisms should be based on in vivo measurements for the drug administered orally. Alternatively, if sufficient data are available, the fraction of the dose available for colonic microorganisms can be calculated as 1 minus the fraction (of an oral dose) excreted in urine. Human data are preferred, but in its absence, non-ruminant animal data are acceptable. In the absence of data to the contrary, it is assumed that metabolites have antimicrobial activity equal to the parent compound. The fraction may be lowered if the applicant provides quantitative in vitro or in vivo data to show that the drug is inactivated during transit through the intestine'.

Using the input data for (MIC_{calc}) as 0.74 μg/ml and the fraction available as 5% then the microbiological ADI with respect to the colonisation barrier can be calculated as:

$MIC_{cals} = 0.74\,\mu g/ml$
Fraction available = 0.05
The equation constants are:
Mass of colonic contents = 220 g
Weight of human = 60 kg

$$ADI = \frac{0.74 \times 220}{0.05 \times 60} = 54.27\,\mu g/kg\ bw$$
$$= 3.26\,mg\ per\ 60\text{-}kg\ person$$

Resistance development

It is important that before any work commences with regard to resistance development there is agreement as to which group (or groups) of bacteria constitute a potential public health concern. It is thus necessary to engage in discussion with the regulatory authorities to identify this sentinel population, as all discussions concerning resistance development studies must subsequently be directed towards this sentinel group. The Guideline states:

> 'Preliminary information regarding the prevalence of resistance in the human intestinal flora, such as daily variation within individuals and the variation among individuals can be useful in developing criteria for evaluating resistance emergence. MIC distributions of sensitive and known resistant organisms of concern can provide a basis to determine what drug concentration should be used in the selective agar media to enumerate resistant organisms in the fecal samples.'

Clearly it is necessary to be able to selectively culture the sentinel population to determine the

potential for resistance development within the overall gastrointestinal environment and as such there needs to be appropriate selective media. If, for example, the *Bacteroides fragilis* group was considered as the sentinel population then it is pertinent to ask if Bacteroides Bile Esculin (BBE) agar is sufficiently selective. In a series of as yet unpublished studies, considerable problems have been encountered with regard to the selectivity of BBE.

BBE agar was first described as a primary plating medium for the selective recovery of the *B. fragilis* group (Livingston *et al*., 1978). The initial description was of a selective medium that also provided for presumptive identification by virtue of esculin hydrolysis resulting in a blackening of the medium. In that first paper which primarily focused on screening pure cultures and a trial with 687 clinical specimens the authors demonstrated good selectivity and presumptive identification. They did, however, demonstrate that other anaerobes, aerobes and yeasts were able to grow to some extent on the media and indeed in some cases also cause blackening.

This was not considered to be a problem with major clinical implications. It has, however, led to media manufacturers and major clinical anaerobic microbiology text books stating, for example, that 'Some strains of *Fusobacterium mortiferum*, *Klebsiella pneumoniae*, enterococci and yeast may grow to a limited extent on this medium' (Engelkirk *et al*., 1992). The utility of BBE in clinical studies is a function of its selectivity by virtue of multiple mechanisms; not only are antimicrobials incorporated into the medium but also bile and esculin hydrolysis serve as selective agents. It is the black pigmentation arising from esculin hydrolysis that differentiates the *B. fragilis* group from other flora.

One of the problems in microbiological ADI determination studies is the need to ensure high microbial loadings on the plates in order to provide the necessary sensitivity when attempting to determine population susceptibility to the selected test drugs. Such high inoculum levels will cause widespread diffusion of the black pigment not only around *B. fragilis* group colonies but also into the body of the medium, thereby removing the benefits of esculin hydrolysis as a distinctive and selective marker.

It appears from a review of the literature that whilst BBE is widely used it has not been appropriately validated for work with faecal flora. This does not detract from its use as it is always clearly stated that counts determined on BBE agar are 'presumptive' *B. fragilis* group. When the media are used for isolation of *B. fragilis* group, the colonies causing blackening would be picked off the plate and confirmed for identity.

Many studies describing the impact of therapeutic doses of antimicrobials on human faecal flora have revealed some interesting findings in that rarely has BBE agar been used in such studies (Brumfitt *et al*., 1984; Enzensberger *et al*., 1985; Pecquet *et al*., 1986, 1987, 1990; Edlund and Nord, 1999). In none of these publications is reference made to the use of BBE agar; in most cases the authors have only determined the total number of anaerobes rather than the *Bacteroides fragilis* population. Only Enzensberger *et al*. (1985) reported on the *Bacteroides* spp. population and in this case they used a medium simply described as 'Kanamycin-vancomycin'. The authors made no reference to the selective properties, although the medium clearly supported significant numbers as they diluted down to 10^{-10}, recording counts of more than 10^9 cfu *Bacteroides* spp. per gram of stool. If colistin at a concentration of 10 μg/ml is incorporated into BBE then it still supports growth of the *Bacteroides fragilis* group population at concentrations commensurate with what would be expected in faecal samples and also inhibits non-*Bacteroides fragilis* group flora.

Assuming that the media chosen to isolate the sentinel group of public health importance is sufficiently sensitive, the next question that arises is whether the normal human flora already contains significant resistant flora. If there is already a significant resistant flora present in the normal human flora then it can be argued, in accordance with step 4 of the Guideline, that there are good scientific reasons for not having to determine a

microbiological ADI with respect to microbial resistance. The real challenge arises when the sentinel population is shown to be susceptible to the test drug.

The type of data that can be observed when the flora of the gastrointestinal tract are exposed to an antimicrobial has been described (Silley, 2007) and results in the challenge as to how we relate this type of data to the important issue of public health. It is vitally important that we continue to debate the question of whether such studies can predict impact upon public health, with particular respect to development of antibiotic resistance.

CVMP/VICH/644/01-FINAL (Harmonised VICH Guideline 27)

Introduction

This Guideline was adopted in Europe by CVMP on 14 January 2004 and came into effect on 15 December 2004. The premise behind it is that the use of antimicrobial agents is likely to lead to selection of resistance irrespective of whether antimicrobial compounds are administered to humans, animals or plants. The Guideline makes the important point that zoonotic organisms can, by definition, be transferred to humans from animals and thus it stands to reason that resistant zoonotic organisms can also be transferred to humans. What remains contentious is the extent of transfer of antimicrobial-resistant non-zoonotic bacteria or their genetic material from animals to humans via the food chain.

The objective of Guideline CVMP/VICH/644/01-FINAL (GL 644) is to provide harmonised technical guidance in the EU, Japan and the US for registration of antimicrobial veterinary medicinal products intended for use in food-producing animals with regard to characterisation of the potential for a given antimicrobial agent to select for resistant bacteria of human health concern.

GL 644 outlines the types of studies and data that are recommended to characterise the potential resistance development as it might occur in the food-producing animal under the proposed conditions of use of the product. This latter phrase, '*under the proposed conditions of use of the product*', is of fundamental importance and is often forgotten when issues of resistance development are being considered.

Whilst there are many examples where well-meaning scientists have argued that the use of antimicrobials in veterinary medicines unnecessarily contributes to the resistance pool, many draw their conclusions from studies that do not take into consideration how products are used in the field. One such example was the study of Delsol *et al*. (2004) in which they concluded:

> '. . . our study has provided direct evidence that enrofloxacin-treated pigs could be entering abattoirs with higher numbers of quinolone-resistant zoonotic bacteria than untreated pigs, increasing the risk of these entering the food chain'.

This conclusion could not be supported by the data (Silley and Froyman, 2004), and more importantly the authors failed to relate the experimental conditions to current understanding of development of the gastrointestinal flora in the pig and to commercial practice within the pig industry. The experiment was completed at approximately 13 weeks, whereas under commercial conditions pigs are slaughtered at 5.5–6 months of age, i.e. at 23–25 weeks of age, which is 10–12 weeks later than the age at which the experimental trial was finished. It was therefore erroneous and highly misleading to state that this trial provided direct evidence that enrofloxacin-treated pigs could be entering abattoirs with higher numbers of quinolone-resistant bacteria compared to untreated pigs and thereby increasing the risk of quinolone-resistant zoonotic bacteria entering the food chain. The authors made no reference to the fact that the trial was performed in young weaner pigs, at an age at which the intestinal flora would still be immature.

Indeed Belœil *et al*. (2003) made the point that there is little information about the age of contamination by ubiquitous *Salmonella* serotypes of growing pigs in subclinically infected herds and

that longitudinal studies following the bacteriological and serological status of pigs should be carried out to determine the typical age of contamination. This is crucial because it is well accepted that the gastrointestinal flora of the young animal changes over time. Understanding the development of the gastrointestinal flora and associated immunological changes in growing pigs is fundamental to making conclusions that extrapolate from a limited study such as that of Delsol *et al*. (2004).

The types of data required by the Guideline include information describing attributes of the drug substance, the nature of the resistance and the potential exposure of the gut flora in the target animal species. It does not account for post-slaughter factors such as processing of food products or kitchen hygiene which affect the potential human health impact. In many respects it is these last-mentioned factors that play an important role with regard to impact upon public health as they are at the interface of animal production and retail meat consumption. I would argue that the post-slaughter factors should be considered when estimating the risk to public health arising from use of antimicrobials in veterinary medicine. After all, the majority of the public are only exposed to such hazards through retail food products.

GL 644 differentiates data into basic and additional. It is expected that a drug sponsor will provide all the 'basic' data. In practice a sponsor will only provide additional data if requested to do so, or if following an initial risk analysis it is clear that such data will need to be provided to allow the regulatory authorities to address issues of public health concern. Much of the basic information is fundamental to understanding the antimicrobial activity of a test compound. The Guideline is very clearly structured and the relevant sections are detailed below.

Basic information

Within this section the sponsor is required to make comment on the antimicrobial class and mechanism and type of antimicrobial action. It is rare for a new antimicrobial class to be first introduced into veterinary medicine, although there are classes that until recently have not been used in human medicine, such as the pleuromutilins.

The pleuromutilins are an interesting class of compounds, having been used exclusively in veterinary medicine for more than 30 years yet not until 2006 was a representative of this class licensed for use in humans. It is interesting to speculate on how the debate might develop with respect to use of this class. Clearly, successful use in veterinary medicine over 30 years has not prejudiced human use, as seen by the successful licensing in Europe and the US, albeit for topical application. It is obviously somewhat simplistic to argue that any future resistance development must be due to use of the drug in man as there are many complex factors to consider, particularly in relation to dosage, route of administration and target indications. We will follow this situation with interest.

Much of the basic information can be taken from the literature, patent information or specific mechanism of action studies undertaken by the sponsor. Literature studies are usually used in order to determine the overall spectrum of activity. Where MICs are determined by the sponsor, there is a need to properly describe the origin of the tested isolates and to use validated and controlled methods. The Guideline specifically cites the CLSI (formerly NCCLS) Standards as being an appropriate internationally agreed and harmonised methodology. MICs of target animal pathogens, as indicated on the label claim, must be provided; this data will also be required for Guideline EMEA/CVMP/627/01-FINAL, and obviously the same data can be used with regard to both Guidelines.

The real focus, however relates to the MICs of the food-borne pathogens and commensal organisms. Again this information may be based on published data or on studies conducted by the sponsor, but in all cases the data must be recent. Isolates less than 5 years old at the time of submission are preferred. This often precludes the use of published data as there is also a need to

provide detailed histories of the isolates used in the study.

The sponsor is expected to provide data for the food-borne pathogens *Salmonella enterica* and *Campylobacter* spp. and for the food-borne commensal organisms *Escherichia coli* and *Enterococcus* spp., and appropriate strains must be selected according to the provided guidance. Wherever possible, the strains included should be selected according to the following recommendations:

- Strains of relevant bacterial species/serotypes should be isolated from the proposed target animal species. When the product is intended for a broad range of animal species, the strains should be from the main food-producing species (e.g. cattle, pigs and poultry).

Information on the tested strains should include identification, at least to the species level and origin, source and date of isolation. Experience suggests that if these criteria are not met then the sponsor will be asked to provide additional data.

The Guideline asks for information on the resistance mechanism(s) and information on the molecular genetic basis of resistance to the antimicrobial agent. Again it is acceptable for this information to come from literature or from studies carried out by the sponsor. It is pertinent to point out that if a sponsor claims to have a common mechanism of action with another compound, yet provides MIC data that differentiates the compound from the comparator, the authorities are likely to ask for additional data that explain the difference in susceptibility data; this is clearly a reasonable request.

The requirement to provide data on the occurrence and rate of transfer of antimicrobial resistance genes is relatively new within the European regulatory system, although once again the data can be from the literature or from studies carried out by the sponsor. Within this section the Guideline suggests that the sponsor may consider including data on target animal pathogens, relevant food-borne pathogens and relevant commensal organisms. The real challenge in the context of this requirement is being able to relate what is usually laboratory-generated in vitro data to what is likely to happen in the field. It is therefore fundamentally important to ensure that the in vitro test conditions do bear some relevance to field conditions.

Information on cross- and co-resistance with other antimicrobial agents needs to be provided. In the past, it was sufficient to provide only phenotypic data, but now the Guideline states that, if available, a genotypic description of strains should be provided. Clearly the choice of antimicrobials for the co-resistance study will be dependent in part on the antimicrobial class of the test compound.

Additional information

A sponsor must decide whether the data listed in this section of the Guideline are likely to be useful in order for the authorities to assess any impact of the active compound on public health. This includes in vitro mutation frequency studies, antimicrobial agent activity in the intestinal tract and other animal studies conducted to help characterise the rate and extent of resistance development associated with the proposed use of the antimicrobial product. Within any antimicrobial dossier there will have been a need to determine pharmacokinetic data. These data need to be available in order to predict the antimicrobial activity of the test compound within the intestinal tract. It is crucial that concentrations of active compound within the intestinal tract are adequately determined such that their potential impact on zoonotic and commensal bacteria can be assessed. Without knowledge of drug concentrations in the gastrointestinal tract it is somewhat difficult to make any sort of assessment of the likely resistance impact on those organisms that inhabit that ecosystem.

With regard to the animal studies the Guideline makes the important point that:

> '. . . the predictive value of the results of such studies is yet to be established with regards to resistance development. Therefore the results

of such studies should be interpreted in the context of all other pre-approval information described in this document'.

Expert report

When all these data have been assembled, the sponsor, normally through an Expert Report, is expected to characterise the potential for the use of the product to select for antimicrobial-resistant bacteria of human health concern. In order to achieve this it is necessary to discuss the information provided in the previous sections in terms of the exposure of food-borne pathogens and commensal organisms to the microbiologically active substance in the target animal after administration of the veterinary medicinal product under the proposed conditions of use. It is crucial that all the data are referred back to the intended use of the test compound in the target animal. Intuitively one can easily see that an antimicrobial compound administered orally in feed to a poultry flock is likely to present a greater challenge to public health than the same compound administered by injection to individual cattle.

As part of this discussion the Expert Report needs to consider how the class of drug is currently used in human medicine and how resistance development in animals might impact this use. If, for example, one were considering a macrolide antimicrobial, the discussion is likely to address the fact that macrolides emerged in the early 1950s in the form of erythromycin, and for more than 25 years erythromycin was used to treat upper respiratory and soft tissue infections, often as a second-line therapy. Mention would be made that macrolides were also clinically active against a number of emerging pathogens, such as *Chlamydia* spp., *Legionella* spp., *Campylobacter* spp. and *Helicobacter pylori*.

The important point to make is that a wide range of macrolide antibiotics are available for human use, as parenteral, oral and topical formulations, and that they are primarily active against Gram-positive organisms and generally used to treat common respiratory infections but do show activity against some atypical bacteria.

The discussion would then address the current state of resistance to macrolides in human medicine and how this affects therapeutic choices. It is clearly relevant to consider the role of the class of drug in treating gastrointestinal disease as this is an area where there is overlap with the zoonotic bacteria, those that pass from animal to man, causing disease in the human population. This is where the susceptibility data for the food-borne pathogens and commensal flora need to be related to those same bacteria currently causing disease in man.

Using the macrolide example it has already been described that they are often used as the treatment of choice in the management of atypical respiratory infections. The regulatory authorities would be rightly concerned that use of macrolides in veterinary medicine should not prejudice this use. This is not likely to happen as there is no clear link between organisms such as *Chlamydia* spp., *Rickettsia* spp., *Mycoplasma* spp. and *Legionella* spp. and the microbial flora of the target animals. It is unlikely that any resistance arising from use of macrolides will readily be transmitted to these infectious agents.

Additionally there are a number of options for treatment of atypical infections. Chlamydial infections are generally of low pathogenicity and doxycycline retains good activity. Rickettsial infections can be treated with chloramphenicol and tetracycline and urogenital mycoplasmas with metronidazole and clindamycin. Respiratory mycoplasmas can be treated with tetracyclines.

The discussion would obviously address the major factors influencing the level of exposure of the gastrointestinal flora to the active compound with particular respect to:

- the route of administration;
- concentration and persistence of the active antimicrobial compound within the gastrointestinal tract;
- group or individual treatment of the target animal species.

From this information an assessment would be made as to the likely level of exposure of the gastrointestinal flora to the active antimicrobial, leading to a safety assessment. The risk being assessed is the probability of disease due to infection in man by antibiotic-resistant pathogens arising from the use of the antimicrobial in animals. The susceptibility data detailing resistance profiles in the animal and man thus become important input data for this assessment. Finally, and wherever possible, it is necessary to relate the conclusions to the published literature.

EMEA/CVMP/627/01-FINAL

Introduction

This Guideline relates to the demonstration of efficacy of veterinary medicinal products containing antimicrobial substances for use in all animals and applies all routes of administration, except intramammary administration. This European Guideline was adopted by CVMP in December 2002 and came into effect on 11 June 2003. It aims to ensure that the applicant can demonstrate therapeutic efficacy of an antimicrobial substance for given indications whilst using a therapeutic regimen that minimises the risk of selecting antimicrobial-resistant bacteria. In this context the minimisation of selecting resistant bacteria relates to the target animal pathogens rather than the food-borne pathogen and commensal flora.

As might be expected there is considerable crossover with Guideline CVMP/VICH/644/01-FINAL and issues such as details on antimicrobial class, mode and mechanism of action, antimicrobial spectrum of activity, target animal pathogen MIC data and resistance mechanisms are common between the two. The additional data are intended to assist the authorities in assessing the basis for likely efficacy of the antimicrobial compound in the field and requires the sponsor to provide minimum bactericidal concentration (MBC) data, kinetics of bacterial killing and post-antibiotic effect (PAE) studies.

Whilst these appear to be simple requirements as most microbiologists will be familiar with standard bacterial kill curves and PAE studies, there is something of a sting in the tail. The Guideline clearly states that:

> 'Data on the kinetics of bacterial killing should be provided to enable the action of the antimicrobial against the target bacteria to be characterised'.

When unpacked this relates to characterising the nature of killing, which is of course important to interpretation of the pharmacodynamic nature of the test compound. The resultant data thus need to address questions of bactericidal and bacteriostatic action as well as whether the active compound can be characterised as a time-dependent, concentration-dependent or co-dependent compound. Well-designed in vitro studies that take into consideration the pharmacokinetics of the test compound by the relevant route of administration can adequately address all these questions. The relevance of all this to our considerations is simply to respond to the question, 'Does it work?'. This part of the submission is aimed at ensuring a product reaches the market that does what it says on the box, that it proves to be efficacious at the label dose and does not select for resistant organisms.

There is a section entitled 'Other Information' and under this heading the Guideline makes the comment that some environmental factors may influence the antimicrobial activity of a test drug. This may be especially important with respect to activity of the compound in specific infections. If indeed a claim is being sought for urinary tract infections a sponsor may consider it appropriate to look at in vitro activity in the presence of urine, or for a mastitis claim, activity in milk would be appropriate. Similarly if a claim was being made for activity against abscesses it might be considered appropriate to look at the effect of incubation atmosphere on in vitro susceptibility. Again

the emphasis here is to ensure that the test conditions used to generate laboratory data relate to how the product is likely to be used.

Data interpretation

The remainder of this Guideline refers to how the data should be interpreted and addresses the very important issues of pharmacokinetic-pharmacodynamic (PK-PD) analysis, breakpoints and all the issues relating to clinical studies such as dose-determination, dose-confirmation and field trials. Clearly the latter fall into the realm of the veterinary clinician whereas the former are the subject of the microbiological expert report.

It is not appropriate to discuss PK-PD analysis at length other than to say that it is directed towards obtaining the best correlation between clinical cure and antibacterial activity and can thus be used as one of the contributing factors to selecting an appropriate administration regimen which optimises dosing by maximising efficacy and restricting emergence of resistance. Guideline EMEA/CVMP/627/01-FINAL advises that when the product is aimed at more than one pathogen which is part of the same therapeutic indication, it may be useful, within the context of PK-PD analysis, to identify the bacterial species that is dose-limiting.

As the pharmacokinetic behaviours of various drugs in different animal species are different, it is necessary to address this on a case by case basis and within each animal species to consider the limiting bacterial species claim. It is also necessary to realise that PK-PD principles are antimicrobial class specific and need to be worked through for each 'bug/drug' combination It is also important to be mindful of the important issue which is highlighted in Guideline EMEA/CVMP/627/01-FINAL when it points out that it may be appropriate to use concentrations of the active substance in tissue or other biological fluid rather than those in serum or plasma in PK-PD studies.

Breakpoints

The Guideline highlights the requirement to detail MIC distribution of recent representative isolates of target pathogens indicating the proportion of resistant isolates and the breakpoints used. CLSI uses microbiological, pharmacokinetic and clinical data to establish breakpoints and without such considerations it is not possible to consider what is truly clinically sensitive or resistant. The MIC distribution pattern for a large number of microorganisms often enables identification of two or more populations of microorganisms that can be differentiated by the presence or absence of resistance factors. 'Susceptible' and 'resistant' MIC breakpoints can thus be established to differentiate these populations.

Dudley and Ambrose (2000) highlighted the challenge of combining microbiological, pharmacokinetic and clinical data to produce a single susceptibility/resistance breakpoint and asked the question, 'Should a breakpoint detect resistance or predict the antimicrobial effects of a drug in a patient when the drug is administered as a normal dose?' These are two fundamentally different functions, and it would appear that the considerable disagreement that often emerges in selecting breakpoints stems from a failure to recognise this difference and hence a desire to combine all information into a single function (Dudley and Ambrose, 2000). These authors suggested that the dual objective for breakpoints to detect resistance/predict outcome of therapy will continue to fail in many circumstances, and continue to serve as a source of confusion among clinicians, clinical microbiologists, regulators and researchers.

Moreover, in an age in which clinicians manage infections by empiric therapy of a patient syndrome, the need to consider the distribution of MICs as well as drug exposure when administering safe doses in target populations must be taken into account while establishing MIC breakpoints for susceptibility. Indeed Kahlmeter *et al.* (2003) recently published a review of the state of breakpoints within Europe in an attempt to harmonise

this issue. These authors point out that the success or failure of antimicrobial therapy in bacterial infections is predicted ideally by antimicrobial susceptibility testing (AST), in which microorganisms are divided into treatable and non-treatable categories on the basis of MIC breakpoints. In Europe, the categorisation was traditionally a clinical one and it was made irrespective of whether or not the organism harboured resistance mechanisms.

MIC breakpoints generally divide bacteria into three categories of susceptibility:

- susceptible;
- intermediate or indeterminate; or
- resistant.

These terms can be defined as:

- susceptible (S) – where the antimicrobial activity is associated with a likelihood of therapeutic success;
- intermediate or indeterminate (I) – where the antimicrobial activity is associated with an indeterminate or uncertain therapeutic effect;
- resistant (R) – where the antimicrobial activity is associated with a higher than expected likelihood of therapeutic failure.

MIC breakpoints are used either directly, as in MIC determination and 'breakpoint' susceptibility testing methods in broth or agar, or indirectly when converted into inhibition zone diameters in disc diffusion techniques.

MIC breakpoints are defined against a background of data, including therapeutic indications, clinical response data, dosing schedules, pharmacokinetics and pharmacodynamics, and other microbiological data. The process of determining breakpoints never was, and probably never will be, exact or strictly scientific (Kahlmeter *et al*., 2003). My view is that there should be standardisation on the setting of breakpoints following the established CLSI procedure. The breakpoint is fundamental to establishing whether an antimicrobial is likely to work and if we get this wrong and chose the 'wrong' drug we are faced with clinical failure (or lack of efficacy). Is this an adverse reaction? The issue was well summarised by Bywater *et al*. (2006) and it is appropriate to consider just why we need breakpoints.

The breakpoint MIC for an antimicrobial agent and a bacterial pathogen has traditionally been the threshold above which the pathogen is unlikely to respond to treatment with the specified antimicrobial agent. The reason that breakpoints are becoming contentious is because of the differing and incompatible demands being placed on what has hitherto been a single parameter. In particular, the needs of the clinician and the epidemiologist are different.

A clinician choosing an antimicrobial agent to treat an animal suffering from a specific infection needs to know that the compound chosen should be effective against the pathogen involved (although a clinical result may be affected by several other factors such as formulation and dosage). To this end, the MIC is ideally obtained for the pathogen in vitro, and this is compared with the predetermined clinical breakpoint to determine whether the organism is likely to respond in vivo. The clinical breakpoint should have taken account of the behaviour of the drug following administration, and assumes that if an isolate shows an MIC below the allocated clinical breakpoint for the pathogen, then a clinical response should be obtained if the drug is dosed as recommended, and there are no other factors to affect the outcome. Conversely, an MIC for the target pathogen found to be above the clinical breakpoint indicates resistance and that an alternative treatment should be considered.

Knowledge of the appropriate breakpoint (whether expressed as an MIC or indirectly through an inhibition zone diameter) is even more important as veterinarians are increasingly expected to defend their choice of antimicrobial agent amid concerns about imprudent or indiscriminate use. In reality, however, specific veterinary breakpoints, especially for older compounds, may not be clearly established (Watts and Lindeman, 2006).

The epidemiologist, however, has different needs. The MIC distribution pattern often enables

identification of two or more populations of microorganisms that can be differentiated by the presence or absence of resistance factors.

This is illustrated in Drlica (2000). The wild-type (WT) 'susceptible' subpopulation is assumed to show the antibiogram profile before any resistance has developed or has been acquired, and its distribution can be differentiated clearly from the 'resistant' subpopulation. Where full resistance is achieved by a single step (perhaps through acquisition of a plasmid or a single point mutation), then an isolate may be expected to fall clearly into one of the two major subpopulations – either fully susceptible or, having acquired the plasmid, fully resistant. However, where resistance is achieved in a series of steps, for example combination of efflux mechanisms and point mutations, then an isolate may fall somewhere in between, depending on the number of steps passed.

A dividing or cut-off MIC value can thus be established to indicate the MIC above which the pathogen has some discernable reduction in susceptibility. This value should be based on an adequate number of isolates to give confidence that the WT population has been identified, and will normally be placed close to the WT population. Considering the hypothetical illustration in Drlica (2000), an isolate with an MIC of, say, 4 μg/ml (shown as 'intermediate population') may yet be expected to respond clinically.

Thus a breakpoint set by clinical criteria may fail to identify emerging resistance, although it may be perfectly adequate to predict clinical efficacy. Conversely a breakpoint set by epidemiological criteria may imply that a potential treatment would fail, yet in fact it could respond since it may yet fall below the clinical breakpoint for the particular agent and organism.

The objective of a single universal breakpoint to achieve both (1) detection of the early stages of resistance development among a bacterial population and (2) prediction of outcome of therapy will continue to fail in many circumstances, and will be a source of confusion among veterinary clinicians, clinical microbiologists and regulators. MIC breakpoints for veterinary clinical purposes are defined against a background of data, including therapeutic indications, clinical response data, dosing schedules, pharmacokinetics and pharmacodynamics. Although the process of determining such breakpoints never was, and probably never will be, exact or strictly scientific, clarity of definition is essential. I maintain that the term 'breakpoint' should be retained solely for clinical breakpoints and be distinguished from the 'epidemiological cut-off value', where the latter shows that a change away from the wild-type population may have occurred in a subpopulation.

Universal adoption and understanding of such separate terminology would enable veterinarians to choose appropriate treatment based on information relevant to the individual patient or groups of patients, yet would recognise that epidemiologists need to be aware of small changes in bacterial susceptibility which may indicate emerging resistance, and allow for appropriate control measures to be considered. For further consideration of this issue the reader is referred to the excellent review by Turnidge and Paterson (2007).

Guidance for industry #152

Introduction

The full title of Guidance for Industry #152 is *Evaluating the Safety of Antimicrobial New Animal Drugs with Regard to Their Microbiological Effects on Bacteria of Human Health Concern*. As part of the process of approving an antimicrobial new animal drug application, the FDA must determine that the drug is safe and effective for its intended use in the animal. The Agency must also determine that the new animal drug intended for use in food-producing animals is safe with regard to human health (21 CFR 514.1(b)(8)). The FDA considers an antimicrobial new animal drug to be 'safe' if it concludes that there is reasonable certainty of no harm to human health from the proposed use of the drug in food-producing animals.

Guideline #152 provides guidance for industry with regard to a process for evaluating the potential effects of new antimicrobial animal drugs on non-target bacteria as part of the new animal drug application procedure. The guidance document outlines a risk assessment approach for evaluating the microbial food safety of new antimicrobial animal drugs. It is an assessment of the effect of the transmission of food-borne bacteria of human health concern through the consumption of animal-derived food products. The FDA believes that human exposure through the ingestion of antimicrobial-resistant bacteria from animal-derived foods represents the most significant pathway for human exposure to bacteria that has emerged or has been selected as a consequence of antimicrobial drug use in animals.

The rationale behind this guidance is wholly consistent with the previously described CVMP guidance, CVMP/VICH/644/01-FINAL, although the approach is somewhat different. The major concern, however, is that the use of antimicrobials in veterinary medicine may select for antimicrobial resistance among food-borne pathogen populations present in the target animal population. Consequently antimicrobial resistance may be selected. In itself this is of relatively minor significance. However, resistant pathogens have the potential to infect susceptible persons through routes such as the consumption of improperly handled food products. The consequence is that there is a potential for human illness to arise that has been caused by pathogens that are carrying antimicrobial resistance determinants and that no longer respond to antimicrobial therapy.

Guideline #152 provides guidance for industry with regard to a process for evaluating the potential effects of new antimicrobial animal drugs on non-target bacteria as part of the new animal drug application procedure.

Approach to risk analysis

Guidance #152 makes the point that whilst a sponsor of an antimicrobial new animal drug may use the guidance and described methodology to conduct a qualitative risk assessment, the sponsor is in fact free to demonstrate the safety of their proposed drug product in other ways.

Additionally the FDA does not exclude a sponsor undertaking a quantitative risk assessment in favour of the described qualitative process. The risk analysis process used by the FDA is based on the process described by the Office International des Epizooties (OIE) Ad Hoc Group on Antimicrobial Resistance (Vose *et al*., 2001). The risk assessment comprises a hazard characterisation, release assessment, exposure assessment, consequence assessment and risk estimation. The risk estimation integrates the components of the risk assessment into an overall conclusion, providing a qualitative indication of the potential risk to human health of the proposed use of the antimicrobial new animal drug. The FDA then uses the overall risk estimation ranking, along with other relevant data and information submitted by the sponsor, to determine whether the drug is approvable under specific risk management conditions.

The guidance includes a series of definitions that must be properly understood before attempting to carry out such an analysis. These are detailed below:

- **Hazard**: human illness, caused by an antimicrobial-resistant bacteria, attributable to an animal-derived food commodity, and treated with the human antimicrobial drug of interest.
- **Hazardous agent**: antimicrobial-resistant food-borne bacteria of human health concern that are in or on a food-producing animal as a consequence of the proposed use of the antimicrobial new animal drug.
- **Risk**: the probability that human food-borne illness is caused by antimicrobial-resistant bacteria, is attributable to an animal-derived food commodity and is treated with the human antimicrobial drug of interest.

The guidance also makes it absolutely clear that the overriding FDA concern is the decreased

or lost effectiveness of antimicrobial drugs in humans as a consequence of human exposure to resistant bacteria through ingestion of animal-derived food products. The FDA is also concerned about a range of deleterious effects that resistant bacteria may have on human health. These effects include but are not limited to increased duration of illness, treatment failure and loss of therapeutic options. Due to the difficulties associated with measuring loss of effectiveness, the risk assessment process described in this guidance document estimates the probability of the occurrence of the hazard. We will consider the respective components of the risk analysis.

Hazard characterisation

In effect, Guidance #152 can be considered as a two-part process. Prior to initiating and submitting the risk assessment, the FDA recommends that sponsors first characterise the hazard and the conditions that influence the occurrence of that hazard as a stand-alone 'Hazard Characterisation' document. This submission is first assessed by the FDA and serves to enable the sponsor and the FDA to determine the information that should be included in the complete risk assessment, if indeed a full assessment is needed. On the basis of this hazard characterisation it may be considered in certain cases that completion of a risk assessment is not needed and I can testify that this does happen. Again it is worth reminding ourselves that the hazard is defined as human illness, caused by bacteria with antimicrobial resistance, attributable to an animal-derived food commodity, and treated with the human antimicrobial drug of interest.

The FDA recommends that sponsors address the hazard characterisation step of the risk assessment by submitting information regarding the chemical, biochemical, microbiological and physical properties of the antimicrobial new animal drug that bear on characterising the downstream effects of the drug. It is not surprising to find that this information is common to that required by the European Guidelines, including chemical name, structure, drug class, mechanism and type of action, spectrum of activity, etc. One differentiating factor, although this is thankfully changing, is that the FDA insists on sponsors providing adequate quality control (QC) alongside MIC data. The regulatory authorities in Europe have seemingly been less concerned on seeing this data, yet without adequate and appropriate QC data it is simply not possible to have confidence in susceptibility data from different laboratories.

As is the case in Europe the FDA requires the sponsor to address the relative importance of the drug in human medicine and in this context it provides an appendix to the guidance that ranks antimicrobial drugs used in human medicine. The FDA recommends this ranking be considered when completing the hazard identification and the consequent assessment portions of the qualitative risk assessment. The ranking process attributes drugs as to whether they are critically important, highly important or important to human medical therapy.

The guidance makes clear that this ranking does not necessarily include all antimicrobial drugs or drug classes. The development of new antimicrobials for human therapy, the emergence of diseases in humans and changes in prescribing practices are among the factors that may cause the rankings to change over time. It is the intent of the FDA to periodically reassess the rankings to confirm they are consistent with current circumstances, and so they may be subject to change at any time when information becomes available that would impact those rankings. It therefore becomes apparent that a sponsor may wish to consult with the FDA regarding the ranking relevant to their proposed drug at the time the assessment is made.

In developing criteria for ranking antimicrobial drugs with regard to their importance in human medicine, the FDA considers broad issues associated with the efficacy of drugs in human medicine and factors influencing the development of antimicrobial resistance. Specific factors include:

- the usefulness of the drug in food-borne infections;
- the types of infections treated;
- the availability of alternative therapies; the uniqueness of the mechanism of action;
- the ease with which resistance develops and is transferred between organisms.

It is important to understand that multiple factors may be applicable to some products, illustrating their considerable importance to human medicine.

As well as importance in human medicine the sponsor must consider what is known with regard to bacterial resistance. In particular it is important to consider the bacterial species and strains for which resistance acquisition has potential human health consequence and the known resistance determinants or mechanisms associated with the antimicrobial drug(s) of interest. There is a tacit acknowledgement that scientific understanding does not stand still and as such the FDA encourages sponsors to consider data gaps and emerging science that may be relevant to the microbial food safety assessment for the proposed conditions of use.

The presented data and current scientific knowledge need to be summarised in light of the relative importance of the drug in human medicine, such that a case can be made with regard to whether a full risk assessment is needed. Only if the FDA decides there is a case to be made should a sponsor proceed with a full risk assessment.

Qualitative risk assessment

After submission and review of the hazard characterisation, and prior to completing the risk assessment, the sponsor is encouraged to consult with the FDA regarding recommendations on additional information to complete the risk assessment. As already discussed, the complete risk assessment has to include a release, exposure, consequence and risk assessment, with a final section addressing risk management considerations. These will be considered in turn.

Release assessment

The release assessment estimates the probability that the proposed use of the antimicrobial new animal drug in food-producing animals will result in the emergence or selection of resistant bacteria in the animal. It is important to understand that the boundaries of the release assessment span from the point the antimicrobial new animal drug is administered to the food-producing animal, to the point the animal is presented for slaughter or the animal-derived food is collected.

Human exposure to the hazardous agent is addressed in the exposure assessment. A number of relevant factors are suggested for consideration in completing the release assessment, and the sponsor is asked to provide an estimate of whether each factor would have a high, medium or low likelihood of favouring resistance emergence. These rankings can then be integrated into an overall release assessment ranking of high, medium or low. It is recommended that the sponsor provide a detailed discussion of the conclusions as well as presenting the conclusions in summary format. If there is insufficient information regarding respective factors, the most conservative estimate should be assumed.

The outcome of the release assessment is intended to estimate the probability that resistant bacteria will emerge or be selected for as a consequence of the proposed drug use in animals. The FDA recommends that the sponsor use the conclusions obtained from assessing all relevant factors to derive an overall qualitative ranking for the release assessment. This overall conclusion may be expressed in terms of a high, medium or low probability that resistant food-borne bacteria will occur in animals as a consequence of the proposed use of the drug.

Exposure assessment

The exposure assessment describes the likelihood of human exposure to food-borne bacteria of human health concern through particular exposure pathways, in this case animal-derived food products. The exposure assessment should provide a qualitative estimate of the probability of this exposure occurring.

The division of the qualitative risk assessment into 'release' and 'exposure' components effectively produces a natural placement of animal and animal treatment factors into the 'release assessment component and food-chain and human factors within the 'exposure assessment component'. The FDA recognises that there are many factors that may affect the bacteria of interest between the time animals are presented for slaughter (or the animal-derived food is collected) and the time the final food product is consumed. For the purposes of this qualitative risk assessment, the FDA assumes the probability that bacteria in or on the animal at slaughter may be used as an estimate of the probability of human exposure to that bacterial species in the food commodity derived from that animal.

The FDA also recognises that food-borne human exposure to antimicrobial-resistant bacteria is complex and often involves contributions from other sources of exposure. However, it is believed that evaluating antimicrobial new animal drug safety relative to the food-borne pathway is the best way to qualitatively assess the risk of antimicrobial drug use in food-producing animals. Uncertainties regarding the contribution of other exposure pathways may be considered during the development of appropriate risk management strategies.

It is important to emphasise that the exposure assessment is independent of the use of the antimicrobial drug under review and may be estimated by considering the relative amount of relevant bacterial contamination of the food product and the relative quantity consumed by humans.

The FDA recommends that the sponsor derives the exposure assessment ranking by integrating the ranking of the probability of human exposure (through food) to the bacteria in question with the ranking of consumption of the animal-derived food commodity. The qualitative probability should be expressed in terms of high, medium or low, and examples are presented in the guidance document, although it is emphasised that these are for illustrative purposes only. Sponsors are encouraged to reference current data sources which best characterise human exposure to bacteria of human health concern via animal-derived foods.

The FDA also recommends that sponsors reference the most reliable data available at the time that the assessment for their product is conducted. Food commodity consumption data, e.g. per capita meat consumption data, are published by the United States Department of Agriculture (USDA) Economic Research Service and current data can easily be sourced.

The outcome of the exposure assessment is intended to estimate the probability that humans will be exposed to the hazardous agent through consumption of animal-derived food commodities. The FDA recommends that the sponsor uses the outcome of the integration process described in the guidance to reach an overall qualitative rank of a high, medium or low probability of human exposure to the hazardous agent.

Consequence and risk assessment

While antimicrobial agents are important for the treatment of infectious disease in humans, the FDA considers certain antimicrobial agents to be of greater importance to the therapy of infectious diseases in humans than are others. Therefore, it is assumed that the human health consequences associated with bacteria that are resistant to drugs of greater importance are more significant than the consequences associated

Table 16.2 Possible risk estimation outcomes based on the integration of release, exposure and consequence assessment rankings (from CVM Guidance for Industry #152).

Release	*Exposure*	*Consequence*	*Risk estimation*
Low	Low	Important	Low
Low	Medium	Important	Low
Medium	Low	Important	Low
Low	Low	Highly important	Low
Low	High	Important	Medium
High	Low	Important	Medium
Medium	Medium	Important	Medium
Medium	High	Important	Medium
High	Medium	Important	Medium
High	High	Important	Medium
Low	Medium	Highly important	Medium
Low	High	Highly important	Medium
Medium	Medium	Highly important	Medium
Medium	Low	Highly important	Medium
Medium	High	Highly important	Medium
High	Low	Highly important	Medium
High	Medium	Highly important	Medium
Low	Low	Critically important	High
High	High	Highly important	High
Low	Medium	Critically important	High
Medium	Low	Critically important	High
Low	High	Critically important	High
High	Low	Critically important	High
Medium	Medium	Critically important	High
Medium	High	Critically important	High
High	Medium	Critically important	High
High	High	Critically important	High

with bacteria that are resistant to drugs of lesser importance.

The FDA recommends that the sponsor bases the consequence assessment conclusion on the human medical importance ranking and that this be expressed as critically important, highly important or important. This ranking will be integrated along with the outcomes of the release and exposure assessments to derive an overall risk estimation. The risk estimation integrates the results from the release, exposure and consequence assessments into an overall risk estimation associated with the proposed conditions of use of the drug. It also recommends that the risk estimation ranks drugs as high, medium or low risk. The risk rankings represent the potential for human health to be adversely impacted by the selection or emergence of antimicrobial-resistant food-borne bacteria associated with the use of the drug in food-producing animals.

Table 6 of the guidance document, reproduced here as *Table 16.2*, provides a method of integrating the outcomes of the release, exposure and consequence assessments into a single risk estimation ranking. The distribution of risk estimation rankings listed provides an initial indication as to the integration of rankings. Refinement of the risk estimation ranking may be

appropriate for specific cases based on available information.

Risk management considerations

The guidance makes it clear that possible risk management steps range from denying the approval of a drug application (i.e. the drug is unsafe or not shown to be safe) to approving the application under various use conditions that assure the safe use of the product.

The Federal Food, Drug and Cosmetic Act (FFDCA), Sec. 512(d), and regulations promulgated thereunder (see 21 CFR 514.111), provide possible grounds for denying the approval of a new animal drug application. The statutory grounds for denying approval include the results of tests that show the drug is unsafe or the determination that there is insufficient information as to whether the drug is safe. Consequently, denying the approval of an antimicrobial drug application is one possible outcome of an overall safety evaluation which could include the qualitative antimicrobial resistance risk assessment process described above.

Approval of the use of the drug under those conditions for which safety and effectiveness have been demonstrated is another possible outcome of an overall safety evaluation that could include the qualitative antimicrobial resistance risk assessment process described above. Drugs considered to be of high concern (with regard to potential human health impact) would typically be associated with more restricted use conditions. Drugs considered to be of lower concern would typically be associated with less restricted use conditions in food-producing animals.

The guidance details a number of risk management steps or conditions that may be appropriate based on the outcome of the qualitative antimicrobial resistance risk assessment process including limitations with regard to marketing status limitations, extra-label use prohibition and extent-of-use limitations. It is also important to understand that antimicrobial new animal drugs intended for use in food-producing animals may be subject to monitoring through a post-approval process, such as the National Antimicrobial Resistance Monitoring System (NARMS).

Within the US regulatory system, the FDA may choose to convene an advisory committee, the Veterinary Medicine Advisory Committee (VMAC), to discuss the application. The experience of such VMAC meetings has been such that this is not a path that would be generally favoured by a drug sponsor.

The integration of all the described processes results in an estimation of the risk that the use of an antimicrobial new animal drug will adversely impact human health. The outcome of the risk estimation (high, medium or low) can be used to help identify steps necessary to manage the risks associated with the proposed conditions of use for an antimicrobial new animal drug.

An issue for consideration – the impact of dose upon resistance development

Irrespective of regulatory considerations and jurisdiction there is a need to consider the important issue of what is meant by selecting an antibiotic dose that minimises resistance development. The stated objective of the *CVMP Guideline for the Demonstration of Efficacy for Veterinary Medicinal Products Containing Antimicrobial Substances* (EMEA/CVMP/627/01-FINAL) is absolutely clear. It makes clear that it is:

> '. . . to specify the data required to demonstrate the therapeutic efficacy of an antimicrobial substance for a given indication(s) using a therapeutic regimen that aims to minimise the risk of selecting antimicrobial resistant bacteria'.

The question to be asked then is whether it is possible to optimise drug exposure in order to ensure efficacy, i.e. that we eradicate the infecting organism, and minimise selection of antibiotic resistance development. In simplistic terms this must on the face of it be achievable if we are only considering the infecting organism. If the drug

regimen were to completely eradicate the infecting organism then there would be no opportunity for resistance to develop and so the way forward would be to increase the dose such that the total population of the infecting organism were eradicated. Alas, life is not so simple and it is rarely possible to achieve such a scenario and this assumes that drug concentrations can be sufficiently high to kill those bacteria carrying resistance determinants.

This introduces the concept of Mutant Prevention Concentration (MPC). In many respects prevention of resistance development is centred on the ability of the dosage regimen to prevent clonal expansion of any mutant population. Drlica and Malik (2003) made the point that a successful strategy to restrict mutant selection is to ensure that drug concentrations are high enough to prevent the growth of selected mutants. Baquero and Negri (1997) forwarded the idea that there was a dangerous concentration range in which mutants were most frequently selected; this is considered to be the window between the MIC and the MPC. The MPC is defined as the drug concentration at which no mutant is recovered when more than 10^{10} cells are applied to an agar plate (Dong *et al*., 1999).

Zhao and Drlica (2001) argued for a general strategy restricting the selection of antibiotic-resistant mutants and presented the case for the 'mutant selection window' or MSW. Drlica (2003) emphasised that the mutant selection window is an antimicrobial concentration range extending from the minimal concentration required to block the growth of wild-type bacteria (MIC) up to that required to inhibit the growth of the least susceptible, single-step mutant. The upper boundary is also referred to as the MPC.

Thus the window is the drug concentration range between the MIC and the MPC. Placing antimicrobial concentrations inside the window is expected to enrich resistant mutant subpopulations selectively, whereas placing concentrations above the window is expected to restrict selective enrichment. Since window dimensions are characteristic of each pathogen–antimicrobial combination, they can be linked with antimicrobial pharmacokinetics to rank compounds and dosing regimens in terms of their propensity to enrich mutant fractions of bacterial populations. This is an extremely important concept and needs to be considered carefully.

It is clear that if drug concentrations are below the MIC no mutant will be enriched because selective pressure is absent, and if drug concentrations are above the MPC then the likelihood that mutants will be selected is very low because, in the case of a fluoroquinolone, for example, a double mutation will be required for growth. Dosage regimens that place drug concentrations in the mutant selection window are likely to lead to development of resistance. Whilst such concentrations will normally clear infections in immunocompetent patients, repeated exposure in the selection window, either with a single patient or spread over many patients, will gradually enrich the mutant fraction of the population (Zhao and Drlica, 2001). It also becomes clear that if drug concentrations exceed the MPC of infecting organisms then the expansion of resistant mutants will be restricted.

Drlica (2000) suggested that the mutant selection window can be visualised in an idealised pharmacokinetic plot. This plot demonstrates that if the window can be closed mutants will not be selected.

It is clear from this and well argued by Zhao and Drlica (2001) that there are two means of closing the window. The first is to minimise the time at which the drug concentration lies inside the window. This can be accomplished if the administered drug rapidly exceeds the MPC, remains above the MPC throughout the treatment period and is then rapidly eliminated once it falls below the MPC. The rapid elimination is less important especially for a drug that rapidly kills and thus the number of wild types and resistant mutants are likely to have been greatly reduced during the time the drug is above the MPC.

The second mechanism is one in which the difference between MIC and MPC is greatly reduced. It thus becomes clear that with respect to minimising resistance development the most effective

compound may not always be the one with the lowest MIC. Whilst Drlica (2003) emphasised that the hypothesis still awaits validation there is good experimental support for it (Firsov *et al*., 2003). It may therefore be important to provide MPC data for new drug evaluations, depending of course on the class of drug and likely resistance mechanisms, thereby allowing an assessment of the propensity of a new active compound to give rise to resistance development.

The relationship between the MIC and the MPC is fundamentally important with respect to minimising resistance development. The optimal situation is for a drug to have a very low MIC and an equally low MPC. A suitable index to evaluate a drug with high intrinsic activity (low MIC) and low potential to enhance resistance (low MPC) may well be to consider the value of the MIC × MPC.

Whilst the definition that the MPC is approximated experimentally as the lowest drug concentration that allows no colony growth when about 10^{10} cells are applied to drug-containing agar plates has been widely accepted, there is no agreed and standardised method for determining MPCs. The choice of 10^{10} cells is based on several considerations:

1. 10^{10} is large enough for mutant subpopulations to be present for testing.
2. Infections rarely contain more than 10^{10} organisms.
3. Testing more cells is often logistically difficult.

Wetzstein (2005) determined MPCs for pradofloxacin and other fluoroquinolones in a series of highly detailed studies. Stephan *et al*. (2007) similarly determined the MPC of a *Porphyromonas gingivalis*, as far as I am aware this being the first instance of MPC data being made available for anaerobic organisms. In the absence of standardised protocols these studies addressed the many variables that are inherently associated with this type of study.

The authors used plates with a culturable surface area of about ten times that of standard plates which would clearly minimise effects restricting colony formation of the surviving cells. This additionally extended the upper limit of detection by about one $\log_{10}$, thereby enhancing the precision of determining counts. The large surface area would also reduce the density of cell debris and the fraction of drug non-specifically bound and therefore inactivated by cell debris. They used incubation times mostly of 5–7 days. Whilst it remains as yet clinically untested, an MPC value lower than serum concentrations should allow a fluoroquinolone to severely restrict the selection of resistant mutants (Schentag *et al*., 2003). Indeed Drlica (2003) has argued that compounds that cannot meet the MPC pharmacokinetic criterion will enrich resistant mutants.

The concept of the MSW is not equally applicable to all antibiotic–organism combinations, as discussed by Olofsson and Cars (2007). For MPC determination to be useful in the approach to restricting the selection of antibacterial resistance, the resistance mechanisms observed in the in vitro MPC studies must be the same as the resistance mechanisms observed in vivo. The MPC has mostly been studied for antibiotics for which resistance primarily develops by stepwise chromosomal point mutations, especially the fluoroquinolones. The application of MPC determination to other drugs has raised questions regarding the relevance of mutational events for such drugs as the β-lactams and the aminoglycosides and whether MPC measurements can be performed for drugs with other resistance mechanisms (e.g. efflux and β-lactamases).

The significance of these concerns is based on whether the MSW hypothesis addresses the first step in resistance development (i.e. spontaneous mutations or horizontal DNA acquisition) or the second step (i.e. the selective enrichment of mutant subpopulations). If the second step is the most important, then the individual resistance mechanisms are of less relevance for the MSW concept.

The fact that resistance development can be affected by antibiotic dosing regimens is evident from the results of a number of in vitro and animal experiments and has been described by

Olofsson and Cars (2007), from which they concluded that clinical and experimental studies indicate that the choice of dose and treatment duration can influence the selection of antibiotic-resistant mutants.

The important point, however, is that our knowledge base regarding optimal dosing strategies to treat bacterial infections while simultaneously preventing the selection and emergence of resistance is still poor. This issue reaches even greater significance when considering therapeutic regimens in veterinary medicine because the issue here is more than that of a dosage regimen that is efficacious and minimises resistance development in the target pathogen. It is also necessary to consider what is happening with respect to antimicrobial exposure of the commensal and food-borne pathogens found in the gastrointestinal tract. These issues are addressed by the regulatory guidelines as has been discussed and are worked out in practice in terms of risk management.

The plethora of 'new' guidelines addressing antimicrobial resistance will play a part in addressing antimicrobial resistance development, but it is crucial to be mindful that antimicrobial resistance is not in itself a disease. Infectious disease is caused by virulent bacteria, and is largely independent of the susceptibility status of the infecting organisms. Appropriate use of antimicrobials relates to appropriate therapy and the overriding need is to ensure disease is treated by the correct antimicrobial regimen. If we can be successful in this important area we will go some way to combating the challenge of antimicrobial resistance.

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17 Adverse effects of veterinary pharmaceutical products in animals

K.N. Woodward

Introduction

Animals, like humans are susceptible to the side effects of medicines and some species may be particularly sensitive to the toxic effects of some specific drugs (and other chemicals), to overdosing or to idiosyncratic effects. For example, the cat has a very low capacity to conjugate paracetamol (acetaminophen) because of its low glucuronyl transferase activity. Hence, cats are extremely sensitive to the toxic effects of paracetamol (and other xenobiotics whose metabolism is dependent on this route), and what is a therapeutic dose in other species may prove to be a lethal dose in the cat (Campbell, 2000a). Cats are also more susceptible to the toxic effects of permethrin because unlike some other mammalian species they lack the necessary detoxification pathways (Volmer *et al*., 1998; Meyer, 1999; Gray, 2000; Martin and Campbell, 2000; Richardson, 2000). Dogs appear to be more sensitive to the effects of non-steroidal anti-inflammatory drugs (NSAIDs) on the gastrointestinal tract than do other species (Campbell, 2000b).

Many drugs are metabolised by the liver where they, or their metabolites, can cause hepatic damage. For example, in the dog, paracetamol, other non-steroidal anti-inflammatory drugs such as carprofen, closantel, some anticonvulsant drugs, 5-flurouracil, halothane, imidocarb, isoniazid, mebendazole and sulphonamides have caused hepatotoxicity, as has the sweetening agent xylitol (Yeary, 1975; Ndiritu and Weigel, 1977; Toth and Derwelis, 1980; Polzin *et al*., 1981; Bunch *et al*., 1982, 1984, 1987; Grenn and Bulmer, 1982; Swanson and Breider, 1982; Gaunt *et al*., 1984; Tams, 1984; Giger *et al*., 1985; Hjelle and Grauer, 1986; Francavilla *et al*., 1989; Dorman *et al*., 1990; Dayrell-Hart *et al*., 1991; Kock and Kelly, 1991; Jones *et al*., 1992; Bunch, 1993; Dill-Macky, 1995; McEntee *et al*., 1995; Noli *et al*., 1995; Villar *et al*., 1995, 1998; Cribb *et al*., 1996; Watson *et al*., 1996; Fuentealba *et al*., 1997; Twedt *et al*., 1997; MacPhail *et al*., 1998; Twedt, 1998; Barton, 2001; Boothe, 2001a; MacNaughton, 2003; Moreau *et al*., 2003; Boomkens *et al*., 2004; Dunmayer, 2004; Elsinghorst, 2004; Murphy, 2004; Watson, 2004; Hoskins, 2005; Nakagawa *et al*., 2005; Woodward, 2005; Dunmayer and Gwaltney-Brant, 2006; Bischoff and Ramaiah, 2007). Such findings underline the importance of reporting, recording and investigating adverse drug reactions in treated animals, and where necessary amending the terms of marketing authorisations, licences or approvals.

Suspected adverse reactions in animals

Companion animals

Gastric effects are the major adverse drug reaction associated with certain NSAIDs, particularly in the dog, although aspirin may be toxic to cats, albeit at relatively high doses and salicylate has induced seizures in a dog (Herrgesell, 1967; Zontine and Uno, 1969; Schubert, 1984; Lees *et al*., 1985). The major effect of many of these drugs, including aspirin, naproxen and ibuprofen and some in the coxib class such as deracoxib, is gastric ulceration resulting from inhibition of prostaglandins and loss of cytoprotection (Taylor and Crawford, 1968; Bolte *et al*., 1980; Stewart *et al*., 1980; Van Ryzin and Trapold, 1980; Roudebush and Morse, 1981; Schiltz, 1982; Smith, 1982; Dean and Reid, 1985; Boulay *et al*., 1986; Spaulding, 1986; Spyridakis *et al*., 1986; Gilmour and Walshaw, 1987; Marlow, 1987; Elliott *et al*., 1988; Tempowski, 1989; Kore, 1990; Ohkubo *et al*., 1990; Spellman, 1992; Murtaugh *et al*., 1993; Smith and Taylor, 1993; Vollmar, 1993; Knight *et al*., 1996; Dye, 1997; Poortinga and Hungerford, 1998; Hawkey, 1999; Bertolini *et al*., 2001; Boothe, 2001b; Lee and Morris, 2001; Waller *et al*., 2001; Ramesh *et al*., 2002; Neiger, 2003; Tjälve; 2003; Bergh and Budsberg, 2005; Lascelles *et al*., 2005a).

Ibuprofen induces vomiting in dogs which may reduce the severity and extent of the gastric damage and other areas of toxicity which otherwise might be expected (Stephenson, 1988; Yeatts, 1988), although gastrointestinal effects have been seen with this drug in dogs (Jackson *et al*., 1991). Some of these drugs may also cause disruption of thyroid function in dogs (Daminet and Ferguson, 2003). Ibuprofen is toxic to ferrets, resulting in severe lethargy, coma, apnoea and ultimately death (Cathers *et al*., 2000). Two non-steroidal inflammatory drugs, meclofenamic acid and phenylbutazone, have been associated with the induction of aplastic anaemia in dogs (Weiss and Klausner, 1990). Anaemia, thrombocytopenia and pancytopenia have also been reported in dogs following treatment with phenylbutazone (Watson *et al*., 1980).

Carprofen has been reported to be associated with a neutrophilic dermatitis in dogs accompanied by immune-mediated haemolytic anaemia and thrombocytopenia (Mellor *et al*., 2005). There are potential drug interactions with non-steroid anti-inflammatory drugs, particularly with compounds such as heparin leading to prolonged bleeding times (Trepanier, 2005). This was almost certainly idiopathic in nature and had much in common with Sweet's syndrome in humans (Sweet, 1964; Fye *et al*., 2001; Khan Durani and Jappe, 2002). Pentosan polysulphate, which is not a NSAID but is used in the treatment of osteoarthritis in dogs, has a low order of toxicity and adverse effects are limited to lethargy and changes in demeanour (Hannon *et al*., 2003).

The adverse gastric effects of the NSAIDs, combined with their other adverse effects (nephrotoxicity, hepatotoxicity and effects on bleeding times) have led to advice being published on their safe and effective use in dogs. Recommendations include screening for high-risk patients, and ensuring that other drugs of the same class are not given concomitantly. There is also a recommendation for a suitable washout period of 5–7 days between the end of administration of one drug and replacement with an alternative (Lascelles *et al*., 2005b).

Synthetic pyrethroids such as permethrin and deltamethrin (*Figure 17.1*) are extremely effective against fleas and other ectoparasiticides in the dog (Endris *et al*., 2000, 2002, 2003), but because of their adverse effects in cats, they are usually contraindicated for this species (see 'Introduc-

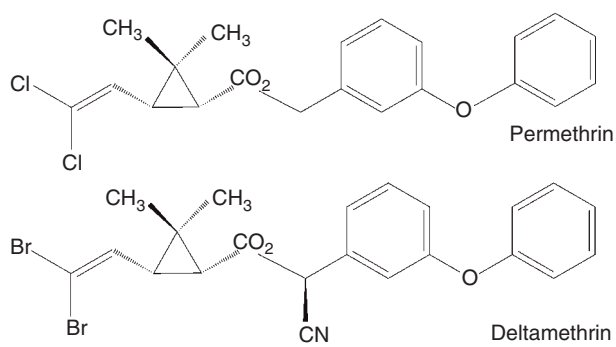

Fig. 17.1 Deltamethrin and permethrin.

tion'). Their use and misuse in cats can result in severe toxicity and fatalities (Valentine, 1990; Mount *et al*., 1991; Volmer *et al*., 1998; Meyer, 1999; Gray, 2000, 2001; Martin and Campbell, 2000; Richardson, 2000; Sutton *et al*., 2007a, b; Gleadhill, 2004; Woo and Lunn, 2004; Merola and Dunmayer, 2006; Linnett, 2008). A significant proportion of cats affected with permethrin toxicity have to be euthanised (Sutton *et al*., 2007b).

The newer ectoparasiticides for use on dogs and cats such as imidacloprid, fipronil and lufenuron have low toxicity (Stansfield, 1997; Hovda and Hooser, 2002), although there has been a report of adverse effects in a cat treated with imidacloprid. However, the animal also had a thymoma and was subsequently treated with other drugs (Godfrey, 1999). Fipronil may be toxic to very young rabbits, probably as a result of overdosing where it resulted in anorexia, lethargy and death (Webster, 1999).

Hypersensitivity reactions to a number of drugs have been widely reported in the literature (Yeary, 1975; Giger *et al*., 1985; Ballarini, 1994; Noli *et al*., 1995) along with diarrhoea and vomiting (Kunkle *et al*., 1995). Diarrhoea, vomiting, loss of appetite and depression are common in dogs (and cats) with a variety of antimicrobial drugs (Kunkle *et al*., 1995). Hypersensitivity reactions have been reported in dogs treated with oxytetracycline (Abdullahi and Adeyanju, 1985; Srinivasan *et al*., 1991). The drug has been associated with renal toxicity in dogs (Stevenson, 1980).

Metronidazole may be toxic to cats and dogs resulting in neurological signs and in severe cases, death (Wright and Tyler, 2003; Olson *et al*., 2005). Sulphonamides and potentiated sulphonamides have also been implicated in hepatic necrosis and keratoconjunctivitis sicca in dogs (Morgan and Bachrach, 1982; Sutton and Roach, 1988; Gray, 1990; Bunch, 1993; Twedt *et al*., 1997; Trepanier *et al*., 2003; Trepanier, 2004). Streptomycin and dihydrostreptomycin may cause death in dogs after large intravenous doses, while the aminoglycoside antibiotics are ototoxic (Martinez-Salgado *et al*., 2007), and may be nephrotoxic in animals (Riskaer *et al*., 1956; Yeary, 1975; Yakota *et al*., 1984; Mealey and Boothe, 1994; Riviere and Spoo, 2001). Fosfomycin, a methyl oxiranyl derivative of phosphonic acid, also appears to be nephrotoxic in the cat (Fukata *et al*., 2008).

Colitis may occur in dogs after antimicrobial treatments (Willard *et al*., 1998). Blood dyscrasias have been reported in dogs and cats following chloramphenicol treatment (Watson and Middleton, 1978; Baig *et al*., 2002). These are characterised by a dose-related reversible bone marrow depression with leukopenia (Yeary, 1975; Clark, 1978; Watson and Middleton, 1978; Papich and Riviere, 2001). Chloramphenicol may also result in prolongation of barbiturate-induced anaesthesia in dogs, with hypotension and bradycardia (Mercer, 1980; Houston *et al*., 1989; Sangiah and Burrows, 1989). The plasma half-life of chloramphenicol can be significantly extended in cases of pre-existing hepatic disease (Tams, 1984).

Epilepsy has occurred in dogs following large experimental doses of penicillin (Currie *et al*., 1970), while amphotericin B may be nephrotoxic (Ndiritu and Enos, 1977; Ceylan *et al*., 2003). Sulphonamide drugs may disrupt thyroid function in dogs when given at high doses (Daminet and Ferguson, 2003).

Toxic epidermal necrolysis (TEN), a serious condition occasionally seen in humans treated with drugs, is also occasionally seen in animals. It has been observed in dogs treated with certain antibiotics including gentamicin, cephalexin, chloramphenicol and potentiated sulphonamides (Roosje, 1991; Scott and Miller, 1999). TEN has been noted in cats given cephaloridine, hetacillin or ampicillin (Scott and Miller, 1998a).

Also rare it seems is the possibility or more likely the recognition that companion animals may be pregnant when being treated (Landsbergen *et al*., 2001), although many veterinary medicinal products carry warnings and contraindications for pregnancy, largely because they have not been tested for reproductive safety rather than due to any actual risk. Nevertheless, unfavourable reproductive outcomes do not feature in reports by the UK's Veterinary Medicines Directorate (VMD), and are rare in the open literature.

Calcipotriol and compounds related to vitamin D are toxic to dogs and may cause renal, splenic, gastric and myocardial mineralisation (Campbell, 1997, 2000c; Fan *et al*., 1998; Durtnell, 1999; Hare *et al*., 2000; Torley *et al*., 2002; Welch, 2002; Mellanby *et al*., 2005). Acepromazine may induce aggression in dogs (Waechter, 1982; Meyer, 1997).

Hepatotoxicity and cholestasis have been reported in dogs treated with phenytoin in combination with phenobarbital and primidone as anticonvulsant therapy (see Chapter 18; Bunch *et al*., 1987). Similar effects have previously been reported in dogs treated with phenytoin (Nash *et al*., 1977; Bunch *et al*., 1982, 1984; Bunch, 1993). Phenytoin treatment has led to dermal atrophy in the cat (Barthold *et al*., 1980). Primidone has resulted in ataxia and collapse in the dog (Shield, 1987). The benzimidazole drug mebendazole and the anaesthetic methoxyfluorane have resulted in hepatic injury in the dog (Ndiritu and Weigel, 1977; Polzin *et al*., 1981; Swanson and Breider, 1982). Fenbendazole has caused pinnal necrosis in the dog (Nuttall *et al*., 2005). This appeared to result from a drug-induced thrombo-ischaemic episode leading to the observed necrosis.

Treatment of dogs with oestrogens is associated with a number of adverse drug reactions including effects on haematology (Acke *et al*., 2003). However, the most common serious adverse effect is the induction of pyometra (Bowen *et al*., 1985; Wheaton *et al*., 1989; Niskanen and Thrusfield, 1998). A recent literature review (Whitehead, 2008) suggests that this may be more common than previously thought, and that around 85% of pyometras that occur within 4 months of treatment with oestradiol benzoate may be drug related.

Heartworm infection in dogs caused by *Dirofilaria immitis* tends not to be a major problem in northern Europe, but it can be a major parasitic condition in warmer geographical regions. In the past this condition was treated with diethylcarbamazine and a severe adverse reaction, similar to hyovolaemic shock, was frequently reported (Sasaki 1986, 1989). This was accompanied by tissue damage, and particularly by hepatic injury

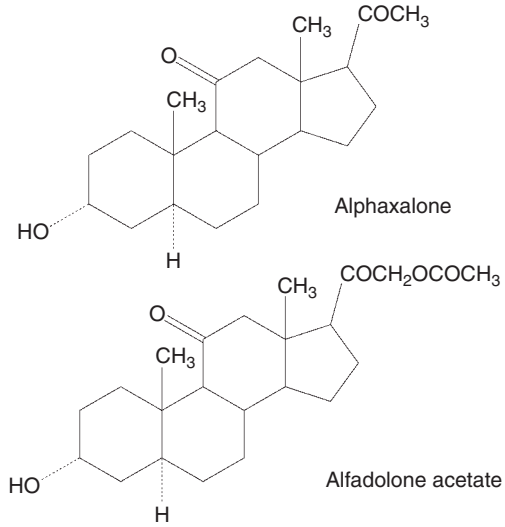

Fig. 17.2 Alphaxalone and alfadolone acetate.

(Powers *et al*., 1980; Palumbo *et al*., 1981; Desowitz *et al*., 1984; Rawlings *et al*., 1986). The mechanism is unknown but appears to be related to parasite burden. The reaction can be partly blocked by diazepam (Palumbo *et al*., 1981; Desowitz *et al*., 1984). The drug has now been largely displaced by more modern treatments for heartworm, including ivermectin, although this too may be toxic at high doses in dogs (Hopkins *et al*., 1990).

Piperazine is widely used in small animal medicine for the treatment of *Ascaris* where it appears to act as a GABA agonist. It has induced toxic effects in cats and dogs, usually when given in overdose (Stoffman and Braithwaite, 1976; Darke, 1987; Gray and Millar, 1987; Hartigan, 1987; Lovell, 1990).

Saffan is an injectable veterinary anaesthetic for use in cats; it contains two steroidal anaesthetic agents, alphaxalone and alfadolone acetate (*Figure 17.2*). In humans (as Althesin) the product produces hypernoea on administration, and apnoea in overdose (Hunter, 1973). Oedema in the ears and paws has been reported in cats following its administration (Alcarez and Stone, 1980; Abou-Madi and Blais, 1987). Adverse effects in cats appear to be due to the release of histamine or histamine-like substances caused by a solubilising agent Cremophor EL, a polyethoxylated castor oil derivative used in the formulation

(Stogdale, 1978). Laryngeal oedema and pulmonary oedema have been reported (Stogdale, 1978; Harding, 1980). These may occasionally be severe and can result in death (Corbett, 1976; Stogdale, 1978; Dodman, 1980; McDonald, 1980), but adverse reactions to the product appear to be rare (Carroll, 1982). Saffan can also lead to marked depression of cardiopulmonary function in cats (Dyson *et al*., 1987).

There has been an isolated report of an anaphylactoid reaction to xylazine in the cat, but most adverse events to this drug appear to be due to overdose which can be treated with tolazoline, doxapram or yohimbine (Arnbjerg, 1979; Jensen, 1985; van Metre, 1992; Raptopoulos *et al*., 1993). Unilateral papillary dilation has been reported in cats following Saffan administration (Fogle, 1987). Propofol has been reported to lead to convulsions in dogs on rare occasions (Helin *et al*., 2001). However, the drug has a good safety profile in cats (Bley *et al*., 2007).

The antifungal drug griseofulvin has been shown to produce birth defects in laboratory animals. It produced teratogenic effects in rats when given oral doses of 250 mg/kg per day from days 6 to 15 after mating. No malformations were noted with 125 mg/kg per day (Klein and Beall, 1972). Similar results were noted in other studies in rats (Aujezdska *et al*., 1978; Steelman and Kocsis, 1978). An in vitro study with rat embryos also suggested teratogenic potential (Bechter and Schmid, 1987).

Therapeutic treatment of pregnant cats with griseofulvin for ringworm resulted in malformations in the offspring including cleft palate, exencephaly, caudal displacement and hydrocephaly, along with multiple skeletal abnormalities including cranium bifidium, spina bifida and abnormal vertebrae. Cyclops and anophthalmia also occurred (Scott *et al*., 1975). Similar cases in cats have been reported (Gillick and Bulmer, 1972; Gruffydd-Jones and Wright, 1977; Turner, 1977). Cats appear to be more susceptible to the toxic effects of griseofulvin (Kunkle and Meyer, 1987), but it is not known if this species is also more susceptible to the teratogenic effects of the drug.

Enrofloxacin treatment has been associated with irreversible retinal degeneration and blindness in cats (Davidson, 2001; Gelatt *et al*., 2001; Abrams-Ogg *et al*., 2002; Crispin *et al*., 2002; Watson, 2002; Wiebe and Hamilton, 2002). Data from the UK's VMD suggest that this is related to overdosing or misdosing (Dyer *et al*., 2007). Retinopathy has been reported in a guanaco following enrofloxacin treatment (Harrison *et al*., 2006).

Salinomycin has resulted in polyneuropathy in cats after oral intake through contaminated cat food (van der Linde-Sipman *et al*., 1999). 5-Fluorouracil has been associated with neurotoxicity in dogs (Harvey *et al*., 1977; Henness *et al*., 1977).

Glucocorticoids are associated with liver toxicity in cats as well as dermatological effects (Lowe *et al*., 2008).

Interestingly, the predominance of adverse reactions in cats and dogs has been reported in other countries such as Australia and France (Keck and Lorgue, 1990; Maddison, 1992). There were other similarities too; for example, the occurrence of pyrethroid toxicity arising from the use of ectoparasiticides in cats and gastrointestinal effects resulting from the use of non-steroidal anti-inflammatory agents in dogs (Maddison, 1992).

Although not indicated for use in hamsters, clindamycin and lincomycin have been shown to result in enterocolitis in this species (and in guinea-pigs) (Small, 1968; Lusk *et al*., 1978; Onderdonk *et al*., 1981). This is associated with one or more bacterial toxins, including clostridial toxins (Bartlett *et al*., 1978; Knoop, 1979; Toothaker and Elmer, 1984; Merrigan *et al*., 2003). Lincomycin and clindamycin cause pseudomembranous colitis in humans (Scott *et al*., 1973; Lee and Morris, 2001), and the hamster has been suggested as a model for the human disease (Price *et al*., 1979).

Other therapeutic agents and drugs of abuse that have caused adverse effects in companion animals include lidocaine, levothyroxine, loperamide, diazepam, flurazepam, marijuana, cocaine, minoxidil, methamphetamine, caffeine,

promethazine, quaternary ammonium salts, terfenadine and serotonin and its precursors (Foor, 1975; Trapani *et al*., 1982; Dumonceaux and Beasley, 1990; Hansen *et al*., 1992; Otto and Greentree, 1994; Staley and Staley, 1994, 1995; Center *et al*., 1996; Beier and Bischoff, 1997; Bischoff *et al*., 1998; Gwaltney-Brant *et al*., 2000; Wismer, 2000; DeClementi *et al*., 2004; Bates, 2007; Lemo *et al*., 2007). Albendazole has resulted in pancytopenia in dogs and cats (Stokol *et al*., 1997).

Several chemotherapeutic agents, but particularly the aminoglycosides and some anticancer drugs, are ototoxic in dogs and cats (Pickrell *et al*., 1993; Merchant, 1994). A number of drugs have been reported to result in idiosyncratic skin reactions in cats including phenytoin, cimetidine, doxycycline and econazole (Scott and Miller, 1998a) and in dogs including cephalexin, enalapril, amitriptyline and potentiated sulphonamides (Scott and Miller, 1999).

Large animals

Many animals are specifically intolerant to the microbiological effects of some antimicrobial drugs (Keck and Ibrahim, 2001). For example, rabbits, hamsters, ruminants and horses rely significantly on the gut flora for digestion of plant material, particularly cellulose, and if the gut flora is inhibited or otherwise disrupted by some antibiotics, morbidity and death can occur (Killby and Silverman, 1967; DeSalva *et al*., 1969; Milner, 1975; Olfert, 1981; Keen and Livingston, 1983; Rollin *et* al., 1986; Gray, 1989, 1993). Adverse effects to antimicrobial drugs were reported relatively frequently in horses (Gray *et al*., 2003). There was little description of these effects, but gastrointestinal disturbances, including diarrhoea, have been reported after treatment of horses with a number of antibiotics, as has the development of *Clostridium difficile* colitis in mares following treatment of their offspring with erythromycin and rifampicin (Keen and Livingston, 1983; Wilson *et al*., 1996; Baverud *et al*., 1998; Stratton-Phelps *et al*., 2000; Brumbaugh, 2001).

Ototoxicity and nephrotoxicity were observed in calves given neomycin (Crowell *et al*., 1981), while cardiotoxicity and pulmonary oedema were noted in calves accidentally given a large overdose of doxycycline (Yeruham *et al*., 2002). Pharyngeal and lingual paralyses have been reported in calves after doxycycline treatment (Chiers *et al*., 2004).

Procaine penicillin has resulted in toxicity in pigs. Animals became pyrexic and lethargic, with vomiting, inappetance and cyanosis of the extremities. Swelling of the vulva, mucous discharge and abortion occurred (Nurmio and Schulman, 1980; Embrechts, 1982).

Hypersensitivity reactions and effects on the gastrointestinal tract have been described following tetracycline or penicillin treatment of cattle (Balasubramanyam, 1980; Sakar, 1993; Thirunavukkarasu, *et al*., 1995). Chloramphenicol has resulted in hypersensitivity reactions in large animals (Sudhan *et al*., 1990; Bhat *et al*., 1995).

Adverse effects, suggestive of anaphylaxis, have been reported in horses treated with penicillin, and signs of procaine toxicity after treatment with procaine penicillin have also been noted (Eyre and Lewis, 1973; Farmer, 1980; Marshall, 1980; Owen, 1980; Xu and Liu, 1985; Allpress and Heathcote, 1986; Nielsen *et al*., 1988; Chapman *et al*., 1992; Kemble, 1995). In one report, of 11 horses treated with penicillin, 5 died. One had post-mortem findings suggestive of anaphylaxis, while in the others, the clinical signs suggested procaine toxicity (Nielsen *et al*., 1988).

Sudden death has been reported in a pony after treatment with a number of drugs including procaine penicillin and neomycin (McCann, 1995). There have been a number of cases of penicillin-induced immune-mediated haemolytic anaemia, one with hepatic failure, reported in horses (Blue *et al*., 1987; Step *et al*., 1991; McConnico *et al*., 1992; Robbins *et al*., 1993). Intravenous injection of trimethoprim-sulphonamide products has been associated with fatalities in the horse, while neomycin has resulted in nephrotoxicity (Alexander and Collett, 1975; Edwards *et al*., 1989; Gray, 1989; Rohner and Demuth, 1994).

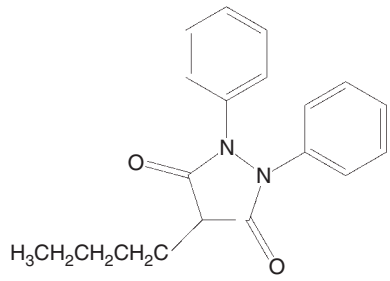

Fig. 17.3 Phenylbutazone.

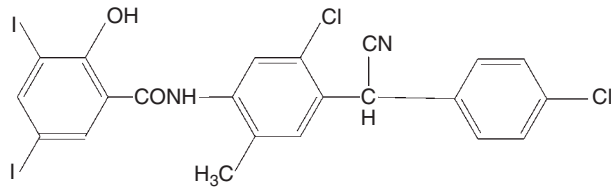

Fig. 17.4 Closantel.

Trimethoprim and sulphonamides has been reported to cause diarrhoea in horses, but its prevalence was similar to that noted following other antimicrobial drugs including penicillin (Wilson *et al*., 1996). Erythromycin also causes diarrhoea in the horse (Stratton-Phelps *et al*., 2000). On the other hand, the cephalosporin drug ceftiofur sodium appears to be relatively safe in the horse, at least after intramuscular administration (Mahrt, 1992), while several drugs, including potentiated sulphonamides and β-lactams, have resulted in skin reactions (Scott and Miller, 1998b).

Xylazine can occasionally result in extreme excitation in horses (Groenendyk and Hall, 1989).

Phenylbutazone (*Figure 17.3*) is one of the most commonly used anti-inflammatory drugs in horses. It may induce gastric ulceration in this species; there have also been reports of decreased bone mineralisation in cortical bone following phenylbutazone administration, while eltenac resulted in dose-dependent NSAID toxicity. Death may occur after doses in excess of those recommended (Jeffcott and Colles; 1977; Lees and Michell, 1979; MacKay *et al*., 1983; Hamm *et al*., 1997; Rohde *et al*., 2000; Brumbaugh, 2001). Major adverse effects include gastric ulceration and renal papillary necrosis.

There are a number of reports of the induction of optic neuropathy and retinopathy in sheep and goats and other animals following treatment with the salicylanilide drug closantel (*Figure 17.4*) (Button *et al*., 1987; McEntee *et al*., 1995; Gill *et al*., 1999; Barlow *et al*., 2002; Ecco *et al*., 2006; van der Lugt and Venter, 2007). This generally appears to follow overdosing with the drug (Borges *et al*., 1999; van der Lugt and Venter, 2007). Overall, the drug has relatively low toxicity (Van Cauteren *et al*., 1985).

Levamisole is an anthelmintic drug used in animals which has also found a role in the treatment of cancer and rheumatoid arthritis in humans because of its immunomodulatory effects (Robens, 1984; Laurie *et al*., 1989; Forman, 1994; Holcombe *et al*., 1998; Moore and Haller, 1999; Yip *et al*., 2000; Zlotta and Schulam, 2000; Chen *et al*., 2007). In humans, it may induce adverse effects including agranulocytosis and hypersensitivity reactions (Secher *et al*., 1977, 1978; Mielants and Veys, 1978; Prieur *et al*., 1978; Symoens *et al*., 1978; Runge and Rynes, 1983). In animals, adverse effects are seemingly rare; the drug is well tolerated by a number of species, including birds (Buys and van der Made, 1977; Reinemeyer and Courtney, 2001). However, in the past, levamisole has been reported to be toxic in domestic animals, and indeed a report published in 1980 claimed that levamisole caused the greatest number of adverse effects reported to the FDA, with pigs and cattle being the major species affected (Hsu, 1980).

The use of levamisole is now much less widespread, often because of levamisole-resistant parasites and the alternative use and rotation of other anthelmintic drugs such as the benzimidazoles and the avermectins and related compounds. There have been reports of levamisole toxicity in treated animals (Babish *et al*., 1990; Cawley *et al*., 1993; Sarma and Sarma, 2002) including the kiwi (Gartrell *et al*., 2004), and the drug may be more toxic when given with other medications such as diazinon (Ford and Abdelsalam, 1983; Abdelsalam and Ford, 1987). Diazinon toxicity has been reported in cattle and

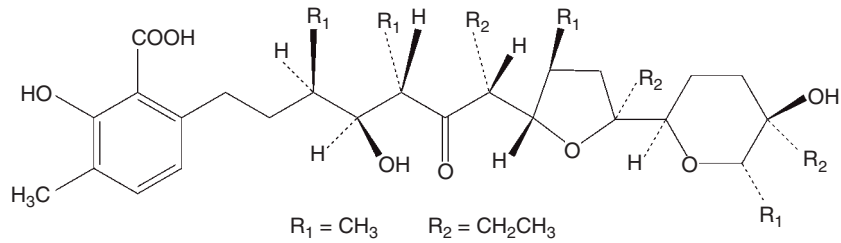

Fig. 17.5 Lasalocid A.

sheep following the use of unlicensed or out-of-date veterinary medicinal products, possibly due to the formation of more toxic breakdown products including sulfotepp and monothiono-TEPP (Sharpe *et al*., 2006). Showering sheep with ectoparasitic product contaminated with *Pseudomonas aeruginosa* resulted in rhinitis and otitis (Watson *et al*., 2003).

The ionophore antibiotics such as lasalocid (lasalocid A; *Figure 17.5*), maduramicin, monensin, narasin and salinomycin are widely used in poultry for the prevention and treatment of coccidiosis caused by *Eimeria* species (Lindsay and Blagburn, 2001). They have narrow therapeutic indices and are toxic to turkeys and mammals at relatively low doses (Todd *et al*., 1984; Oehme and Rumbeiha, 1999; Lindsay and Blagburn, 2001).

Ionophore toxicity, often with fatalities and frequently as a result of accidental treatment or misuse, has been reported in a number of species including rabbits, dogs, cats, pigeons, quail, chickens, turkeys, ostriches, goats, pigs, sheep, cattle, camels and horses (Matsuoka, 1976; Collins and McCrea, 1978; Malone, 1978; Donev *et al*., 1980; Howell *et al*., 1980; Wilson, 1980; Hanson *et al*., 1981; Nuytten *et al*., 1981; Halvorson *et al*., 1982; Newsholme *et al*., 1983; Van Vleet *et al*., 1983; Wagner *et al*., 1983; Anderson *et al*., 1984; Todd *et al*., 1984; Gad *et al*., 1985; Reece *et al*., 1985; Bourque *et al*., 1986; Galitzer *et al*., 1986; Potter *et al*., 1986; Van Vleet and Ferrans, 1986; Egyed *et al*., 1987; Rollinson *et al*., 1987; Chalmers, 1988; Ficken *et al*., 1989; Dalvi and Sawant, 1990; Drumev *et al*., 1990; Groom and Beck, 1990; Kavanagh and Sparrow, 1990; Sawant *et al*., 1990; Gregory *et al*., 1992; Hazlett *et al*., 1992; Mousa and Elsheikh, 1992; Novilla, 1992; Andreasen and Schleifer, 1995; Lehel *et al*., 1995; Plumlee *et al*., 1995; Bernáth *et al*., 1996; Baird *et al*., 1997; Hoop, 1998; Oehme and Pickrell, 1999; Roder and Stair, 1999; Van der Linde-Sipman *et al*., 1999; Bila *et al*., 2001; Jones, 2001; Agaoglu *et al*., 2002; Condon and McKenzie, 2002; Peek *et al*., 2004; Segev *et al*., 2004; Carpenter *et al*., 2005; Litwak *et al*., 2005; McGuirk and Semrad., 2005; Sharpe and Livesey, 2005).

Cardiomyopathy, with dilated heart or petechial and ecchymotic haemorrhages have been noted in cattle poisoned with lasolocid and monensin (Potter *et al*., 1984; Galitzer *et al*., 1986; Mathieson *et al*., 1990; Bastianello *et al*., 1996; Basaraba *et al*., 1999). Cardiomyopathy and myopathies have been seen in other species with ionophore poisoning (Wilson, 1980; Hanrahan *et al*., 1981; Muylle *et al*., 1981; Pressman and Fahim, 1983; Anderson, *et al*., 1984; Novilla, 1992).

The toxicities of ionophores may be potentiated by other substances and notably by the antimicrobial drugs tiamulin and enrofloxacin (Miller, 1981; Wanner, 1984; Miller *et al*., 1986; Mitema *et al*., 1988; Pott, 1990; Bartov, 1994; Wendt *et al*., 1997; Basaraba *et al*., 1999; Szucs *et al*., 2000; Sureshkumar *et al*., 2004; Carpenter *et al*., 2005). In vitro studies with mouse fibroblasts suggest that monensin induces early mitochondrial damage with consequential effects on energy balance in the cell (Souza *et al*., 2005).

Etorphine (Immobilon; *Figure 17.6*), usually in combination with other drugs such as acepromazine or thiopentone, has been used as an analgesic and capture drug in wildlife and other animals.

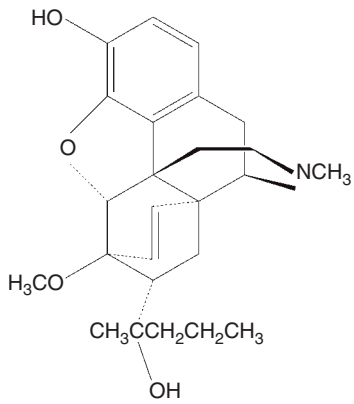

Fig. 17.6 Etorphine.

In horses and donkeys, etorphine results in a dramatic rise in blood pressure and heart rate, with pronounced muscle tremors, although this can be controlled with the use of other drugs such as thiopentone (Dobbs and Ling, 1972; Hebeler and Budd, 1974; Van Laun, 1977). Its use in fallow dear may lead to fatalities (Low, 1973), while administration to rhinoceros can lead to hypoventilation, hypoxaemia and metabolic acidosis (Wenger *et al*., 2007). Butorphanol produced similar effects in the rhinoceros (Wenger *et al*., 2007). Equally poor results have been noted in pigs (and wolves) and other drugs such as azaperone may be safer (Symoens and van der Brande, 1969; Symoens, 1970; Callear and van Gestel, 1973; Tobey and Ballard, 1985; Henrikson *et al*., 1995).

Fish

Fish meat is an increasingly important source of protein, and aquaculture is an increasingly important method of providing the fish (Brown, 1987; Liu and He, 1987; Shell, 1991; Sargent and Tacon, 1999; Naylor *et al*., 2000; Tidwell and Allan, 2001; Pauly *et al*., 2002). Medicines are used systematically in aquaculture. These generally take the form of antibiotics for bacterial infections, antifungal drugs, some of them unauthorised, and ectoparasitic drugs to combat parasitic infections, particularly sea lice in farmed salmon (Alderman and Clifton-Hadley, 1988; Brown, 1989; Inglis *et al*., 1993; Roth *et al*., 1993; Burka *et al*., 1997; Fernandes *et al*., 2000; Papoutsoglou, 2000; Costello *et al*., 2001; Ramstad *et al*., 2002; Revie *et al*., 2002; Ritchie *et al*., 2002; Lillehaug *et al*., 2003; Campbell *et al*., 2006a, b; Gustafson *et al*., 2006). The products used may be either liquid formulations or those integrated into fish feed.

One of the major economic and animal welfare problems associated with salmon farming is sea lice (Thomassen, 1993). These are ectoparasitic copepods including *Lepeophtheirus salmonis* and *Caligus elongatus* which feed on the skin of Atlantic salmon, *Salmo salar* (Wootten *et al*., 1982; Bron *et al*., 1991; Johnson and Albright, 1991; Pike and Wadsworth, 1999). They cause economic and welfare problems in farmed fish (Richards, 1983; Roth *et al*., 1993, 1996; Brocklebank, 1995; Stone *et al*., 1999 Bowers *et al*., 2000). In New Brunswick, loss to sea lice affecting aquaculture was of the order of $20 million in 1995 (MacKinnon, 1997). Several products containing a number of compounds have been used to treat this condition in the UK, including azamethiphos, dichlorvos, hydrogen peroxide, emamectin benzoate, cypermethrin, teflubenzuron and diflubenzuron (Roth *et al*., 1993; Thomassen, 1993; Burka *et al*., 1997; Bishop, 1998; Roth, 2000; Anonymous, 2006).

Signs of organophosphorus toxicity have been reported in salmon following dichlorvos poisoning or treatment with trichlorfon which is converted to dichlorvos in water; this may also lead to excess residues of the drug in salmon tissues (Horsberg and Høy, 1989; Horsberg *et al*., 1989, 1990). Hydrogen peroxide, also used for the treatment of ectoparasitic disease in fish, may cause gill damage (Clayton and Summerfelt, 1996; Arndt and Wagner, 1997; Kiemer and Black, 1997; Rach *et al*., 1997; Tort *et al*., 2002a, b).

Ivermectin, which has been used experimentally (and sometimes illegally) to treat sea lice in salmon and other species (Sutherland, 1990; Roth *et al*., 1993; Palmer *et al*., 1997) has also caused gill damage after oral and intraperitoneal administration to sea bass (Athanassopoulou *et al*., 2002). In experimental studies, levamisole proved toxic to Atlantic salmon smolts (Munday and

Zilberg, 2003). Teflubenzuron did not result in adverse drug reactions when tested in clinical trials in salmon (Ritchie *et al*., 2002; Campbell *et al*., 2006c). Lack of efficacy with several chemotherapeutic agents, probably due to drug resistance in sea lice, has been reported (Jones *et al*., 1992; Treasurer *et al*., 2000).

Off-label use

Many drugs are either used infrequently or are used off-label, usually under the cascade, and consequently they are involved in few adverse reaction reports. For example, with the exception of dogs, cyclosporin is not widely used in veterinary medicine and the numbers of adverse events associated with it are small. Nevertheless, there are a number of cases where adverse reactions have been reported and these involve anorexia, alopecia, vomiting and nausea and hirsutism in dogs, and seizures, diarrhoea, anorexia, vomiting and constipation in cats (Ryffel, 1982; Rosenkrantz *et al*., 1989; Seibel *et al*., 1989; Kyles *et al*., 1999; Robson, 2003).

There are few, if any, antineoplastic drugs authorised for use in veterinary medicine, and animals with tumours tend to be treated with products authorised for the treatment of human neoplastic diseases including cyclophosphamide, lomustine (CCNU), vincristine, doxorubicin, epirubicin, etopside, melphalan, L-asparaginase, cytosine arabinoside, cisplatin, carboplatin, ifosfamide, actinomycin D and gemcitabine (a radiosensitising agent) (Dernell *et al*., 1998; Fox; 2000; Moore and Kitchell, 2003; Fujino *et al*., 2004; Alvarez *et al*., 2006; Cave *et al*., 2007; Kim *et al*., 2007; Lana *et al*., 2007; Saba *et al*., 2007; Sauerbrey *et al*., 2007; Seo *et al*., 2007; Skorupski *et al*., 2007; Wilson *et al*., 2007). Hence, regulatory agencies are unlikely to receive many, if any, adverse reaction reports.

Nevertheless, there are published reports of adverse effects associated with the use of cyclophosphamide, melphalan, lomustine, 5-fluorouracil, gemcitabine and other antineoplastic drugs in companion animals (Gralla, 1975; Ndiritu and Enos, 1977; Page *et al*., 1988; Fan *et al*., 2002; Charney *et al*., 2003; LeBlanc *et al*., 2004; Thamm and Vail, 2007). Studies in healthy dogs suggest that depression of haematopoiesis may be a major concern with cyclophosphamide (Jalil and Pandey, 1987), while hepatotoxicity has been reported with lomustine chemotherapy (Kristal *et al*., 2004).

Discussion

Many of the adverse effects noted in treated animals can be directly associated with the toxicological and pharmacological properties of the drug. Thus the gastrointestinal effects of the non-steroidal anti-inflammatory drugs in dogs, the effects of pyrethroids in cats, and the toxicity seen with certain organophosphorus-based medicines in fish arise from the toxicological properties of the compounds concerned and so, to a large extent, they are predictable.

Hypersensitivity reactions and anaphylaxis are idiosyncratic in nature. In animals, they occur with some groups of compounds known to induce these effects in humans. These include certain antimicrobial drugs, notably the tetracyclines in dogs and the β-lactam antibiotics in horses, and many of the cutaneous effects noted in a range of species.

Not surprisingly, off-label uses, in species that have not been the subject of rigorous pharmaceutical testing with the drug administered, have resulted in adverse reactions in animals.

Together with the evidence from spontaneous reporting schemes, the available evidence suggests that the frequencies of adverse reactions to veterinary medicines are relatively low, although under-reporting, as with adverse reactions to human medicines, must be acknowledged. However, the magnitude of any under-reporting is difficult to quantify. The adverse reactions that do occur must be seen against the benefits of drug treatment, and the consequences of not treating sick animals or animals at significant risk of becoming sick if prophylactic treatments are

withheld. Better target animal safety studies and a greater understanding of the genomics of domestic animals may lead to enhanced design of veterinary medicinal products, and hence may result ultimately in fewer adverse reactions or at least may give more predictability of the likelihood of adverse events (Carakostas and Colaianne, 1996; Witkamp, 2005).

The value of information available in the literature would often be enhanced if it could be seen in conjunction with data generated by spontaneous reporting schemes. Unfortunately, much of the latter is published in anonymised form where not only have the details of the manufacturer been obscured, but so too have the details of the pharmacologically active substance (Woodward, 2005). A higher degree of transparency in these reports would facilitate a broader understanding of the nature of adverse reactions in the veterinary context.

Pharmacoepidemiological studies are frequently expensive to conduct, thus limiting their utility in the investigation of adverse effects in veterinary medicine. Nevertheless, where these have been conducted, the results have provided valuable insights into the nature and biological characteristics of the drugs of interest and their future employment is to be encouraged. However, it should also be recognised that lack of efficacy, with increased (or at least non-reduced) morbidity, is a major adverse effect associated with the use of veterinary pharmaceutical products (Dyer *et al*., 2004–2007).

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18 Adverse drug reactions in dogs – toxic hepatic responses

K.N. Woodward

Introduction

As discussed elsewhere in this work, and in other citations, the application of the principles of pharmacovigilance to veterinary medicinal products is an issue of growing scientific and regulatory importance (Boothe, 2001; Keck and Ibrahim, 2001; Woodward, 2005a, b). Moreover the liver is both the major anatomical site for drug metabolism and it is often the major target organ for hepatotoxicity in experimental studies and as a result of clinical drug use in both animals and humans (Wallace Hayes *et al*., 1982; Timbrell, 1983; Neumann *et al*., 1992; Perry, 1992; Steinberg and Oesch, 1992; Døssing and Sonne, 1993; Zimmermann and Lewis, 1995; Farrell, 1997; Routledge, 1999; Shah, 1999; Grover *et al*., 2000; Larrey, 2000; Lee, 2003; Larrey and Pageaux, 2005; Maddrey, 2005; Peters, 2005; Navarro and Senior, 2006; Hunt *et al*., 2007; Halegoua-De Marzio and Navarro, 2008; Matsuda *et al*., 2008; Meropol *et al*., 2008). As a consequence, iatrogenic hepatic disease in animals treated with veterinary medicinal products is both a distinct possibility and a focus for drug safety.

The liver is a large organ in most mammalian species and it has a number of functions. In fact, it has both exocrine and endocrine roles. The exocrine function is to secrete bile and thus to supply bile acids and salts for emulsification of fats in the intestines. Its endocrine function is to secrete most of the critical plasma proteins with the exceptions of the immunoglobulins. The liver also carries out a number of other critical functions including the regulation of lipid metabolism, the recycling of plasma proteins, and, through the mediation of Kupffer cells (sinusoidal macrophages), the recycling of unwanted red blood cells (Hinton and Grasso, 2000). However, one of its major roles is in the metabolism of xenobiotic compounds through a number of different pathways which have presumably evolved phylogenetically in response to the necessity to detoxify both exogenous foreign compounds as well as endogenous substances derived from catabolism. The major family of foreign compound metabolising enzymes, the microsomal cytochrome P450 group, can be traced back to ancient prokaryotic lineages which predate vertebrates including mammals and, most notably, predate the use of man-made drugs (Lewis, 2001). It seems likely, that the major purposes of these and many other enzymes were the detoxification of noxious substances found in or associated

with food of plant origin, although molecular oxygen may have been the first toxic substance targeted.

Cytochrome P450 enzymes catalyse a number of reactions involving metabolism of foreign compounds including acetylation, hydroxylation, dealkylation, dehalogenation, oxidation and epoxidation. In fact many isoforms of cytochrome P450, each with a preferred substrate, are involved in these reactions. The cytochrome P450 enzymes constitute the major group of biological catalysts involved in what is known as Phase 1 metabolism – the chemical modification of generally hydrophobic substances. Many products of Phase 1 metabolism are then subjected to Phase 2 reactions where they are conjugated by way of other enzyme systems to yield glucuronides, sulphates, amino acid derivatives or glutathione conjugates, and are then subjected to urinary excretion.

The system is designed to be protective to the organism in question by facilitating the deactivation and excretion of potentially toxic substances. Unfortunately, it can 'go wrong' – be inactivated or even be circumvented, leading to organ toxicity and, notably, to hepatotoxicity and other conditions and may ultimately result in fulminant hepatic failure (Kraus and Costa, 1963; Heneghan and Lara, 2003; Vaquero and Blei, 2003; Villeneuve and Pichette, 2004; Dorne, 2007; Ma and Lu, 2007; Masubuchi and Horie, 2007; Johnson, 2008). Indeed, rather than deactivation, some materials can be activated to toxic metabolites. As this occurs primarily in the liver, then the liver, including the canine liver, is uniquely susceptible to their adverse effects (Malone, 1969; Prescott, 1983; Timbrell, 1983; Fallon and Boyer, 1990; Badr, 1991; Britton, 1996; Sturgill and Lambert, 1997; Bradham *et al*., 1998; Amacher *et al*., 2001; Barbare *et al*., 2001; Luster, *et al*., 2001; Amacher, 2002; Brown and Desmond, 2002; Goodman, 2002; Jaeschke *et al*., 2002; Parra and Reddy, 2003; Park *et al*., 2005; Aithal, 2007; Wijnen *et al*., 2007; Ozer *et al*., 2008; Zuin *et al*., 2008). Hepatotoxicity is also modulated, at least in humans, by a number of other factors including age, obesity, gender and the use of recreational drugs (Larrey, 2000; Hines, 2007).

The liver is made up of a number of cell types any one of which may be affected by toxic substances or materials converted to toxic moieties in vivo. The major functional cell of the liver is the hepatocyte. This carries out the majority of the critical hepatic functions including xenobiotic metabolism, bile formation, protein synthesis and nutrient homeostasis. The liver has a huge functional reserve and where damage does occur, it can be repaired if the repair process is not compromised. Clearly, hepatocyte damage has the potential to lead to several areas of dysfunction. There are two major types of hepatocyte damage – fatty liver (steatosis) and death, caused by foreign compounds including many drugs. The former is frequently reversible, while the latter, necrosis, is irreversible, although these phenomena may be dose-related, with the former occurring at low doses and the latter at higher doses, as is the case with carbon tetrachloride. Hepatic necrosis may be focal, zonal, i.e. centrilobular (the terminal hepatic vein area), periportal or panacinar (across the hepatic lobule). Hepatocellular necrosis may be accompanied by apoptosis, although the role of this in xenobiotic-induced necrosis is disputed. It probably does occur with obstructive cholestasis, although the predominant phenomenon is necrosis (Jaeschke *et al*., 2004).

Other types of hepatic damage, sometimes involving other hepatic cell types, are known, and these and agents known to have caused these in laboratory species and/or humans are shown in *Table 18.1*.

In addition, the Kupffer cells may undergo toxic damage, especially after uptake of toxic or radioactive particulate matter or in response to endotoxin, while the sinusoidal endothelial cells are susceptible to direct toxic action of substances that also affect hepatocytes (Moslen, 1996; Hinton and Grasso, 2000). However, there is evidence to suggest that some toxic materials may specifically target these cells. These include allyl formate, urethane and alloxan (Zerbe and Gresner, 1988).

Table 18.1 Foreign compounds and their involvement in hepatic damage in laboratory species and/or humans (Rodriguez Olleros *et al*., 1969; Timbrell, 1983; Zimmerman, 1990; Vahlquist, 1992; Døssing and Sonne, 1993; Kowalski *et al*., 1994; Lahoti and Lee, 1995; Reddy and Schiff, 1995; Zimmerman and Lewis, 1995; George and Crawford, 1996; Selim and Kaplowitz, 1999; Shah, 1999; Grover *et al*., 2000; Shahidi, 2001; Brown and Desmond, 2002; Thiim and Friedman, 2003; Thole *et al*., 2004; Chitturi and Farrell, 2007; Constantinou *et al*., 2007; see also references in the text).

Effect	*Non-pharmaceutical*	*Pharmaceutical*
Necrosis	Dimethylformamide, ethanol	Isoniazid, hydralazine, halothane, paracetamol (acetaminophen), cyclophosphamide
Fatty liver	Carbon tetrachloride, ethanol	Valproic acid, amiodarone, corticosteroids, methotrexate
Cirrhosis	Vitamin A, ethanol, thioacetamide	Synthetic retinoids, androgens
Tumours	Aflatoxins, vinyl chloride, benzidine, *o*-anisidine	Androgens
Cholestasis	Ethanol, 1,1-dichloroethylene	Chlorpromazine, cyclosporine A, oestrogens, griseofulvin, piroxicam

Some xenobiotics, and notably 4, 4′-methylene dianiline, are toxic to the bile duct lining cells and may cause necrosis followed by bile duct proliferation. These include tilidine fumarate, dibutyltin and oxamniquine, largely in rats and mice although sporidesmin has caused these effects in sheep, and dogs are affected by arsenic (III) or (IV) compounds.

Hepatotoxicity is accompanied by changes in serum enzymes and other biomarkers due to hepatic leakage and following the induction of cholestasis in the majority of species studied, including humans and dogs (Tapia *et al*., 1973; Leonard *et al*., 1984; Sutherland, 1989; Zimmerman and Lewis, 1995; Kedderis, 1996; Moslen, 1996; Farrell, 1997; Hoffman *et al*., 1999; Routledge, 1999; Shah, 1999; Solter and Hoffman, 1999; Hinton and Grasso, 2000; Amacher, 2001, 2002; O'Brien *et al*., 2002; Wiedmeyer *et al*., 2002; James *et al*., 2003; Gaskill *et al*., 2005; Navarro and Senior, 2006; Bischoff and Ramaiah, 2007; Chitturi and Farrell, 2007; Ramaiah, 2007; Ozer *et al*., 2008).

Damaged areas of liver are replaced by regeneration of the liver parenchyma. However, wherever there is repeated toxic insult, fibrotic changes are likely to occur, possibly as a result of the synthesis of collagen by Ito (fat storing) cells following stimulation by factors released from Kupffer cells (Hopwood and Nyfors, 1976; Ballardini *et al*., 1983; French *et al*., 1988; Davis *et al*., 1990; Loreal *et al*., 1993; Moslen, 1996; Bissell, 1998; Hinton and Grasso, 2000). This fibrosis, and an increase in reticulin fibres, probably occurs in the space of Disse, the space between the hepatocyte and the fenestrated endothelium which permits intercellular exchange. With continuing periods of hepatic necrosis the fibrotic cycle continues until there is a progressive loss of hepatic structure, normal architecture and function as the liver becomes organised into areas of normal hepatocytes separated by areas of connective tissue or cirrhosis. In humans, treatment with methotrexate is sometimes associated with an increased risk of fibrosis, possibly due to its effects on Ito cells (Hopwood and Nyfors, 1976; Farrell, 1997).

Adverse hepatic drug reactions in dogs

A number of drugs have been reported to cause adverse hepatic effects in dogs. These may be observed with drugs used therapeutically in dogs, in canine experimental studies or in dogs

that have inadvertently consumed medications intended for human use.

Non-steroidal anti-inflammatory drugs (NSAIDs)

Paracetamol (acetaminophen)

Paracetamol is not a classical anti-inflammatory drug. In fact it is an analgesic and antipyretic drug with only weak anti-inflammatory activity, but it is included here for convenience. From a veterinary pharmacovigilance perspective, the majority of true NSAIDs are perhaps best known for their adverse effects on the gastrointestinal tract, especially in dogs (Stewart *et al*., 1980; Jones *et al*., 1992; Poortinga and Hungerford, 1998; Neiger, 2003; Woodward, 2005b). However, paracetamol has no effects on the gastric mucosa, but it is hepatotoxic at doses in excess of the recommended therapeutic dose in a number of species, including rodents and humans. In fact human hepatotoxicity of paracetamol is well documented and the drug is the leading cause of drug-induced hepatic failure in humans (Black, 1980; Prescott, 1980; Mulcahy and Hegarty, 1993; Tolman, 1998; McClain *et al*., 1999; Bolesta and Haber, 2002; Rumack, 2002; Bromer and Black, 2003; James et al., 2003; Hinson *et al*., 2004; Larrey and Pageaux, 2005).

In normal human individuals, at therapeutic doses, the drug is inactivated by sulphation and glucuronidation, with only small amounts being activated through the cytochrome P-450 pathway. At toxic doses this activation to a toxic metabolite, N-acetyl-*p*-benzoquinine imine, leads to depletion of glutathione, the formation of active nitrogen species and covalent binding to hepatic proteins including mitochondrial proteins resulting in mitochondrial dysfunction, disruption of calcium homeostasis and even DNA damage with subsequent apoptosis and ultimately hepatocellular necrosis (Cousins *et al*., 1989; Bursch *et al*., 1992; Thomsen *et al*., 1995; Kedderis, 1996; Manautou *et al*., 1996; Jaeschke *et al*., 2002).

In fact the severe depletion of glutathione prevents subsequent conjugation and excretion of the imine (Corcoran *et al*., 1980; Savides and Oehme, 1983; Nelson, 1990; Holtzman, 1995; Kretzschmar, 1996; McClain *et al*., 1999; Hinson *et al*., 2004; Jaeschke and Bajt, 2006). The toxicity of paracetamol is potentiated by substances that activate cytochrome P450 or deplete glutathione, including barbiturates and ethanol (Neumann *et al*., 1992; Kretzschmar, 1996; Makin and Williams, 1997; McClain *et al*., 1999; Prescott, 2000; Riordan and Williams, 2002; Suchin *et al*., 2005).

Paracetamol is hepatotoxic in the dog and the dog has been suggested as a model of human fulminant hepatic failure (Ortega *et al*., 1985; Francavilla *et al*., 1989; Kelly *et al*., 1992). In one of these studies, doses of 250 mg/kg bodyweight as a 90-minute infusion produced focal to massive hepatocellular necrosis depending on the time of sacrifice after infusion (Ortega *et al*., 1985). In the other, doses of 500 mg/kg bodyweight paracetamol given to dogs pre-treated with buthionine sulfoximine to deplete glutathione produced massive hepatocellular necrosis of the centrilobular or midzonal areas (Kelly *et al*., 1992). Fractionated doses of paracetamol, to a total dose of 1,150 mg/kg body weight consistently produced hepatic failure in dogs (Francavilla *et al*., 1989). These models, while demonstrating the toxicity of paracetamol in the dog, and serving as models of human paracetamol overdosage, have limitations in illustrating more general aspects of fulminant hepatic failure, e.g. of viral aetiology, and other substances show more promise, for example galactosamine in the dog (Diaz-Buxo *et al*., 1997; Patzer *et al*., 2002), largely because of slightly better reproducibility (death from liver failure) and freedom from complications associated with paracetamol-induced methaemoglobinaemia (Newsome *et al*., 2000; Filipponi and Mosca, 2001).

Perhaps not surprisingly, cases of inadvertent toxicity resulting from paracetamol ingestion have been reported in dogs (Jones *et al*., 1992). The normal therapeutic oral dose of paracetamol in dogs is approximately15 mg/kg bodyweight three times daily. However, dogs can tolerate up to 45 mg/kg bodyweight per day orally without apparent adverse effects (Villar *et al*., 1998;

Campbell and Chapman, 2000), although the use of paracetamol in companion animals is probably infrequent (Watson *et al*., 1996) possibly due to the availability of more effective and safer alternatives. Higher doses lead to hepatotoxicity, methaemoglobinaemia and, eventually, encephalopathy associated with liver failure. Cardiovascular failure may also occur (Villar *et al*., 1998).

Interestingly, although paracetamol is frequently cited as a cause of hepatic damage, including fulminant hepatic failure in dogs, there are few actual reports of toxicity in dogs. This probably reflects its low usage and those cases that are reported tend to involve consumption rather than treatment, for example, a Dalmatian that consumed an unknown quantity of 500 mg paracetamol tablets. This animal failed to develop liver damage, possibly because of supportive therapy including administration of the antidote N-acetylcysteine, although the actual dose may have been low (500 mg paracetamol equates to only 20 mg/kg body weight for this 25-kg dog and so several tablets may not have amounted to a hepatotoxic dose). However, methaemoglobinaemia and haemoglobinuria did develop (MacNaughton, 2003). In fact, methaemoglobinaemia and other haematological abnormalities have been reported by others following paracetamol administration (Savides *et al*., 1984; Harvey *et al*., 1986; Schlesinger, 1995), while mildly impaired renal function has been noted in another report (Colletti *et al*., 1999).

The apparent discrepancy between hepatotoxic and even lethal doses of paracetamol in dogs and humans is probably at least partially explained by the magnitude of the dose required for extensive hepatotoxicity in dogs (300–1000 mg/kg body weight, although doses of 300–500 mg/kg have been ingested without effect) (Campbell and Chapman, 2000) compared with the large intentional doses often taken by humans in suicide attempts. Differences in toxicity in individual dogs to paracetamol may arise for a number of reasons, but glutathione *S*-transferase, responsible for catalysing conjugation reactions, is low in some dogs and this could increase the toxicity of some hepatotoxic substances including paracetamol (Watanabe *et al*., 2004).

Interestingly, cats are more susceptible than dogs to the toxic effects of paracetamol due to their lower capacity to form glucuronides and the saturation of the sulphate conjugation pathway. However, hepatotoxicity, although it can occur, may not always be a major feature of paracetamol-induced toxicity in the cat, whereas methaemoglobinaemia is relatively common (Leyland, 1974; Leyland and O'Meara, 1974; Steele, 1974; Finco *et al*., 1975; Black, 1980; Christiansen, 1980; St. Omer and McKnight, 1980; Davis, 1985; Judson, 1985; Lugten, 1985; Savides *et al*., 1985; Walker, 1985; Hjelle and Grauer, 1986; Ilkiw and Ratcliffe, 1987; Malley, 1987; Villar *et al*., 1998; Campbell and Chapman, 2000; Allen, 2003). It is advisable not to administer paracetamol to cats (Prasuhn, 1983).

Other NSAIDs

Adverse hepatic reactions to other NSAIDs in dogs are relatively rare. In one review, although ibuprofen, aspirin and indomethacin were often associated with ulcerogenic effects, there were no reports of hepatic damage with these drugs (Jones *et al*., 1992). Hepatotoxicity has been observed in dogs treated with the drug carprofen (Moreau *et al*., 2003; Nakagawa *et al*., 2005). In one report, 21 dogs with clinical signs of hepatotoxicity following carprofen treatment were studied. Of these, eight had received carprofen doses in excess of that recommended. Biopsy specimens revealed multifocal to extensive hepatocellular necrosis, with some secondary inflammation and cholestasis (MacPhail *et al*., 1998).

Analysis of pharmacovigilance data from the UK's veterinary regulatory agency (the Veterinary Medicines Directorate; VMD) suggests that hepatic injury arising from carprofen is rare. Only 1.9% of carprofen-related adverse reactions in dogs were related to the drug (Hannon *et al*., 2003). Hepatic damage in dogs related to NSAID usage has been reported to the Center for Veterinary Medicine in the US (Hampshire *et al*., 2004).

In humans, although rare, hepatic adverse reactions have been reported with a range of NSAIDs, including aspirin, indomethacin, diclofenac, ibufenac, sulindac, naproxen, piroxicam, nimesulide, phenylbutazone and benoxaprofen (Gallanosa and Spyker, 1985; Zimmerman, 1990; Simon, 1991; Biour *et al*., 1992, 2004; Rabinovitz and van Thiel, 1992; Døssing and Sonne, 1993; Scully *et al*., 1993; Boelsterli *et al*., 1995; Fry and Seeff, 1995; Zimmerman and Lewis, 1995; Farrell, 1997; Bjorkman, 1998; Tolman, 1998; Shah, 1999; Grover *et al*., 2000; Boelsterli, 2002; Chitturi and George, 2002; O'Connor *et al*., 2003; Teoh and Farrell, 2003; Rubenstein and Laine, 2004). These reactions are considered to be idiosyncratic (see later) in nature and determined by a number of individual predisposing factors. In humans, for example, idiosyncratic, or type B reactions may be due to underlying conditions, immune-related factors, genetic differences in drug metabolism and activation, and other mechanistic factors (Uetrecht, 2007; Ulrich, 2007). Similar considerations apply in both animal models and treated animal patients. This appears to be the case with carprofen (and probably other NSAIDs) in the dog, although the mechanism is unknown (MacPhail *et al*., 1998).

Anticonvulsant drugs

Phenytoin is a member of the hydantoin class of drugs that is widely used for the treatment of partial and tonic-clonic seizures in human epilepsies (McNamara, 1996; Walia *et al*., 2004). Along with chemically related drugs and other anticonvulsive agents, it is known to be hepatotoxic under some conditions in humans, causing hepatic cholestasis and necrosis (Parker and Shearer, 1979; Mullick and Ishak, 1980; Egerton-Vernon *et al*., 1983; Powell-Jackson *et al*., 1984; Aaron *et al*., 1985; Plumber *et al*., 1986; Dreifuss and Langer, 1987; Lisker-Melman and Hoofnagle, 1989; Stephens and Levy, 1992; Roy *et al*., 1993; Altuntas *et al*., 2003; Walia *et al*., 2004). Phenytoin and related substances such as primidone and phenobarbital may also potentiate the hepatotoxicity of other drugs including halothane, paracetamol and potentiated sulphonamides (Jenner *et al*., 1990; Brackett and Bloch, 2000; Ilario *et al*., 2000; Cook *et al*., 2006; Suchin *et al*., 2005). For some drugs at least, this potentiation of hepatotoxicity by phenytoin and related substances is probably due to induction of cytochrome P450 isozymes and subsequent activation to active metabolites in the liver (Brackett and Bloch, 2000).

A similar pattern of liver injury has been reported in dogs given phenytoin, primidone or phenobarbital for prolonged periods (up to 3 years) for the control of seizures (Nash *et al*., 1977; Bunch *et al*., 1982, 1984, 1987; Dayrell-Hart *et al*., 1991; Bunch, 1993). There were areas of hepatic necrosis, small foci of bile duct hyperplasia and intracanalicular bile casts and other features associated with hepatic cholestasis. In one study of three dogs with hepatotoxicity and cholestasis associated with phenytoin treatment, other drugs had been administered, but the adverse effects appear to have commenced prior to the treatment with these other drugs. Consequently, it is not possible to determine from this or other reports whether phenytoin potentiated the toxicity of other chemotherapeutics. However, its potential to do so should not be excluded.

Another drug used in the control of seizures in human medicine, valproic acid, is also hepatotoxic (Walia *et al*., 2004; Koenig *et al*., 2006; Gerstner *et al*., 2008), but the use of this drug in dogs is limited due to an inability both to achieve therapeutic concentrations and thus to control seizures (Boothe, 2001).

Halothane

Halothane is a halogenated hydrocarbon used as an inhalation anaesthetic in human and veterinary medicine. Until recently, it was an extremely popular anaesthetic, but its use has declined following the introduction of other gaseous anaesthetics including isoflurane and

enflurane as well as several injectable anaesthetic agents.

Halothane has been shown to be hepatotoxic in experimental dogs (Stephen *et al*., 1958). It was reported to have induced severe centrilobular hepatic necrosis in a 10-year-old Dachshund (Gaunt *et al*., 1984), but reports of liver damage in dogs with this agent are extremely rare. Hepatic necrosis with the related halogenated agent methoxyflurane has been reported in dogs (Ndiritu and Weigel, 1977; Thornburg *et al*., 1983).

In humans, halothane induces a mild hepatotoxicity probably by way of a metabolite(s) and through a direct mechanism and a major hepatotoxic reaction, probably mediated by an immune mechanism, which is frequently fatal (Takaki and Haber, 1970; Davies, 1973; Brown and Sipes, 1977; Brown, 1981; Neuberger and Kenna, 1987; Gelman and Van Dyke, 1988; Neuberger and Williams, 1988; Ray and Drummond, 1991; Bird and Williams, 1992; Elliott and Strunin, 1993; Gut *et al*., 1993; Holt *et al*., 1995; Kenna and Jones, 1995; Bourdi *et al*., 1996, 2001; Marshall and Longnecker, 1996; Mikatti and Healy, 1997; D'Arcy, 2000; Kharasch *et al*., 2000; Lee, 2003; Anders, 2005; Bjornsson *et al*., 2005).

However, there is inadequate information to comment on the mechanisms or types of toxicity in canine patients. Furthermore, if major immune-mediated hepatotoxicity does occur in the dog, then it is likely to be rarely reported if its frequency is similar to that occurring in human patients – 1 in 10,000 to 1 in 30,000 (Bird and Williams, 1989; Neuberger, 1990; Marshall and Longnecker, 1996). However, it is worth noting that halothane has been identified as a potential health risk for veterinarians and laboratory personnel working with animals (Woodward, 2005b). It is also worth noting that isoflurane and methoxyflurane have occasionally been reported as hepatotoxic in human patients, emphasising the need for caution in veterinary anaesthesia from both a human and animal perspective (Brown, 1989; Dyson, 1992; Sinha *et al*., 1996; Turner *et al*., 2000).

Antimicrobial substances

In humans, antimicrobial drugs are a major cause of drug-induced liver injury after paracetamol (Westphal *et al*., 1994; Norris *et al*., 2008) and it is interesting to see how they fare in the dog.

Sulphonamides

The sulphonamide drugs are among the oldest of the antibacterial agents and they are generally regarded as extremely safe. However, there have been a number of reports of liver toxicity in dogs given these drugs therapeutically, although the numbers involved are low. These hepatic reactions are idiosyncratic in nature. In fact sulphonamide drugs or more specifically potentiated sulphonamides produce a number of characteristic idiosyncratic effects in dogs including polyarthropathy, blood dyscrasias, skin eruptions and ocular effects in addition to the hepatotoxicity (Cribb and Spielberg, 1990; Trepanier, 2004). A number of other clinical effects have also been noted in dogs treated with sulphonamide drugs, including proteinuria, nephrotic syndrome and facial nerve palsy (Scott *et al*., 1976; Giger *et al*., 1985; Trepanier, 2004; Vasilopulos *et al*., 2005).

Approximately 50% of dogs affected by potentiated sulphonamide-induced toxicity die. Hepatic necrosis, acute cholestasis and jaundice are characteristic findings (Toth and Derwelis, 1980; Anderson *et al*., 1984; Rowland *et al*., 1992; Dodds, 1997; Trepanier, 2004). Duration of therapy prior to signs of toxicity varied from 4 to 30 days (Twedt *et al*., 1997), while rechallenge in surviving animals may re-elicit the hepatic reactions (Thornburg *et al*., 1983; Giger *et al*., 1985). The mechanism of toxicity is poorly understood and not all sulphonamide drugs elicit these responses. Sulfadiazine and sulphamethoxazole have been implicated in the induction of canine liver toxicity, but not all substances possessing a sulphonamide moiety are active in this respect (Giger *et al*., 1985; Cribb and Spielberg, 1990; Twedt *et al*., 1997; Trepanier, 2004).

Sulphonamides are known to induce idiosyncratic reactions in humans treated with these drugs, although the mechanism of action is again unclear. However, although there are some similarities in the clinical entities that may occur, sulphonamide toxicity in human tends to show as multi-organ failure. Nevertheless, adverse hepatic effects, which are sometimes fatal, have been observed following treatment with potentiated sulphonamides and with the sulphonamide drug sulfasalazine (Dujovne *et al*., 1967; Abi-Mansur *et al*., 1981; Ransohoff and Jacobs, 1981; Horak *et al*., 1984; Jennings *et al*., 1986; Ribe *et al*., 1986; Berg and Daniel, 1987; Cribb and Spielberg, 1990; Hautekeete, 1995; Noli *et al*., 1995; Cario *et al*., 1996; Cribb *et al*., 1996; Mandell and Petri, 1996a; Uhari *et al*., 1996; Rieder *et al*., 1997; Mahboob and Haroon, 1998; Ilario *et al*., 2000; Choquet-Kastylevsky *et al*., 2002; Mainra and Card, 2003; Thiim and Friedman, 2003; Zaman *et al*., 2003; Karpman and Kurzrock, 2004; Bjornsson *et al*., 2005).

Isoniazid

Isoniazid, isonicotinic acid hydrazide, is one of the major drugs used in the treatment and prophylaxis of tuberculosis in humans, usually in combination with other drugs such as rifampin or streptomycin (Salpeter, 1992; Mandell and Petri, 1996b; Stuart and Grayson, 1999; Saltini, 2006). It has also been used in the treatment of tuberculosis in some animals (Leask *et al*., 1964; Castagnino *et al*., 1973; Wolf *et al*., 1988; Langenegger *et al*., 1991; Boothe, 2001).

In humans, treatment with isoniazid may lead to the development of hepatotoxicity and in some cases fulminant hepatic failure (Garibaldi *et al*., 1972; Timbrell, 1979; Westphal *et al*., 1994; Vasudeva and Woods, 1997; Stuart and Grayson, 1999; Navarro and Senior, 2006; Preziosi, 2007; Kaneko *et al*., 2008; Tafazoli *et al*., 2008). This arises as a result of the toxicity of acetylhydrazine, a metabolite of isoniazid which undergoes metabolic activation by the hepatic cytochrome P450 isozyme system to generate a reactive intermediate. This binds covalently to hepatic macromolecules, resulting ultimately in centrilobular hepatic necrosis and a dose-dependent decline in hepatic cytochrome P450 (Mitchell *et al*., 1976; Nelson *et al*., 1976; Timbrell *et al*., 1977; Wright and Timbrell, 1978; Timbrell *et al*., 1980; Woodward and Timbrell, 1984). The toxicity has been modelled in rats, although in this species it only occurs following hepatic enzyme induction with phenobarbital (Bahri *et al*., 1981). Further acetylation of acetylhydrazine gives rise to the less hepatotoxic diacetylhydrazine (Timbrell, 2000). However, hydrazine itself may play a role in the hepatotoxicity of isoniazid (Tafazoli *et al*., 2008).

In dogs, 100% fatalities occurred in a group of experimental animals given 75 mg/kg bodyweight (BW) isoniazid, while 50 mg/kg BW produced fewer fatalities. Hepatotoxicity was noted in these experiments (Chin *et al*., 1978). The oral LD_{50} of isoniazid in the dog has been estimated to be around 50 mg/kg BW (Rubin and Burke, 1953) and dogs are therefore at risk from consumption of isoniazid tablets intended for human use, particularly from those containing higher quantities of the drug (300-mg tablets). Toxic doses of isoniazid produce a spectrum of effects in dogs that are extremely similar to those observed in humans exposed to high doses, including seizures, salivation, diarrhoea, vomiting, incoordination, metabolic acidosis and tachycardia or bradycardia as well as hepatotoxicity (Villar *et al*., 1995; Frank *et al*., 2002).

It has recently been suggested that combination therapy with drugs including isoniazid be used for the treatment of tuberculosis in dogs (and cats) (Boothe, 2001). Clearly, this will require a degree of caution and adequate dose determination. In humans, susceptibility to hepatotoxicity of normal therapeutic doses of the drug is influenced by acetylator phenotype, with slow acetylators being exposed to greater systemic amounts of acetylhydrazine because of a lower capacity to detoxify this by converting it to diacetylhydrazine (Peretti *et al*., 1987; Timbrell, 2000). Acetylator phenotype may also contribute to the toxicity of other drugs including sulphonamide hepatotoxicity (Larrey and Pageaux, 1997; Shah, 1999; Maddrey, 2005), but, without evi-

dence, this remains unclear. It is also unclear whether genetic polymorphism plays any role in the toxicity of isoniazid in the dog (or other animal species likely to be treated therapeutically with the drug), but dogs apparently have no or very little ability to N-acetylate xenobiotics in general (Poirier *et al*., 1963; Lakshmi *et al*., 1995; Dalvie *et al*., 1996; Whysner *et al*., 1996; Savidge *et al*., 1998; Yabuki *et al*., 2003) and isoniazid in particular (Sharer *et al*., 1995).

Other antimicrobial drugs

Tetracyclines, including chlortetracycline, tetracycline, oxytetracycline and minocycline, have a long history of safe use in veterinary medicine, including use in the dog. They have been used for a number of indications (Riviere and Spoo, 1996). In human medicine, hepatotoxicity has been reported, particularly after large doses, usually in excess of 2 g/day (Schultz *et al*., 1963; De Jonge, 1973; Westphal *et al*., 1994; Kapusnik-Uner *et al*., 1996). There have been no comparable reports in animals, including the dog. Hepatotoxicity is rare with erythromycin in humans (Braun, 1969) and has not been reported in animals. Similarly, human hepatotoxicity with cephalosporins is also rare (Thompson and Jacobs, 1993; Hautekeete, 1995).

The fluoroquinolone antimicrobial drugs are widely used in human and veterinary medicine and are recognised for their favourable safety profiles. There have been no reports of significant hepatotoxicity in humans, although elevations of hepatic enzymes and foci of centrilobular necrosis and steatosis have been reported in one patient treated with norfloxacin (López-Navidad *et al*., 1990; Hooper and Wolfson, 1993; Rubinstein, 2001). There have been no similar reports in animals despite extensive use of this class of drugs.

Discussion

It is important to emphasise that, from the data reviewed here at least, iatrogenic hepatotoxicity in dogs is relatively rare. This said, it must also be appreciated that there is considerable underreporting of adverse reactions in both human and veterinary medicine and this may contribute to the seemingly low incidence (Gray and Evans, 1988; Alvarez-Requejo *et al*., 1998; Moride *et al*., 1997; Edwards, 2001; Gray and Knivett, 2002; Van der Heijden *et al*., 2002; Gough, 2005; Thiessard *et al*., 2005; Woodward, 2005b; Hazell and Shakir, 2006). Furthermore, as many causes of death and morbidity may go without further biochemical or pathological investigation, cases of hepatotoxicity may be missed or unconfirmed. For some drugs, hepatotoxicity may be rare and infrequently reported and there are only isolated reports of toxicity with thiacetarsemide, diethylcarbamazine, primidone, mibolerone and ketoconazole (Bunch, 1993).

Several of the drugs known to have caused hepatotoxicity in humans have also resulted in the condition in dogs. Thus, when drugs developed for human use are extended into the veterinary sector, due attention should be paid to their ability to induce not only hepatotoxicity in humans, but also other forms of adverse effects. Moreover, when hepatotoxicity occurs in both humans and dogs, there is a probability that similar mechanisms may be involved, especially if the target cells are the same in each. In fact, where the toxicity has a classical basis, for example, as with paracetamol, rather than an idiosyncratic or immune basis, it is realistic to extrapolate between the species. Preclinical testing results, while not infallible, are also useful in predicting some forms of hepatotoxicity, although unfortunately not for idiosyncratic reactions (Kaplowitz, 2005; Peters, 2005). A number of drugs produce idiosyncratic and thus unpredictable reactions in humans (Oehme, 1977; Larrey and Pageaux, 1997; Kaplowitz, 2005; Stickel *et al*., 2005; Walgren *et al*., 2005).

With this information, future problems may be anticipated and possibly ameliorated. For example, there is a growing demand for alternative or complementary therapies for use in veterinary medicine and in some countries herbal preparations are becoming more commonly used.

For example, in the UK, veterinary medicinal products containing garlic, saw palmetto, valerian, celery, fenugreek, skullcap and other herbal preparations are authorised for use in companion animals (NOAH, 2007). Many herbal products are hepatotoxic in humans and animals including dogs (Larrey and Pageaux, 1995; Kaplowitz, 1997; Chitturi and Farrell, 2000; Stickel *et al*., 2000; Stedman, 2002; Barnes *et al*., 2002; Pak *et al*., 2004; Woodward, 2005c; Cooper and Webster, 2006; Furbee *et al*., 2006) and their use in veterinary medicine should be carefully monitored.

Drugs newly introduced into human medicine are sometimes subsequently found to be hepatotoxic, with recent examples being the antiarrhythmic drug amiodarone, some of the antiretroviral drugs and the antihyperglycaemic drug troglitazone. All of these drugs could conceivably be used in veterinary medicine, and especially in the treatment of conditions in companion animals, and all are hepatotoxic to some degree in humans (Flaharty *et al*., 1989; Lewis *et al*., 1990; Styrt and Freiman, 1995; Subramaniam, 1999; Brown, 2000; Kohlroser *et al*., 2000; Yamamoto *et al*., 2001; Kaneko *et al*., 2002; Boelsterli, 2003; Kontorinis and Dieterich, 2003; Tolman and Chandramouli, 2003; Hug *et al*., 2004; Abrescia *et al*., 2005; Nunez and Soriano, 2005; Watkins, 2005; Chitturi and Farrell, 2007; Fux *et al*., 2007; Jaeshchke, 2007; Chan *et al*., 2008).

In fact the latter drug is worth some consideration. Diabetes mellitus in dogs and cats is treated using similar approaches to those employed in human therapy, depending to some extent on whether the condition is insulin or non-insulin dependent (Hoenig, 1996; Boothe, 2001). Oral hypoglycaemic agents such as the sulphonylureas (glipizide) and the biguanides (metformin) have been used, along with other agents, to successfully manage the condition in animals and in humans. The thiazolidinedione drug troglitazone was introduced into human medicine in the US in 1997, with between 1 and 2 million patients being treated until the drug was withdrawn at the beginning of 2000; it has been estimated that there were at least 90 cases of liver failure (Gale, 2001; Scheen, 2001a; Isley, 2003; Smith, 2003). It has been claimed that troglitazone produced an idiosyncratic form of hepatotoxicity, but there is doubt over this and the drug may be regarded as a classical hepatotoxic material (Smith, 2003).

The episode has also raised the question as to whether the adverse effects noted with troglitazone are a class effect. In vitro studies with related compounds including rosiglitazone and pioglitazone, as well as clinical experience in humans, suggests not or if these are hepatotoxic, then not to the same degree as troglitazone (Tolman, 2000; Scheen, 2001b; Lloyd *et al*., 2002; Bae *et al*., 2003). Troglitazone also potentiates the hepatotoxicity of paracetamol, probably through the induction of cytochrome P450 3A (Kaneko *et al*., 2002).

All of these factors suggest that these drugs should be used with considerable care in veterinary treatments. Although troglitazone may no longer be available, and the other members of the group may be seemingly either less or not hepatotoxic, this may not necessarily hold true in veterinary use and caution should be exercised.

This is true too for older drugs. For example, many antineoplastic agents are used in companion animal oncology, and the vast majority, if not all, involve the off-label use of human medicines including the alkylating agents (e.g. cyclophosphamide and melphalan), the mitotic spindle inhibitors (e.g. vincristine and vinblastine), the antimetabolites (e.g. methotrexate and 5-fluorouracil) and the antitumour antibiotics (e.g. daunorubicin, doxorubicin and bleomycin) (Rogers and Coppoc, 1996, 2001; Barton, 2001). The majority of these substances have a long history of use in human cancer treatment, but many are relatively new to veterinary use. A number of them are hepatotoxic to humans, including some of the alkylating agents, the antitumour antibiotics, the antimetabolites and the spindle inhibitors (Hayes *et al*., 1977; Gralla *et al*., 1979; Kevat *et al*., 1988; Dorman *et al*., 1990; Perry, 1992; King and Perry, 1995, 2001; West, 1997; Ahern *et al*., 1998; Sachs *et al*., 2002; Floyd *et al*., 2006), while other antimetabolite drugs used in the treatment of other medical conditions, for example propylthiouracil used in the treatment

of hyperthyroidism, may also be hepatotoxic (Levy, 1993; Westphal, 1994).

To date there is little evidence to suggest that the majority of antineoplastic agents used in human medicine are hepatotoxic when used in veterinary medicine. Indeed, it is claimed that there is little evidence of adverse effects in dogs treated with cyclophosphamide (Goldberg and Lidsky, 1985; Stanton and Legendre, 1986; Miller, 1997), while azathioprine may occasionally be hepatotoxic (Beale, 1988). However, the nitrosourea alkylating agent lomustine (CCNU) has resulted in hepatotoxicity in dogs. Of 179 dogs with tumours, lomustine was given at doses in the range 50–110 mg/m^2 body surface area for varying treatment durations. After the treatments, 11 dogs developed hepatotoxicity, although the median number of doses and the median cumulative dose with lomustine were higher in dogs that developed hepatotoxicity (Kristal *et al*., 2004). Hepatotoxicity has also been reported in the dog after treatment with methotrexate (Pond and Morrow, 1982; Bunch, 1993). These reports emphasise the need for caution when using medicines intended for use in humans (or indeed in any other species), off-label in a second species, particularly when it is recognised that they *are* hepatotoxic in humans. Conversely, doxorubicin, at least at 10 mg/m^2 weekly, is well tolerated in dogs (Ogilvie *et al*., 1991; Simon *et al*., 2007).

Other older drugs with a long history of safe use in veterinary (and human) medicine have on occasions also shown evidence of canine hepatotoxicity. These include glucocorticoids, closantel, ketoconazole, imidocarb, mebendazole, oxibendazole, novobiocin, nitrofurazone and certain oestrogens (Yeary, 1975; Rogers and Ruebner, 1977; Polzin *et al*., 1981; Swanson and Breider, 1982; Van Cauteren *et al*., 1983, 1985; Abdullah *et al*., 1984; Kock and Kelly, 1991; Castellan *et al*., 1993; McEntee *et al*., 1995; Boothe, 2001).

Any conclusions on the relationship between an episode of hepatotoxicity in the dog or other animal and an administered drug must also take into account other possible chemical aetiological agents. Hepatotoxic substances found in or around domestic and industrial premises to which dogs may be exposed include carbon tetrachloride and other haloalkanes, hexachlorophene, some pesticides and other miscellaneous chemicals, including the sugar alcohol xylitol which has resulted in fatalities (Rechnagel and Glende, 1973; Maddy and Winter, 1980; Plaa, 1988; Bruckner *et al*., 1989; Moore *et al*., 1989; Poppenga *et al*., 1990; Williams and Burk, 1990; den Besten *et al*., 1994; Murphy, 1994a; Vörös *et al*., 1997; Papaioannou *et al*., 1998; Weber *et al*., 2003; Dunmayer, 2004; Anonymous, 2006; Dunmayer and Gwaltney-Brant, 2006).

Dogs and other animals are also susceptible to the hepatotoxic effects of many plants and fungi (Vogel *et al*., 1984; Murphy, 1994b; Lorgue *et al*., 1996; Hollinger and Ekperigin, 1999; Oehme and Rumbeiha, 2000; Beltman, 2005; Sharma *et al*., 2007). In one incident in Australia, dogs died as a result of hepatotoxicity after being fed horse meat from animals contaminated with indospicine, a toxic amino acid derived from a leguminous plant, *Indigofera linnaei* (Hegarty *et al*., 1988).

Drugs of abuse are frequently found around domestic and other premises and some of these are hepatotoxic in humans. These include cocaine and ecstasy and they should be treated as potentially hepatotoxic to exposed animals including dogs (Shuster *et al*., 1988; van Thiel and Perper, 1992; Jones and Simpson, 1999; Selim and Kaplowitz, 1999; Garbino *et al*., 2001).

Similarly, some dog breeds are seemingly susceptible to metabolic disease predisposing to copper toxicosis. These include Doberman Pinschers (Johnson *et al*., 1982; Crawford *et al*., 1985; van den Ingh *et al*., 1988; Röcken *et al*., 1991; Speeti *et al*., 1998; Mandigers *et al*., 2004 a, b, 2005), Dalmatians (Grenn and Bulmer, 1972; Nalley, 1996; Napier, 1996; Cooper *et al*., 1997; Noaker *et al*., 1999; Webb *et al*., 2002), West Highland terriers (Thornburg and Crawford, 1986; Thornburg *et al*., 1986; Thornburg *et al*., 1996), Bedlington terriers (Twedt *et al*., 1979; Eriksson, 1983; Robertson *et al*., 1983; Colvin *et al*., 1984; Hyun and Filippich, 2004) and Skye terriers (Haywood *et al*., 1988; Elsinghorst, 2004). There

has also been a report of a suspected case in a Kerry Blue terrier (Thornburg *et al*., 1981), and other breeds are also affected (Savage, 1987; Rolfe and Twedt, 1995; Twedt, 1998; Hoskins; 2005). The condition is likely to involve a genetic component; in Bedlington terriers, for example, there is an autosomal defect in copper metabolism (Johnson *et al*., 1980; Su *et al*., 1982). Depending on the breed, the effects include apoptosis, hepatic necrosis, subacute and chronic hepatitis, and cirrhosis (Hardy, 1985; van den Ingh and Rothuizen, 1994; Rolfe and Twedt, 1995; Fuentealba *et al*., 1997; Thornburg, 2000; Sterczer *et al*., 2001), but there are differences, although all probably result from oxidative stress (Rolfe and Twedt, 1995; Thornburg, 2000; Spee *et al*., 2006).

There are a number of similarities with these metabolic conditions in dogs and their counterparts in humans. This is particularly so with the entity in Bedlington terriers and Wilson's disease, although recent evidence suggests a different genetic background (Fuentealba and Aburto, 2003; Wu *et al*., 2006). Wilson's disease is a metabolic disease in humans with an autosomal recessive manner of inheritance characterised by copper deposition and accumulation. It has a number of clinical manifestations including hepatic damage (Müller *et al*., 1998; Fuentealba and Aburto, 2003; Tao and Gitlin, 2003; Schilsky and Fink, 2006; Das and Ray, 2006; Merle *et al*., 2007).

Finally, infectious diseases may result in liver conditions whose pathology mimics the effects of drug-induced toxicity, such as hepatitis, necrosis, fibrosis, hepatocyte vacuolation, proliferation of Kupffer cells, bile duct proliferation and cirrhosis. Diseases such as infectious canine hepatitis, leishmaniosis, *Helicobacter canis*, *Dirofilaria immitis*, clostridial and leptospiral infections, and infection with canine adenovirus 1 and canine acidophil cell hepatitis are associated with hepatic changes which can mimic some of the changes seen with hepatotoxic drugs and which may result in chronic hepatitis in some species, including humans (Wright, 1967; Gocke *et al*., 1970; Tams, 1984; Jarret and O'Neil, 1985; Eaton and Rosol, 1989; Bornand-Jaunin *et al*., 1993; Dill-Macky, 1995; Fox *et al*., 1996; Adamus *et al*., 1997; Ishak, 2000; Lucena *et al*., 2001; Boomkens *et al*., 2004; Hendrix, 2004; Watson, 2004; Hoskins, 2005; Rallis *et al*., 2005). It is also important to realise that in laboratory animals at least, including the beagle, spontaneous lesions arise, and this can include focal necrosis and other pathologies (Foster, 2005) and hepatic diseases are common in older dogs (Hoskins, 2005).

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19 Adverse reactions to vaccines

K.N. Woodward and L.A. Toon

Introduction

Vaccines and other biological agents intended for veterinary use are highly regulated products usually used for the prevention of diseases. In the European Union (EU) they are regulated as veterinary medicinal products and consequently are controlled under veterinary medicines legislation whereas in other countries, and notably the United States, they are regulated separately from pharmaceuticals and by a separate regulatory authority (Woodward, 2000, 2005a, b). Regardless of these considerations, veterinary vaccines and other biologics are reviewed and assessed prior to approval on the basis of their safety, quality and efficacy in exactly the same way as are veterinary pharmaceuticals (Brinley Morgan, 1983; Lee, 1989; Espeseth and Greenberg, 1993; Draayer *et al*., 1997; Espeseth, 1997; Goodrich, 1997; Woodward, 2000; Cowan, 2002; Rutter, 2003; Woodward, 2005a, b).

In the EU the regulatory pathways open to applications for both human and veterinary vaccines are identical to those for pharmaceuticals and the philosophy of testing requirements similar for manufacturing control and quality, efficacy and safety (Cartwright, 1991; Woodward, 1991, 1996, 1997a, 2000, 2005a; Lee, 1993; van Oirschot, 1994; Jefferys, 1995; Hendriksen, 1996; Webster, 1996; Brunko, 1997; Makie, 1998; McVey *et al*., 2003; Roberts and Lucken, 1996; Verschueren and Brown, 1997; Grein *et al*., 2007; Jones *et al*., 2007; De Clercq *et al*., 2008; Heldens *et al*., 2008).

The scientific quality and efficacy testing components of an application for a licence, marketing authorisation or approval for a veterinary vaccine are similar to those for veterinary pharmaceuticals. Although they may (and do) differ in their nature and detail they include aspects of manufacture and control, stability and clinical trials. In the EU, non-biological components intended for use in food-producing animals must also be included in one of the Annexes of Regulation (EEC) 2377/90, the MRL Regulation, or be shown not to have pharmacological activity and be exempt from the scope of the Regulation (Woodward, 1993, 1997b, 2000, 2004). In reality, the majority of chemical components of veterinary vaccines including solvents, adjuvants, stabilisers, antioxidants, preservatives and colorants are contained in Annex II of the Regulation (*Table 19.1*).

However, whereas pharmaceutical product active components are subject to extensive toxicity testing, this is rarely appropriate, with the exception of some excipients as discussed above, for vaccines and their constituents, which tend to be complex derivatives of cellular components,

Table 19.1 Components or potential components of vaccines in Annex II of Regulation (EEC) No. 2377/90.

Alkali metal phosphates and diphosphates	Lactic acid and lactates
Aluminium hydroxide and aluminium salts	Lecithin
Amino acids	Montanide
Ammonium chloride	Nitrogenous bases
n-Butanol	Orgotein
Calcium hydroxide and calcium salts	Orotic acid
Benzoic acid and benzoates	Permitted colours
Benzyl alcohol	Poloxamers
Betaine	Poloxalene
Butylated hydroxyanisole	Polyethylene glycols
Butylated hydroxytoluene	Polyoxyethylene derivatives
Carnitine	Polyoxyl castor oils
Cetrimide	Polysorbates
Chlorocresol	Propylene glycols
Dimethyl sulphoxide	Salts of mono-/diglycerides
Ethanol	Sorbitol, mannitol, xylitol and derivatives
Ethyl lactate	Sorbic, lactic, adipic, malic and citric acids
Folic acid	Thiomersal
Formaldehyde	Thymol
Gluconates	Titanium dioxide
Glutaraldehye	Tocopherols
Glycerol formal	Urea
Iron salts	Vitamins (A, B1, B3, B5, B6, B12, C, D, E)
Isopropanol	

constructs, proteins and other antigenic materials and adjuvants (Phillips and Schultz, 1992; Francis, 1993; Tatner, 1993; Meloen *et al*., 1998; Vinitnantharat *et al*., 1999; Williams, 2002; Chalmers, 2006; Mutwiri *et al*., 2007; Schijns and Degen, 2007; Hirao *et al*., 2008).

Adverse reaction reports to vaccines are common in both the British and Australian adverse reaction reporting schemes (National Registration Authority (Australia), 2000; Gray and Knivett, 2002; Gray *et al*., 2003; Australian Pesticides and Veterinary Medicines Authority, 2004–2006; Dyer *et al*., 2004–2006).

Adverse reactions to vaccines

Adverse reactions to veterinary vaccines have been classified into a number of categories (Martinod, 1995), namely:

- injection site reactions;
- systemic reactions;
- allergic reactions;
- effects on the immune system;
- residual pathogenicity;
- inadequate inactivation;
- genetic recombination;
- contamination.

This continues to form an adequate basis for the classification of adverse events following vaccination and it will be used here. Many of the problems associated with vaccines arise from manufacturing faults, and for human vaccines these have resulted in a number of major disasters. These have included use of virulent rather than attenuated organisms (e.g. the Lübeck Disaster of 1930 where the virulent Kiel strain of *Mycobacterium tuberculosis* was employed to manufacture vaccine), the increased virulence of

a strain of organism (meningo-encephalitis following use of a serially passaged Dakar strain of yellow fever vaccine), failure of inactivation (the Cutter Disaster resulting from live poliovirus) and presence of extraneous agents (hepatitis as a result of infected human serum in yellow fever vaccine) (Beale, 1992). All of these offer possibilities for problems with animal vaccines.

Injection site reactions

Injection site reactions are common in both human and veterinary medicine following vaccination. In fact in human medicine, swellings and abscesses at the injection site are the commonest adverse reaction to vaccination (Brooks, 1991; Jefferson *et al*., 2004; Casey and Pichichero, 2005; Scheifele *et al*., 2005; Kohl *et al*., 2007a, b). Injection site reactions may be sterile abscesses or areas of oedema, possibly caused by allergic mechanisms, or they can be frequently induced by bacterial as well as viral vaccines in companion animals, farm animals and fish (Tittes-Ritterhaus *et al*., 1980; Lund, 1988; Littledike, 1993; Martinod, 1995; Evensen, 2003; Mutoloki *et al*., 2006, 2008).

In Atlantic cod (*Gadus morhua* L.), inflammation occurred rapidly after intraperitoneal injections of oil-adjuvanted vaccines. However, overall, they were well tolerated in this species. Granulomatous reactions have been reported in chickens (Droual *et al*., 1990) and sterile peritonitis in trout (Simko *et al*., 1999). Occasionally, these may lead to blemishes in meat (Vannier, 1986; Apley *et al*., 1994; Dexter *et al*., 1994; George *et al*., 1995; Midtlyng, 1996; Roth, 1999; Mutoloki *et al*., 2004, 2006). Vaccines frequently contain mineral oils as adjuvants and these and other adjuvants including aluminium hydroxide have been implicated in at least some of these lesions (Straw *et al*., 1990; Cox and Coulter, 1997; Macy, 1997; Aucouturier *et al*., 2001, 2006; Spickler and Roth, 2003; Mutuloki *et al*., 2006; Sesardic, 2006; Day *et al*., 2007). Aluminium-based adjuvants may result in granulomas, particularly in sheep (Macy, 1997; Meyer, 2001).

An elevated frequency of sarcomas at the injection site in cats has caused some concern. These have been reported in the UK, USA and elsewhere, and, although relatively rare (Coyne *et al*., 1997; Tennant, 2000), the frequency has steadily risen in recent years (Hendrick *et al*., 1992, 1994a, b; Dubielzig *et al*., 1993; Esplin *et al*., 1993; Kass *et al*., 1993, 2003; Thornburg, 1993; Doddy *et al*., 1996; Lester *et al*., 1996; Hendrick 1998a, b, 1999; Leveque, 1998a, b; Starr, 1998; Brearley, 1999, 2001; Macy, 1999, 2004; Macy and Couto, 2001; McEntee and Page, 2001; Morrison *et al*., 2001; Gaskell *et al*., 2002; Gray and Knivett, 2002; O'Rourke, 2002; Gray *et al*., 2003; Hauck, 2003; Dyer *et al*., 2004, 2005, 2006; Tjälve, 2003, 2004; Vaccine-Associated Feline Sarcoma Task Force, 2005; Dean *et al*., 2006).

There are several case reports of vaccine-associated sarcomas in cats (Gruffyd-Jones and Sparkes, 1994; Rudmann *et al*., 1996; Burton and Mason, 1997; Sandler *et al*., 1997; Briscoe *et al*., 1998; Gemmill, 1998; Brearley, 2003; Lewis, 2003; Martin, 2003; Sauvage, 2003; De Man and Ducatelle, 2007). They are usually fibrosarcomas, but there has been a report of a rhabdomyosarcoma at a vaccine site in a cat (Chang *et al*., 2006).

A working group of the Veterinary Products Committee (VPC) has recently reported its findings on feline and canine vaccination. It reached a number of conclusions, the most important one being that vaccination plays a major role in protecting the health of cats and dogs. It concluded that the induction of injection-site sarcomas in cats was relatively rare, and aluminium used as an adjuvant may contribute to sarcoma formation. As a result of considering the balance of the undoubted benefits of feline vaccines versus the very small risks involved in their use, the working group recommended that all feline vaccines as a group should carry a generic warning as to the potential problems involved (Veterinary Products Committee, 2001; Gaskell *et al*., 2002). The Committee for Veterinary Medicinal Products has also drawn attention to this problem and has made it clear that it will continue to monitor the issues (Committee for Veterinary Medicinal Products, 2003), although at this time it has not

made any regulatory recommendations, but these are under consideration (European Medicines Agency, 2007).

A study in the USA found an increased incidence of tumours at the injection site in vaccinated cats, but there was no association with any particular brand of vaccine or antigen class and, in fact, there was also an association between tumour case animals and a long-acting penicillin preparation and a methyl prednisolone medication, thus providing even more evidence that the tumorigenic effects could be non-specific (Kass *et al*., 2003). Over recent years the number of sarcomas in cats in reports submitted to the Veterinary Medicines Directorate (VMD) has steadily risen (Gray and Knivett, 2002; Gray *et al*., 2003; Dyer *et al*., 2004–2006) as shown below:

2001 – 17
2002 – 27
2003 – 35
2004 – 43
2005 – 34
2006 – 39

These tumours, unsurprisingly, show various morphological and subcellular abnormalities, as do spontaneous fibrosarcomas (Mayr *et al*., 1991, 1996; Madewell *et al*., 2001; Carroll *et al*., 2002; Couto *et al*., 2002). They are fast growing and aggressive in nature, spreading locally along fascial planes and frequently metastasising to remote sites (Davidson *et al*., 1997; Martano *et al*., 2005; Dean *et al*., 2006).

Treatment of spontaneous soft tissue tumours in cats (and dogs) is itself problematic and involves resection, chemotherapy, radiotherapy and even electrotherapy (Hilmas and Gillette, 1976; Bostock and Dye, 1979; Mauldin 1997; Mauldin *et al*., 1988; Hammer and Couto, 1990; Miller *et al*., 1991; Thrall and Gillette, 1995; Davidson *et al*., 1997; Mir *et al*., 1997; Couto and Macy, 1998; Cronin *et al*., 1998; Withrow, 1998; Barber *et al*., 2000; Novosad, 2003; Davis *et al*., 2007) with varying degrees of effectiveness. Treatment of vaccine-related sarcomas is similar (Séguin, 2002). Surgical treatment alone had limited beneficial effects on survival time (Hershey *et al*., 2000), whereas surgery and doxorubicin or surgery, doxorubicin and radiation therapy appeared to achieve a moderate degree of success, although the efficacy of doxorubicin is unclear (Bregazzi *et al*., 2001; Martano *et al*., 2005).

There has been a report of the occurrence of malignant lymphomas developing following treatment for vaccine-associated sarcomas (Madewell *et al*., 2004). The cats had been treated with surgery and carboplatin, radiation plus surgery, surgery plus radiation, radiation alone or surgery alone and so no single treatment-related cause or any other causal factor could be identified.

The cause (or causes) of vaccine-associated fibrosarcomas is unclear, although they may be related to aluminium used as an adjuvant (or other adjuvants; see previous discussion). However, no major contributory factor has been identified (Kass, 2004), although significantly less inflammation with non-adjuvanted vaccines has been noted in experimental studies (Day *et al*., 2007). It is not known if general localised tissue damage and inflammation has any role in sarcoma formation and no single type of vaccine has been implicated (Kahler, 1993; Hendrick *et al*., 1994b; Bergman, 1998; Macy, 1999; McNeil, 2001; Jelínek, 2003).

Even if inflammation does have a role in sarcoma development, it does not explain why otherwise normal cells undergo neoplastic change and subsequent clonal expansion to give rise to these tumours in the absence of materials known to be sarcomagenic. There is no firm evidence for the involvement of feline immunodeficiency virus in vaccine-associated sarcoma development (Kidney *et al*., 2000), but feline leukaemia virus has been found in one ultrastructural study (Madewell *et al*., 2001). As noted above, they have also been associated with the administration of pharmaceuticals, suggesting that injection injury itself, rather than vaccination, is responsible and a similar effect has been noted in a cat at the site of a non-absorbable suture (Buracco *et al*., 2002). Possible vaccination site fibrosarcomas have been reported in dogs and ferrets (Murray, 1998; Vascellari *et al*., 2003).

Similar tumours have also been reported under experimental conditions in rodents given cadmium sulphate and cadmium chloride, chromium compounds, yttrium, 4-hydroxyaminoquinoline-1-oxide, nitrosoethylurea or other potentially carcinogenic substances, natural and synthetic vitamin E, nickel sulphide, food colourings and surfactants (Haddow *et al*., 1964; Graf and Lafuma, 1965; Grasso and Goldberg, 1966; Gangolli *et al*., 1967; Hooson and Grasso, 1970; Grasso *et al*., 1971; Grasso and Crampton, 1972; Hooson *et al*., 1973; Shibata and Enomoto, 1977; Shabad and Ol'shevskaia, 1980; Waalkes *et al*., 1988; Shibata *et al*., 1989; Nitta *et al*., 1991; O'Brien *et al*., 2003).

Subcutaneously implanted plastic materials have led to sarcomas in rats (Dewan *et al*., 1995). Intramuscular injections of iron compounds have been associated with the induction of soft tissue tumours in humans (Fielding, 1977; Weinbren *et al*., 1978). Although some of these substances are known or suspected carcinogens, others are clearly not, suggesting that carcinogenesis associated with injection sites is not restricted to cats or indeed to vaccination.

While the evidence suggests that vaccination of cats is associated with a small increased risk of sarcomagenesis, and while vaccination guidelines need to be continually reviewed, the undoubted benefit of vaccination versus the small degree of risk should be kept in mind (Ford, 2001; O'Rourke, 2004; European Medicines Agency, 2006; Kirpensteijn, 2006). Indeed, and as discussed in Chapter 2, this is a fundamental tenet of pharmacovigilance in particular and of medicine in general.

Systemic reactions

Various adverse effects including vomiting, neurological signs, reduced milk yields, anorexia and necrotic oopheritis, and effects on pulmonary function and hyperthermia have been reported in a number of animal species following vaccination (Soós, 1987; George *et al*., 1988; Smith *et al*., 1990; Dalgleish and Love, 1993; Yeruham *et al*., 1994, 2001; Dixon *et al*., 1996; Ellis and Yong, 1997; Twigg *et al*., 1997; Gaskell *et al*., 2002; McLean, 2003; Newton *et al*., 2005; Ramsay *et al*., 2005). Hepatocellular necrosis has been reported in a dog following subcutaneous administration of a *Bordetella bronchiseptica*-canine parainfluenza virus vaccine which was intended for intranasal administration (Toshach *et al*., 1997). Other effects include alopecia and juvenile cellulitis in dogs and muscle necrosis and spinal cord inflammation in lambs (Wilcock and Yager, 1986; Perl *et al*., 2003; Horvath *et al*., 2007).

Panniculitis occurred in cats following vaccination (Scott and Miller, 1998). However, in a US study of 2,560 cases in the period 2002–2005, lethargy, with and without fever, was the most frequent adverse reaction to vaccination in cats (Moore *et al*., 2007). An epidemiological study into vaccination and general ill-health in dogs in the UK used a questionnaire approach to question owners about post-vaccination adverse effects. A total of 4,040 questionnaires out of 9,055 distributed by mail were returned and analysed. The results suggested that vaccination had no significant adverse effects on the general health of dogs in the period up to 3 months post vaccination (Edwards *et al*., 2004). Any systemic effects of vaccination noted may be due to residual endotoxin, excipients used in the products, e.g. saponins and adjuvants, and the pyrogenic effects of the antigens (Soós, 1987; Martinod, 1995; Ellis and Yong, 1997).

Allergic reactions

Several components or contaminants of vaccines may give rise to allergic reactions. These include cells and cellular debris, serum, foreign protein, excipients such as preservatives including antibiotics, and the antigens themselves (Erdös *et al*., 1975; Nyerges *et al*., 1982; Martinod, 1995), and adverse reactions are relatively common (Tjälve, 2004; Dyer *et al*., 2005, 2006; Müntener *et al*., 2005; Woodward, 2005b).

Allergic reactions, including anaphylaxis and hypersensitivity reactions, accounted for around

8% of suspected adverse reactions in cats reported to the UK's VMD in the period 1985–1999 and over 20% in dogs in the same period (Gaskell *et al*., 2002). In a retrospective cohort study of over 1.2 million dogs involving nearly 3.5 million doses of vaccine, 4,678 adverse events occurred, with the majority of these being allergic or possibly allergic reactions (Moore *et al*., 2005). Reactions noted in dogs are frequently indicative of type I hypersensitivity, including skin reactions, hypotensive shock, dyspnoea, facial oedema, pruritus and diarrhoea (Greene, 1998; Ohmori *et al*., 2002, 2005a). This is supported by laboratory findings that vaccinated dogs that developed immediate-type allergic reactions have IgE reactivity to vaccine components (Ohmori *et al*., 2005b). Dogs selected for high skin reactivity to grass and other plant pollens had elevated IgE antibodies which were increased by vaccination for canine distemper (Frick and Brooks, 1983). In humans, at least, vaccination is a risk factor in the recurrence of anaphylaxis (Mullins, 2003) and this may also be true of veterinary vaccines.

Anaphylaxis has been reported in several species, including dogs and ferrets vaccinated against distemper (Reddy *et al*., 1994; Greenacre, 2003). Allergic reactions in cattle have been noted following vaccination against foot-and-mouth disease (Lorenz and Straub, 1971; Black, 1977; Yeruham *et al*., 2001). These may be due to antigens arising from BHK cells used in the production of the vaccine (Jensen, 1969; Bauer *et al*., 1970; Eyal and Mayer, 1971; Black, 1975; Knudsen *et al*., 1979; Sharma *et al*., 1985). Allergic reactions have been observed in pigs vaccinated against swine erysipelas (Domán, 1975). A horse given two vaccines, one containing killed eastern equine encephalomyelitis, western equine encephalomyelitis, tetanus toxoid and influenza and the other containing modified equine herpesvirus-1, suffered an anaphylactic-like reaction (Zimmel *et al*., 2000).

Effects on the immune system

Idiopathic arthritis or idiopathic immune-mediated arthritis is a relatively common disease in dogs (Pedersen, 1976a, b; Bennett and Day, 1999). In 1996, it was first suggested that vaccination may play a role in the development of this and other immune diseases (Duval and Giger, 1996). Polyarthritis has developed or has been suspected of developing after vaccination of dogs (Kohn *et al*., 2003; Clements *et al*., 2004). Autoimmune disease has been induced in beagles using a multivalent canine vaccine (Hogenesch *et al*., 1999). It has been reported as a suspected adverse reaction to the UK's VMD (Gray, 1998).

The mechanisms involved in the development of these conditions are largely unknown. However, multivalent vaccines have been shown to suppress lymphocytes and lymphocyte responses to mitogen. The response was not evident with individual vaccine components but occurred when canine adenovirus type 1 or type 2 were combined with canine distemper vaccine (Phillips *et al*., 1989). Vaccination has also been followed by haemolytic anaemia in the dog (Duval and Giger, 1996). In humans, at least, vaccination is a risk factor in the recurrence of anaphylaxis (Mullins, 2003) and this may also be true of veterinary vaccines.

Glomerular disease in mink has been associated with vaccination against distemper, botulism, mink viral enteritis and *Pseudomonas aeruginosa*. Deposition of immunoglobulin, but not complement, was more frequent in mink given the vaccine than in those administered with saline or monovalent canine distemper vaccine (Newman *et al*., 2002).

Residual pathogenicity

Bacteria or viruses used to manufacture vaccines are passaged in culture to achieve attenuation. If attenuation is only partly effective, then disease can occur. For example, bovine herpesvirus (BHV) infection has occurred after vaccination of cattle with partly attenuated infectious bovine rhinotracheitis-parainfluenza vaccine, and necrotic oophoritis has resulted from a BHV-1 vaccine. A live BHV-1 virus caused infectious bovine keratoconjunctivitis in cattle. Signs of

respiratory disease have occurred in birds following administration of live infectious laryngotracheitis vaccine (Picault *et al*., 1982; George *et al*., 1988; Smith *et al*., 1990; Bryan *et al*., 1994; Yeruham *et al*., 1994).

Inadequate inactivation

As part of the manufacturing process, vaccines are subjected to inactivation with compounds such as formaldehyde, binary ethylenimine, β-propiolactone and hypochlorite (Bahnemann, 1990; Martinod, 1995). If this inactivation is inadequate, surviving pathogens may then be found in the finished product. This may have dramatic consequences for disease in humans and animals. The most notorious examples of these include outbreaks of poliomyelitis in humans and foot-and-mouth disease in Europe attributable to inadequately inactivated vaccines (Nathanson and Langmuir, 1963, 1995; Barteling *et al*., 1983; Fedida *et al*., 1986; Soós, 1987; Brown, 1991, 1993; Offit, 2005). An outbreak of equine encephalitis in Venezuela was also probably due to inadequate inactivation (Brown, 1993).

Genetic recombination

Increasing numbers of vaccines derived from genetically modified organisms are becoming commercially available in veterinary medicine, including those derived from recombinant and deletant strains and vector-delivered antigens. Reversion to virulence can occur in these organisms, and indeed in naturally occurring organisms, resulting in disease rather than in disease prophylaxis. Similarly, organisms with deleted genes can reacquire these, potentially resulting in increased virulence (Keck *et al*., 1988; Henderson *et al*., 1990; Katz *et al*., 1990a, b; Kusters *et al*., 1990; Mettenleiter *et al*., 1994; Martinod, 1995). However, there is little field evidence for these phenomena (Kusters *et al*., 1990).

Contamination

Contamination of vaccines and other biological products with adventitious pathogens is considered to be rare (Nims, 2006). Vaccines are tested prior to release for the presence of adventitious contaminants (Sheets, 2006; Whiteman, 2006). However, there have been reports of the contamination of human vaccines, including smallpox and polio (Chastel, 2005; Cutrone *et al*., 2005; Thu *et al*., 2006). Concerns have been expressed over the contamination of polio vaccine with simian virus 40 (SV40) and the induction of lymphoproliferative disease in humans (Vilchez *et al*., 2003; Thu *et al*., 2006) and whether the human immunodeficiency virus originated in polio vaccine (Elswood and Stricker, 1994; Cohen, 2000). Pestivirus RNA has been detected in several vaccines intended for human use (Giangaspero *et al*., 2001). All of this underlines the importance of testing for and avoiding the ingress of adventitious pathogens into biological products (Lecatsas, 2000; Krause, 2001).

There have been a number of reports of the contamination of veterinary vaccines, including:

- live pseudorabies virus contaminated with pestivirus (Vannier *et al*., 1988);
- cell lines from US culture collection contaminated with hog cholera (Bolin *et al*., 1994);
- Marek's disease vaccine contaminated with reticuloendotheliosis virus (Bagust *et al*., 1979; Fadly and Garcia, 2006; Fadly *et al*., 2006);
- Marek's disease vaccine contaminated with avian leukosis virus (Zavala and Cheng, 2006a, b);
- contamination of cell lines and vaccines with bovine diarrhoea virus (Wellemans and Van Opdenbosch, 1987; van Wuijckhuise *et al*., 2001);
- detection of bovine diarrhoea virus in fetal calf serum intended for vaccine production (Makoschey *et al*., 2003);
- detection of bovine diarrhoea virus in a live bovine herpes virus type-1 marker vaccine (Brusche *et al*., 2001);

- bovine leucosis virus contamination of an in vivo-produced vaccine against babesiosis and anaplasmosis (Rogers *et al*., 1988);
- vaccine contaminated with bluetongue virus (O'Toole *et al*., 1994);
- contamination of cattle and pig vaccines with pestiviruses (Harasawa, 1995);
- detection of mycoplasmas in a number of veterinary vaccines (Thornton, 1986).

In addition, there have been a number of occurrences of disease associated with vaccine contamination, including:

- bovine diarrhoea in cattle from a contaminated bovine herpes virus type-1 marker vaccine (Falcone *et al*., 2000; Barkema *et al*., 2001);
- experimental infection of calves with bovine diarrhoea from a contaminated batch of bovine rhinotracheitis vaccine (Falcone *et al*., 2003);
- fatal bovine herpes virus type-1 infection in calves administered contaminated live bovine rhinotracheitis parainfluenza-3 vaccine (Bryan *et al*., 1994);
- bovine diarrhoea in piglets contaminated with swine fever vaccine (Wensvoort and Terpstra, 1988);
- bluetongue in dogs arising from a contaminated live canine vaccine (Akita *et al*., 1994);
- abortion and death in pregnant bitches as a result of a bluetongue virus contaminated vaccine (Wilbur *et al*., 1994);
- hog cholera antibodies detected in pigs given a contaminated Aujeszky vaccine (Jensen, 1981);
- border disease in goats from a pestivirus contaminated orf vaccine (Løken *et al*., 1991);
- outbreak of clostridial disease in ruminants; of 202,523 animals in affected herds, 41,767 were infected with *Clostridium sordellii* and 22,189 died (Téllez *et al*., 2006).

These findings emphasise the need for not only stringent manufacturing conditions and controls, but also adequate testing with robust and appropriate methodologies and full characterisation of the cell lines used in production (Dezengrini *et al*., 2006; Téllez *et al*., 2006; Wessman, 2006). To reduce the risks of disease transmission further, full regard should be given to the use of adequately cleaned injection equipment (Makoschey and Beer, 2004), while use of vaccines in vulnerable groups such as neonates should be carefully regulated and monitored (Day, 2007).

Lack of efficacy

This is an additional group to those discussed by Martinod in 1995. However, there needs to be an awareness that vaccine failures, for whatever reason, can and do occur and that lack of efficacy or even reduced potency or specification with vaccines is, on occasions, a distinct possibility (Smitherman, 1997; Gray, 1998; Gaskell *et al*., 2002; Gray and Knivett, 2002; Gray *et al*., 2003; Dyer *et al*., 2004, 2005, 2006; Woodward, 2005b).

Conclusions

Vaccination of humans and animals is intended to prevent and control diseases caused by infectious agents (see, for example, Patel and Heldens, 2008). However, vaccination itself is associated with risks arising from reactions to the components of vaccines, and to infectious disease resulting from inadequately inactivated or attenuated organisms or from pathogenic contaminants. Most of these factors can be and indeed should be controlled by proper and appropriate manufacturing techniques, tests and controls, including the application of the principles of good manufacturing practice. Issues such as the induction of sarcomas in cats clearly need further research in order to understand the aetiological factors involved and to make recommendations that will minimise the risks. To a great extent, the latter wish is dependent on the former research.

The benefits of vaccination and protection from disabling or lethal pathogens and associated diseases are enormous, but the hazards

and associated risks cannot be totally ignored. However, the health and economic benefits from vaccination far outweigh the small risks involved (Schultz, 1998; Day, 2006; Wood and Adams, 2006). There is a significant degree of consumer opposition to the use of vaccines, especially in companion animals, because of the perceived risks (see, for example, Diodati, 2003; O'Driscoll, 2005; Clifton, 2007), and if misconceptions and misinformation of the type displayed and espoused in these publications are to be dispelled, manufacturers, veterinarians and regulators need to be equally vocal about the benefits of vaccination and the low magnitude of the risks involved, while manufacturers and regulators need to work to ensure the provision and supply of safe and effective vaccines (Day, 2006; Horzinek, 2006). These approaches should be coupled with robust pharmacovigilance exercises to ensure adequate and appropriate post-marketing surveillance of veterinary vaccines (Siev, 1999; Wood and Adams, 2006), where necessary introducing measures similar to those employed for pharmaceutical products and for biological products for human use (Ellenberg and Braun, 2002). In the European Union, this is already the case because, as mentioned earlier, vaccines are subject to the same legislation regarding authorisation and pharmacovigilance as all veterinary medicinal products.

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20 Adverse reactions in humans following exposure to veterinary drugs

K.N. Woodward

Introduction

Under normal circumstances, it is the primary users of veterinary drugs that are potentially exposed to the products. These include veterinarians, veterinary nurses, farmers (including fish farmers) and farm workers, and the owners of companion animals (Moore *et al.*, 1993; Park *et al.*, 1994; Douglas, 1995; Bryant and Mycyk, 2002; Thomann, 2003). In the EU, the assessment of user safety is a requirement of Directive 2001/82/EC, as amended by Directive 2004/28/EC, and it forms an important and integral component of the dossier when marketing authorisation applications are submitted to regulatory authorities. These assessments take into account the toxicity of the active ingredient and the excipients, the potential for human exposure and whatever protective clothing and precautions might be needed to ensure safe use of the product (Woodward, 1992, 1996). In the UK, the Health and Safety Executive (HSE), the government agency responsible for occupational health and safety, is represented at meetings of the Veterinary Products Committee (VPC), and veterinary medicinal products must therefore meet the requirements of UK occupational safety legislation (Woodward and Gray, 1989; Woodward and Atkinson, 1992).

As part of applications for EU marketing authorisations, formal user safety assessments are required in the EU and there are specific guidelines in place for both pharmaceutical and immunological products (Woodward, 2004a, 2007). These guidelines require assessments of the hazards of veterinary medicines and appraisals of the risks involved in their use, in order to minimise exposure, reduce risks and provide information on safe use and necessary precautions for safe use, to end users (Woodward, 2008).

Veterinarians, their assistants and animal workers are exposed to a number of occupational hazards including trauma (being crushed, kicked, bitten, scratched, gored), occupational driving accidents, and exposure to chemicals such as anaesthetics, formaldehyde and ethylene oxide (Blair and Hayes, 1980, 1982; Landercasper *et al.*, 1988; Quick, 1990; Gordon and Rhodes, 1993; Moore *et al.*, 1993; bin Zakaria *et al.*, 1996; Meyer, 1999; Thomann, 2003). Aquaculture poses further dangers to veterinarians and others involved in the enterprise, including diving hazards, exposure to hydrogen sulphide, electrical accidents, incidents involving ice and sunburn (Park *et al.*, 1994; Douglas, 1995; Durborow, 1999). Farmers and animal feed workers are likely to suffer from

respiratory diseases and allergies (Chan-Yeung *et al.*, 1992; Jorna *et al.*, 1994; Von Essen *et al.*, 1999; Von Essen, 2001; Omland, 2002; Andersen *et al.*, 2004; Von Essen and Auvermann, 2005; Wyatt *et al.*, 2008).

Hence, exposure to veterinary medicines is yet one more issue to be dealt with and veterinarians and others are trained in their safe use, although this is not necessarily the case for everyone likely to use them, particularly some farm workers and the public. Veterinary medicines can also be misused or abused, leading to adverse outcomes. For example, they may be taken for the treatment of disease in humans and the incidence of this is often higher among those with access to them, including those working with animals. The major reasons for this misuse are convenience, economic need and mistrust of the medical profession (Erramouspe *et al.*, 2002). They may also be given in error. For example, there is a report of blindness in women mistakenly given the anthelmintic drug closantel for gynaecological purposes after it was wrongly identified as a human medicine by physicians (Hoen and Hodgkin, 1993).

Suspected adverse reactions in humans

The issues described above demonstrate a need for regulatory authorities and industry to compile and maintain health records for those using veterinary drugs. This approach has been proposed for exposures to other chemicals in order to facilitate future epidemiological research (Cooke *et al.*, 1999).

The nature and extent of any adverse reactions that do occur depend on the nature and pharmacological properties of the product and on the extent of the exposure. For example, some drugs used in veterinary medicine are known to produce hypersensitivity reactions in humans. Whether or not they elicit such reactions depends on the sensitising potency of the drug, the susceptibility of those exposed and the extent of any human exposure (Woodward, 1991). Medicated animal feeds containing sensitising agents such as β-lactam antibiotics (e.g. penicillins) may be dusty in nature and so user exposure may occur. However, if these products are formulated to reduce dusting potential by the addition of vegetable oil or some other suitable substance such as propylene glycol, or by pelleting, then exposure is much less likely. Indeed, practically all medicated feeds are now formulated so as to reduce dust emissions. Nevertheless, respiratory protective equipment is sometimes recommended for use during mixing, depending on the properties of the active ingredient and the dusting potential of the formulation.

Figure 20.1 shows the number of suspected adverse reactions in humans in the UK for the period 1985–2006, along with those for suspected adverse reactions to organophosphorus (OP) sheep dips (from 2003 onwards, the organophosphorus exposure values are confounded by the addition of exposures to other therapeutic groups). The increase in reporting in the early 1990s is probably related to a number of factors. Many of the cases occurred prior to these dates, but they were only reported after the Suspected Adverse Reactions Surveillance Scheme (SARSS) was given greater publicity by the Veterinary Medicines Directorate (VMD) and after various pressure groups had exerted their not inconsiderable influence. However, what is clear is that there was a considerable effort by various individuals and special interest groups, assisted by sections of the media, to bring anything remotely related to sheep dip use and possibly including other adverse reactions to the notice of the VMD, government and various medical authorities.

The concerns and controversies over sheep dips should not divert attention from the fact that suspected adverse reactions may occur in humans following exposure to other types of veterinary medicinal product. For example, injuries have occurred in workers engaged in vaccinating young chicks (see later). Interestingly, the majority (55%) of suspected adverse reactions in humans in the UK were reported by marketing

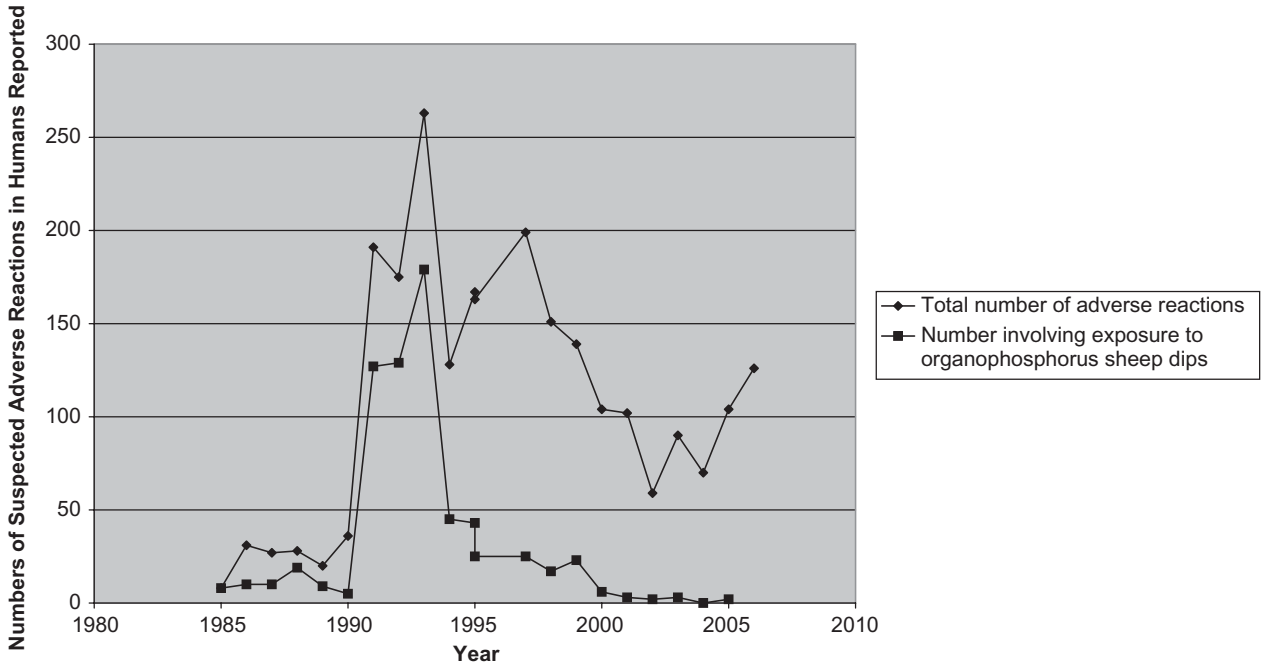

Fig. 20.1 Adverse reactions in humans 1985–2006.

Table 20.1 Reporting of human suspected adverse reactions 1985–2001.

Reporter	*%*
Marketing authorisation holders	57
Farmers	16
Veterinary surgeons	6
Others*	6
General public	6
Physicians and pharmacists	5
NPIS**	4

*Includes staff of the Health and Safety Executive, animal health officers, agricultural merchants and trading standards officers.
**National Poisons Information Service.

authorisation holders, with the remainder being accounted for by farmers, veterinary surgeons and others (*Table 20.1*). A similar pattern was observed for 2004 and 2005 (VPC, 2005, 2006).

In the period 1985–2001, suspected adverse reactions occurring in humans following exposure to ectoparasiticides accounted for 75% of those reported to the VMD. The remainder occurred from the following:

Vaccines	15%
Anaesthetics	2%
Antimicrobials	2%
Anthelmintics	1%
Hormones	1%
Antiseptics	1%
Others	3%

The largest group represented here, after the ectoparasiticides, is the vaccines. The majority of adverse reactions here were accounted for by accidents involving self-injection with inactivated formulations or simple needlestick injuries, the latter also being a relatively common occurrence in human medicine (Bilski, 2005; Smith and Leggat, 2005; Lefebvre *et al.*, 2008), and something that may be avoided through the use of needleguard systems (Sherwood, 2007).

As an illustration, 90 reports of suspected adverse reactions in humans were received by the VMD in 2003, 70 in 2004, 104 in 2005, 126 in 2006 and 138 in 2007 (*Table 20.2*; Dyer *et al.*, 2004–2008). The majority of these involved ectoparasiticides and endectocides, including those for small animal use, and vaccines. Needlestick

Table 20.2 Human suspected adverse reactions received by VMD in 2003–2007 (Dyer *et al.*, 2004–2008).

Product type	*2003*	*2004*	*2005*	*2006*	*2007*
Total number of human reactions	90	70	104	126	138
Ectoparasiticides and endectocides	46	26	45	62	67
OP sheep dips	3	0	2	—*	—*
Vaccines	22	19	29	29	29
Other veterinary medicines	22	35	29	29	42
Needlestick injuries	19	24	—**	—†	—††
Serious adverse reactions	17	8	11	12	6
Deaths	0	0	1	0	0

*Individual values not available.
**90% of reactions involving vaccines and other injectable products were needlestick injuries.
†91% of reactions involving vaccines and other injectable products were needlestick injuries.
††84% of reactions involving vaccines and other injectable products were needlestick injuries.

injuries were relatively common. Serious suspected adverse reactions accounted for approximately 12% of the total received in the period 2003–2006.

As already mentioned, all suspected human adverse reactions to veterinary medicinal products in the UK are considered by the Appraisal Panel for Human Suspected Adverse Reactions to Veterinary Medicines, an independent group that reports periodically to the VPC. The Panel examined these SARs, which fell into three main groups:

- human suspected adverse reactions to dog and cat spot-on products containing imidacloprid;
- ectoparasiticide sprays containing dichlorvos;
- organophosphorus sheep dips.

Human suspected adverse reactions to dog and cat spot-on products containing imidacloprid

Most of these appeared to be skin and eye reactions due to the solvent benzyl alcohol. A number of the reactions reported (18%) to this product were respiratory and it was considered that these were unlikely to be due to the alcohol as it is of low volatility. However, it was possible that the benzyl alcohol, despite its low volatility, might elicit respiratory symptoms in those with asthma and the signs noted were consistent with those of a respiratory irritant. An alternative explanation for at least some of the reactions reported could have been allergy to cats. As a result of these considerations the Panel recommended the wearing of gloves when using the products and recommended that the VPC impose a label change from 'People with known skin sensitivity may be particularly sensitive to this product' to 'This product contains benzyl alcohol which may cause some transient irritation to the skin. Avoid skin contact'.

For some spot-on products, particularly those intended for use on large animals and containing active ingredients such as synthetic pyrethroids, gloves are recommended as the quantities involved are larger than with companion animal products, and larger numbers of animals are likely to be treated.

Imidacloprid has produced neuropsychiatric effects, along with rhabdomyolysis, in a patient poisoned with the substance (Agarwal and Srinivas, 2007).

Ectoparasiticide sprays containing dichlorvos

There were 33 reports of this type of product between 1989 and 1999. These were mainly skin rashes or propellant burns. However, a number of reports concerned longer-term 'generalised'

reactions and the Panel considered these and the associated medical reports and questionnaires sent to and returned by patients or their doctors. It considered that there were no indications of cholinergic effects in these reports. However, one further report included vomiting and three included the occurrence of diarrhoea and these effects may have been due to the product (although the mechanism was unclear). No regulatory action was taken. Dichlorvos causes morbidity and mortality following significant exposures, which usually arise during accidents involving pesticides and as a result of suicide attempts (Yamashita *et al.*, 1997; Ozer *et al.*, 2007; Yurumez *et al.*, 2007). It is also genotoxic in vitro, but the evidence suggests that this is not so in vivo, while carcinogenicity data, including epidemiological studies of agricultural workers, show no evidence of carcinogenicity (Booth *et al.*, 2007; Koutros *et al.*, 2008). Clearly, taking suitable precautions when using veterinary medicinal products containing this active ingredient is a wise course of action.

Organophosphorus sheep dips

Organophosphorus-based sheep dips have long been used for the treatment and attempted eradication of sheep scab (Sargison *et al.*, 2006a, b; Bates, 2007).

It is known that exposure to organophosphorus compounds can produce a number of forms of toxicity in humans including acute toxicity as a result of inhibition of acetylcholinesterase. Signs of acute toxicity are related to muscarinic (cough, wheezing, rhinitis), nicotinic (muscle weakness, tachycardia, mydriasis) and CNS (anxiety, ataxia, hypotension) effects (Heath and Vale, 1992; Koelle, 1994; Karalliede *et al.*, 2000). With some compounds, a specific syndrome of delayed peripheral neuropathy or OP-induced delayed neuropathy (OPIDN) may result (Lotti, 1992; Veronesi, 1992; Johnson and Glynn, 1995; Richardson, 1995; Moretto, 1998).

Acute effects have been noted after sheep dipping (Rees, 1996). However, the ability of low-level exposure to organophosphorus compounds to induce chronic toxicity is more controversial (Jamal, 1997; Ray and Richards, 2001). Some workers have reported subtle adverse effects while others have found no ill effects (Jamal, 1997; Brown and Brix, 1998). Some organophosphorus compounds have been shown to be genotoxic in in vitro and in vivo systems (Garrett *et al.*, 1992) and cytogenetic responses, including sister chromatid exchanges in peripheral lymphocytes, have been reported in farm workers occupationally exposed to diazinon-containing sheep dips (Hatjan *et al.*, 2000), but the identification of frank health-related effects in such workers has proved elusive.

Moreover, the route of exposure is difficult to define. The vapour pressures of organophosphorus compounds are low. Airborne concentrations of diazinon have been shown to be low during sheep dipping – below the limits of detection of the assay used (<0.1 mg m^{-3}) (Niven *et al.*, 1993). Splashing might occur, but the evidence suggests that this is likely to be with the concentrate rather than with diluted material in the dip bath (Niven *et al.*, 1993; Sewell *et al.*, 1999; Pilkington *et al.*, 2001). No significant decreases in erythrocyte or plasma cholinesterase were detected in a study of workers employed in a single sheep dipping session regardless of whether they wore normal or protective clothing (Niven *et al.*, 1993, 1994). Hence, it is difficult to identify any critical route of exposure to organophosphorus compounds during dipping, or to quantify any exposure that might occur.

Over the period 1985–2000 there was a general increase in the numbers of suspected adverse reactions to veterinary medicines in humans reported to the VMD, although the numbers gradually decreased in the period up to and including 2004 (see Chapter 21). Undoubtedly, the major increase in this period and in subsequent years was in the numbers of suspected adverse reactions reported following exposure to sheep dips containing organophosphorus compounds. These products are supplied as emulsion-based concentrates, which are made up as aqueous formulations in the form of a dip bath in which sheep are immersed to treat and protect

against various ectoparasites including biting lice, blowflies, ticks and keds. However, the main clinical and economic ectoparasite of sheep in the UK is *Psoroptes ovis*, which causes sheep scab. Treatment and prevention of scab requires more frequent dipping than the other conditions mentioned, thus increasing the frequency of potential human exposure. In the period 1985–2001, the VMD received a total of 1,967 reports of suspected adverse reactions in humans potentially exposed to veterinary medicines (Woodward and Gray, 1989; VMD, 1993–1997, 2000a, b; Woodward, 1996; VPC, 2001a, b, 2002).

By 2000, the Appraisal Panel for Human Suspected Adverse Reactions to Veterinary Medicines (see Woodward, 2005) had considered a large number of suspected adverse reactions in humans involving organophosphorus-containing sheep dips. It recognised then that there was a similarity between many of the effects reported and chronic fatigue syndrome (CFS), a condition associated with biological, psychological and social factors (Mounstephen and Sharpe, 1997). The majority of the symptoms were:

- chronic headache;
- chronic fatigue;
- myalgia;
- depression;
- arthralgia;
- irritability;
- attention disturbances.

Other signs included:

- sore throat;
- pyrexia;
- memory impairment;
- sleep disorders;
- muscle weakness;
- confusion.

The Panel decided that it would seek the advice of an expert on CFS. The Appraisal Panel later reported that the expert had commented that headaches were typical of CFS, and indeed were typical of normal individuals too, although the rate of headaches in CFS patients was higher. Based on the advice, the Panel concluded that there were no diagnostic features to distinguish those involved in sheep dipping with non-dippers, and that only epidemiology studies or the accumulation of more data from adverse reaction reporting (VPC, 2004) would resolve the issue of whether dipping sheep was related to health problems (VPC, 2003).

Similar signs were reported for large animal pour-on products containing organophosphorus compounds. In fact with these, headache was also the most common symptom reported, along with other CFS-type signs. However, this was not the case with non-OP products. Here, neurological signs were the most frequently reported, particularly paraesthesias. The Panel made no recommendations for the OP pour-on products as they are no longer marketed in the UK.

Studies of sheep farmers whose health problems had been reported to the VMD have examined the association between possible exposure and chronic fatigue (Pilkington *et al.*, 2001; Tahmaz *et al.*, 2003). Many of the subjects investigated reported chronic fatigue as a major problem and higher scores were associated with higher exposures to OP compounds. Only weak evidence of a chronic effect and cumulative exposure to OPs was observed.

The question of adverse reactions to organophosphorus sheep dips was referred to the UK's independent Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT). The COT first considered the issues involved during 1998 and it finally reported in 2000 (Committee on Toxicity, 2000). The COT's report, including appendices, extended to some 250 pages. It largely concerned itself with neurotoxic effects and epidemiology. The report discussed the well-established issues relating to neurotoxicity of this class of chemicals, and specifically the peripheral neuropathy in exposed workers. It examined a number of studies, including those relating to the exposure of sheep dippers to organophosphorus compounds (Stephens *et al.*, 1995, 1996; Davies *et al.*, 1999) and it had access to the data submitted to the VMD.

However, it was not able to draw any firm conclusions on the risks of developing psychiatric illness as a consequence of acute poisoning.

It concluded that the weight of evidence did not support the induction of adverse neuropsychological adverse effects as a consequence of prolonged low-level exposure to organophosphorus compounds, and that the balance of evidence did not suggest that such exposures could result in peripheral neuropathy. Moreover, the studies considered had a number of limitations including:

- differences between control and exposed populations;
- biases due to association between willingness to participate in the studies and health problems;
- small study sizes;
- inclusion of patients with past history of acute organophosphorus poisoning;
- inclusion of patients currently potentially exposed to OPs or with recent exposures.

The COT recognised that there were major gaps in knowledge relating to the effects of these compounds and specifically in the possibility that OPs cause 'disabling neurological or neuropsychiatric disease in a small sub-group of exposed persons'. As a consequence, it suggested recommendations for further research:

- What are the most common patterns of exposure, clinical presentation and clinical course?
- How common is '(sheep) dippers' flu'?
- Does low-level exposure to OPs cause disabling neurological or psychiatric disease in a small subgroup of exposed individuals?
- Do people with chronic disabling disease in a small subgroup differ metabolically from the general population?
- Other than acetylcholinesterase, what mechanisms play a role in the causation of adverse effects?

The COT reviewed sheep dips again in September 2007 (http://cot.food.gov.uk) and in doing so examined the results of some of this work. Although the research provided some interesting results, much of the data were inconclusive and some of the projects were still in progress. The work had gone some way to answering the questions detailed above, but a number of issues remained outstanding. For example, 'dippers flu' did not appear to be a specific syndrome.

Moreover, although there was some evidence of neurological illness in persons who had used organophosphorus compounds, there were associations with the use of other pesticides. There was some evidence of metabolic differences in those suffering chronic disabling illnesses, but this did not correlate with enhanced susceptibility to organophosphorus compound-related toxicity. Recent research has shown that psychological mechanisms may be involved in those suffering neurological symptoms after exposure to sheep dips and to other pesticide active ingredients (Solomon *et al.*, 2007a, b), and so it may prove difficult, if not impossible, to tease out the effects of sheep chemicals from those arising from other substances, from substance combinations and from genetic and phenotypic effects.

However, over the last few years, the labelling for sheep dips, especially organophosphorus-containing products, has been strengthened. These now carry a skull and crossbones symbol with the words TOXIC IF SWALLOWED. There are also warnings and advice on suitable protective clothing, equipment and constitution of the dip bath.

In 1998, the VPC recommended that the purchase of OP sheep dips be subject to a Certificate of Competence Scheme and these have been introduced for all sheep dips including non-OP dips (generally synthetic pyrethroids), following a period of suitable training in their use (Ministry of Agriculture, Fisheries and Food, 1998).

Quite clearly, the active ingredient in this type of sheep dip in the UK, the organophosphorus compound diazinon (*Figure 20.2*) (and until relatively recently chlorfenvinphos), has the ability to induce neurotoxicological effects and under

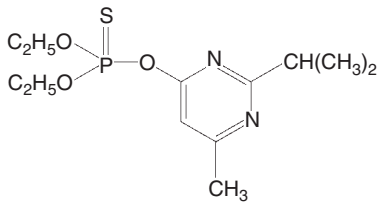

Fig. 20.2 Diazinon.

some circumstances neuropathies. Indeed, cognitive effects and developmental neurotoxicity have been reported in rats (Roegge *et al.*, 2008; Timofeeva *et al.*, 2008). However, many of the effects reported in humans were ill-defined and described as flu-like symptoms, chest tightness, sore throats and general malaise. Although these effects could conceivably have been due to exposure to organophosphorus compounds, other causative factors could not be ruled out. Thus in the early 1990s the VMD established the Appraisal Panel mentioned earlier, composed of medical and scientific staff from the VMD, the Department of Health and the Health and Safety Executive.

It was the remit of this Panel (which at the time was chaired by this author) to assess each human SAR and, in turn, to advise the VPC. Since the late 1990s the Appraisal Panel has been reformed into a committee of independent members with an external chairman (Veterinary Medicines Directorate, 2005a). It has now published a series of annual reports of its findings. The VPC, through the advice of the Panel and other scientific bodies, has made various recommendations on the use of organophosphorus sheep dips. The most recent and controversial came in late 1999 when the marketing authorisations were suspended pending the improvement of container designs. This recommendation arose over concerns regarding exposure to the product concentrates during preparation of the dip bath. Some of these products were allowed to re-enter the UK market in late 2000 after the introduction of taps and other means of reducing exposure to the concentrates. However, the UK authorities only imposed this as an interim measure until late 2001 when they required the introduction of improved systems to further reduce human exposure to the concentrated product (VPC, 1999a–c, 2000, 2001b; Anonymous, 2004a). The products that are now available have been designed to minimise exposure of farm workers to the dip concentrate through the introduction of closed-circuit delivery systems or concentrate-containing water-soluble pouches.

The issue of chronic illness following exposure to low concentrations of organophosphorus compounds has yet to be resolved. There is no doubt that long-term effects can ensue following acute organophosphorus poisoning (Ames *et al.*, 1995; Brown and Brix, 1998; Moore, 1998; Abou-Donia, 2003; Albers *et al.*, 2004). Although some believe that long-term effects can result from low-level exposure in the absence of overt acute effects (Jamal, 1995, 1997; Jamal *et al.*, 2002; Abou-Donia, 2003), others are of the opinion that the results of animal experiments, the analyses of controlled studies and information following accidental exposure show that there are no such chronic effects (Moore, 1998; Lotti, 2002) and that preventing late neurologic effects means preventing acute organophosphorus compound poisoning (Ames *et al.*, 1995).

A telephone survey of persons claiming to have suffered adverse effects following exposure to sheep dips commenced in 2001. Those interviewed were nominated by support groups for those affected by exposure to organophosphorus sheep dips, and a total of 524 eligible participants was identified. The analysis concentrated on 367 individuals with neurological effects who had been screened to include those with contributory diseases (e.g. diabetes) and medications with neurological side effects.

The cumulative exposures to sheep dips varied widely but, overall, the potential exposures were not considered to be unusually high. The participants had been self-selected as having long-term health effects due to organophosphorus exposure. However, on the basis of the data available, there was inadequate information to determine 'whether or to what extent' exposure to these compounds had contributed to ill-health (Fletcher *et al.*, 2005).

A detailed analysis of the VMD's data of 646 reports of suspected adverse reactions in humans to organophosphorus-containing sheep dips noted that a substantial amount of data was missing and 232 respondents had failed to provide information on their potential exposure histories. Many of these reports involved:

- nervous disorders (447);
- general disorders (389);
- psychiatric disorders (203);
- musculoskeletal disorders (192);
- effects on the eyes (50).

The majority of symptoms were headaches, dizziness, paraesthesia, fatigue, influenza-like symptoms, lethargy, depression, amnesia, arthralgias, myalgias and dyspnoea. However, there were no obvious novel patterns of morbidity found in these reports, although nausea and dizziness were associated, as were depression and memory loss.

Short- and long-term exposures were associated with psychiatric disorders and musculoskeletal disorders, notably myalgia. However, these associations were weakened by the confounding effects of age at onset, fear of reporting and missing exposure data. There were no immediately obvious explanations for any of these observations (Dunn, 2002).

A risk assessment conducted by the Health and Safety Executive concluded, among other findings, that for acute toxicity at least the wearing of personal protective equipment was an important factor when handling sheep dips and sheep-dip concentrates (Cocker *et al.*, 2002).

The strategy of replacing organophosphorus sheep dips with those containing synthetic pyrethroids such as cypermethrin looks unlikely in the face of the recent suspension of the marketing authorisations for these products. Their adverse environmental effects, economic impact and potential human toxicity make this an unlikely policy option for the future (Sinclair *et al.*, 2007; Varma and Rayment, 2007).

The biological and medical effects of exposure to organophosphorus compounds are discussed in more detail in Chapter 21.

High-pressure injection injuries

Globally, the use of veterinary vaccines has been associated with injuries to the hands and digits. In the UK, there have been a number of cases of injection injuries arising from the mass administration of vaccines to poultry, pigs or other animals using high-pressure equipment (Neal and Burke, 1991; Couzens and Burke, 1995; O'Sullivan *et al.*, 1997; Christodoulou *et al.*, 2001; Rappold and Rosenmayr, 2001). With poultry vaccines, the inoculant is usually small in volume (around 0.5 ml), but with large animal vaccines, the inoculant volume may be 2 ml or higher, and this may lead to severe tissue injury following accidental high-pressure injection (Burke and Brady, 1996).

Industrial high-pressure injuries to the hand have been recognised for many years. They usually involve the injection of water, oil, grease, paint or industrial solvents into a digit or the palmar area of the hand (Kaufman, 1968; DeCesare and Sprague, 1975; Gelberman *et al.*, 1975; Dickson, 1976; Childress, 1977; LeBlanc, 1977; Craig, 1984; Beguin *et al.*, 1985; Kon and Sagi, 1985; Thakore, 1985; Maxwell and Dixon, 1988; Weltmer and Pack, 1988; Curka and Chisholm, 1989; Pai *et al.*, 1991; Peters, 1991; Flotre, 1992; Goetting *et al.*, 1992; Hogan and Tanglertsampan, 1992; Gutowski *et al.*, 2003; Tempelman *et al.*, 2004; Gonzalez and Kasdan, 2006; Austin and Hankin, 2007), although injection at other sites, with significant tissue injuries, including those in the male genitalia, have been reported (Cohen *et al.*, 2001; Scholten *et al.*, 2005; Akkus *et al.*, 2006; Zickerman and Ratanawong, 2007).

The initial injury may appear trivial and its significance is often unrecognised by the primary physician, leading to delays in specialised treatment (Herrick *et al*, 1980; Harter and Harter, 1986; Karlbauer and Gasperschitz, 1987; Sirio *et al.*, 1989; Fialkov and Freiberg, 1991; Stoffelen *et al.*, 1994; Rosberg, 1995; Schnall and Mirzayan, 1999; Mizani and Weber, 2000; Vasilevski *et al.*, 2000). However, internally, there is often extensive tissue damage which is caused by a combination of the kinetic energy involved in the

high-pressure injection process and the physical and biological properties of the material injected (Scott, 1983; Mrvos *et al.*, 1987; Sirio *et al.*, 1989; Proust, 1993). The injected substance may penetrate fascial planes and tendon sheaths (Mrvos *et al.*, 1987).

The ensuing damage includes:

- haemorrhage;
- vascular pressure and occlusion of digital blood vessels;
- oedema;
- local ischaemia;
- necrosis and inflammation;
- foreign body granulomatous changes

(Williams and Riordan, 1974; Dickson, 1976; Schoo *et al.*, 1980; Hayes and Pan, 1982; Lewis, 1985; Salisbury, 1986).

Secondary infection may follow, including gangrene, and systemic toxicity may occur, depending on the properties of the injected material (Stepanuk, 1976; Schoo *et al.*, 1980; Mrvos *et al.*, 1987; Neal and Burke, 1991). The injuries are often described as 'devastating' and digital amputation is frequently required (Mrvos *et al.*, 1987; Jebson *et al.*, 1993; Pinto *et al.*, 1993; Stoffelen *et al.*, 1994; Lewis *et al.*, 1998; Schnall and Mirzayan, 1999; Christodoulou *et al.*, 2001; Rappold and Rosenmayr, 2001; Gutowski *et al.*, 2003; Valentino *et al.*, 2003). Injuries are usually more severe with paint and solvents when compared with oils and water (Neal and Burke, 1991; Peters, 1991; Obert *et al.*, 2002).

Treatment includes excision of the penetration point, irrigation, debridement, synovectomy, irrigation, decompression, removal of foreign substances and necrotic tissues and, where necessary, amputation as well as antibiotic prophylaxis and treatment with anti-inflammatory drugs (Ramos *et al.*, 1970; Herrick *et al.*, 1980; Hayes and Pan, 1982; Kendrick and Colville, 1982; Salisbury, 1986; Creaser, 1987; Sirio *et al.*, 1989; Fialkov and Freiberg, 1991; Klinger *et al.*, 1991; Neal and Burke, 1991; Taylor, 1992; Jebson *et al.*, 1993; Pinto *et al.*, 1993; Stiles, 1994; Lewis *et al.*, 1998; Vasilevski *et al.*, 2000; del Piñal *et al.*, 2001; Rappold and Rosenmayr, 2001; Obert *et al.*, 2002; Valentino *et al.*, 2003).

Veterinary vaccines are frequently oil-based formulations. However, the small volumes involved in the vaccination of chickens means that the injuries can usually be treated with anti-inflammatory drugs and corticosteroids. Self-injection of a 2-ml dose of vaccine intended for pigs has resulted in amputation of a digit and these larger volumes require the kind of interventions described above for other high-pressure injection injuries (Burke and Brady, 1996), while self-injection of 1 ml of a bovine vaccine into the thigh produced significant muscle damage and long-lasting disability (Gwynne Jones, 1996). Injection of 1 ml of a bovine vaccine into the base of the little finger resulted in signs and symptoms of ischemia and eventual amputation of the digit (O'Neill *et al.*, 2005). Self-injection with a vaccine containing Freund's complete adjuvant (Gudair) for the control of Johne's disease (*Mycobacterium paratuberculosis/Mycobacterium avium paratuberculosis*; paratuberculosis) has resulted in injuries of the hand requiring surgical intervention (Patterson *et al.*, 1988; Shah *et al.* 2001; Richardson *et al.*, 2005; Windsor *et al.*, 2005). Self-injection of a *Salmonella enteritidis* vaccine has led to necrosis of the digits (Ogün *et al.*, 1999). Anaphylaxis has resulted from self-injection with a vaccine intended for use in aquaculture (Leira and Baalsrud, 1992), while concern has been expressed over the possibility that oil-adjuvanted vaccines may induce autoimmune disorders (Kuroda *et al.*, 2004).

Advice regarding the treatment of injuries arising from the administration of veterinary vaccines has been provided in the *British Medical Journal* (Anonymous, 1987) and elsewhere (O'Neil *et al.*, 2005); debridement and irrigation of the effective part with decompression is necessary to result in the most favourable clinical outcome.

In 2003, the VMD published an article to highlight the problems associated with self-injection injuries in the Committee on Safety of Medicine's *Current Problems Information Bulletin* (Anonymous, 2003). This is a periodical widely

circulated to the medical profession and the article is intended to highlight the issues, including suitable and appropriate treatment, to doctors (VPC, 2004).

In 2007, five cases of self-injection were reported to the VMD and treatment of these ranged from irrigation to muscle and skin grafts (Dyer *et al.*, 2008).

Vaccines

Most veterinary vaccines contain killed or attenuated organisms, or antigenic fragments of these, and many of the organisms used are not pathogenic to humans. However, some vaccines do contain zoonotic organisms, or those that have the potential to be pathogenic in immunocompromised patients (Berkelman, 2003).

The major route of human exposure is usually occupational, and may result from simple needlestick injuries, a rare but significant occurrence among veterinarians and fish farm workers (Leira and Baalsrud, 1997; Wilkins and Bowman, 1997). Adverse effects have been reported among zoo veterinarians following needlestick injuries while vaccinating (Hill *et al.*, 1998) and an adverse reaction occurred to a live anthrax vaccine following self-injection, although anthrax virus was not isolated from the subject (Geller, 1990). There have been reports of adverse effects following self-injection of Johne's disease vaccines containing *Mycobacterium paratuberculosis*, but these are restricted to inflammatory reactions (Patterson *et al.*, 1988) and injection injuries (see above). Consumption of milk containing a live Newcastle disease vaccine resulted in no ill effects (Crosby *et al.*, 1986) and self-injection of this virus is unlikely to have any untoward effects in humans.

Many veterinary vaccines contain adjuvants. Their mechanism of action remains unclear, but they may assist in antigen presentation, enhance stability or act as immunomodulatory agents (Cox and Coulter, 1997; Vogel, 1998, 2000). They include oils, aluminium and calcium salts, saponins and nanoparticles (Horzinek *et al.*, 1997; Aucouturier *et al.*, 2001; Spickler and Roth, 2003). These materials may give rise to inflammatory reactions at self-injection sites in patients (Spickler and Roth, 2003).

In sheep and goats, orf (contagious ecthyma, contagious pustular dermatitis, sore mouth, scabby mouth), caused by a parapox virus, is one of the commonest infectious diseases in some parts of the world (Guss, 1980; Moore *et al.*, 1983; Haig and Mercer, 1998; Reid and Rodger, 2007; Sargison *et al.*, 2007). It is also a zoonotic disease and can be contracted from sheep and goats and other animals by direct contact and particularly during bottle feeding of lambs, or following contact with animal products (Leavell *et al.*, 1968; Johannessen *et al.*, 1975, 1980; Kim and Tarrier, 1977; Wilkinson, 1977; Mohr and Katz, 1989; Huerter *et al.*, 1991; Hogan and Tanglertsampan, 1992; Stead *et al.*, 1992; Bassioukas *et al.*, 1993; Chahidi *et al.*, 1993; Bodnar *et al.*, 1999; Ghislain *et al.*, 2000; Gurel *et al.*, 2002; Kuhl *et al.*, 2003).

In humans, it is normally a mild disease which affects the skin and eyes. It produces nodular lesions of the skin which occasionally become exceptionally large, but these resolve over a matter of weeks (Lober *et al.*, 1983; Freeman *et al.*, 1984; Watson *et al.*, 1993; Gurel *et al.*, 2002). It appears to have no adverse effects on pregnancy in humans (Taieb *et al.*, 1988; Watson *et al.*, 1993). In some farming communities a large proportion of the population may have been infected with orf at some stage. For example, in England, up to 15% of farmers reported having orf, while in Wales some 29% reported the disease (Buchan, 1996; Paiba *et al.*, 1999). However, despite its infectivity and geographic spread, and despite the fact that orf vaccines contain live virus, there appears to be no well-documented cases of human infection arising from occupational exposure.

Brucellosis has been contracted from live *Brucella* vaccines (Squarcione *et al.*, 1990; Blasco and Diaz, 1993). A recent study reported on humans exposed to *Brucella abortus* strain RB51

via vaccination. The Centers for Disease Control (CDC) in the United States conducted passive surveillance for accidental injection with exposure to the vaccine and received reports from 26 affected individuals. Of these, 21 subjects had suffered needlestick injuries, while four had received conjunctival spray exposure, and one an exposure to an open wound. There were no clear cases of brucellosis in these individuals, suggesting that this strain might have low pathogenicity to humans, but at present there are insufficient data to determine if the strain can cause systemic brucellosis in humans (Ashford *et al.*, 2004). Exposure to other *Brucella* vaccines can cause brucellosis in humans (McCullough, 1963; Gulasekharam, 1970; Blasco and Diaz, 1993).

Accidental exposure to oral rabies vaccine in eight individuals did not result in adverse effects (Mrvos and Krenzelok, 2007). Those working with animals that might be infected with rabies are subject to, or should be subject to, occupational monitoring (Brookes and Fooks, 2006). Arguably, those working with rabies and other lyssaviruses in the development and manufacture of veterinary vaccines should be subject to the same levels of scrutiny.

Antimicrobial drugs

The major problem following human exposure to antibiotic drugs is sensitisation and subsequent hypersensitivity reactions. This is particularly well recognised with β-lactam antibiotics, which may result in anaphylactic reactions during treatment or prophylaxis of infectious diseases in humans or following inadvertent exposures (de Weck, 1982; Griffin, 1986; Woodward, 1991), and systemic adverse reactions have occurred after the inhalation of penicillin (Reisman and Arbesman, 1968).

During the use of veterinary medicines, and especially medicated feeds, there is potential for worker exposure to antimicrobial drugs and allergic reactions have occurred as a result of such exposures (Mauranges, 1972; Neldner, 1972; Becker, 1976). Dermatitis has also occurred following occupational exposure to penicillin residues in the milk of treated cattle (Erskine, 1958; Zimmerman, 1959; Borrie and Barrett, 1961). The nitrofuran drug furazolidone is known to cause contact dermatitis (Hull and de Beer, 1977; Ancona, 1985; Altamirano and Bondani, 1989) and this has been reported after exposure to veterinary medicinal products containing the drug (de Groot and Conemans, 1990).

Contact dermatitis and urticaria, as a result of either systemic sensitisation or repeated dermal exposure, can also occur, for example, following the ingestion of contaminated foods or due to occupational exposure to penicillin present as residues in milk or to penicillin itself (Erskine, 1958; Vickers *et al.*, 1958; Kautz, 1959; Zimmerman, 1959; Borrie and Barret, 1961; Vickers, 1964; Stewart, 1967a, b; Reisman and Arbesman, 1968; Minkin and Lynch, 1969; Wicher *et al.*, 1969; Mauranges, 1972; Olson and Sanders, 1975; Cany, 1977; Girard, 1978; Lindemayr *et al.*, 1981; Falk *et al.*, 1985; Rudski and Rebandel; 1985; Pigatto *et al.*, 1986; Woodward, 1991; Lisi *et al.*, 1997). There has been a report of a patient who experienced an anaphylactic reaction after a steak dinner. The patient, known to be sensitised to penicillin, developed generalised pruritus, difficulty in swallowing and speaking, and dyspnoea within 20 minutes of eating. The meat was later found to contain penicillin or penicilloyl moieties (Schwartz and Sher, 1984).

A similar event occurred after consumption of beef containing streptomycin residues (Tinkelman and Bock, 1984). There has even been a report of anaphylaxis in a patient after the consumption of a soft drink (Wicher and Reisman, 1980). Although penicillin was detected in the drink, it origins were obscure. There are some limited animal models for penicillin hypersensitivity, including cutaneous anaphylaxis (Kristofferson and Ahlstedt, 1982; Kornbrust *et al.*, 1989; Kubo *et al.*, 1989; Hattori *et al.*, 1997), but it is not possible at present to predict which patients will react, and in which way.

As others have noted, it is difficult to quantify the public health risks of penicillin residues in

foods (Dewdney and Edwards, 1984). Several factors combine to make the risk of adverse reactions to penicillin residues in food very low, including the dose received, oral intake and the low density of antigenic determinants (Dewdney *et al.*, 1991), and, indeed, the literature supports this view; allergic reactions to antibiotic residues are very rare (Dayan, 1993). Nevertheless, the advent of MRLs, the enforcement of withdrawal periods, the conduct of residues surveillance programmes and improved product labelling have almost certainly contributed to the more or less complete disappearance of the occupational and consumer hazards posed by many veterinary drug residues such as penicillin.

Fluoroquinolone antimicrobial compounds are widely used in veterinary medicine (Greene and Budsberg, 1993). Rashes and other dermatological reactions are seen in human patients treated with these drugs (Hooper and Wolfson, 1993), but this does not seemed to have been reflected following human exposure to veterinary medicinal products.

The major group of antimicrobial drugs to have caused skin problems in workers exposed during animal production are the quinoxaline-1,4-di-N-oxides typified by cyadox, carbadox and olaquindox. In some countries these are classified as medicinal products and are regulated as such. However, in the EU they were used as growth promoters in pigs and regarded as zootechnical feed additives and regulated under Directive 70/524/EEC. Consequently, they were not subject to formal veterinary pharmacovigilance requirements.

Concern has long been expressed over the use of these drugs, as carbadox is genotoxic and carcinogenic in experimental animals, while olaquindox is genotoxic, although it has not been shown to be carcinogenic (Sykora and Vortel, 1986; Woodward, 2004b). Olaquindox has been reported to cause allergic and photoallergic dermatitis in farm workers, largely in those involved with pigs, following occupational exposure (Bedello *et al.*, 1985; Francalanci *et al.*, 1986; Schauder, 1989; de Vries *et al.*, 1990a, b; Hochsattel *et al.*, 1991; Fewings and Horton, 1995; Kumar and Freeman, 1996; Schauder *et al.*, 1996; Sanchez-Pedreno *et al.*, 2001; Belhadjali *et al.*, 2002; Sanchez-Perez *et al.*, 2002; Emmert *et al.*, 2007). Their phototoxic potentials have also been demonstrated in animal models (de Vries *et al.*, 1990a, b; Eberlein *et al.*, 1992).

Both carbadox and olaquindox were prohibited in the EU in 1998 because of concerns over their occupational hazards and associated risks (Anonymous, 1998) and particularly over the carcinogenicity of carbadox (Health Council of The Netherlands, 1999).

Following occupational exposure to the macrolide antibiotic spiramycin, there have been reports of dermatitis and bronchial asthma (Hjorth and Weismann, 1973; Davies and Pepys, 1975; Paggiaro *et al.*, 1979; Veien *et al.*, 1980, 1983; Moscato *et al.*, 1984) including reports of occupational asthma in workers in a pharmaceutical company (Nava and Corsico, 1976; Malo and Cartier, 1988). Another macrolide antibiotic tylosin has been reported to cause contact dermatitis and asthma in those occupationally exposed (Verbov and Abell, 1969; Hjorth and Weismann, 1973; Kraemer *et al.*, 1976; Veien *et al.*, 1980; Jung, 1983; Verbov, 1983; Barbera and de la Cuadra, 1989; Gollins, 1989; Lee *et al.*, 1989; Caraffini *et al.*, 1994; Danese *et al.*, 1994; Tuomi and Rasanen, 1995; Pirkis *et al.*, 1997).

There have been several reports of adverse effects in workers who have accidentally suffered a needlestick injury from needles contaminated with the macrolide tilmicosin (*Figure 20.3*). The majority of these were minor local effects, largely dermal, resulting from needle punctures (McGuigan, 1994; Forrester, 2005; Veenhuizen *et al.*, 2006), but there have been reports of cardiac effects in workers who have accidentally injected themselves with significant quantities of the medicine. These have included chest pains, electrocardiographic abnormalities and intraventricular conduction delays (Crown and Smith, 1999; Von Essen *et al.*, 2003; Forrester, 2005). There has been a report of a death following accidental intravenous injection (Kuffner and Dart, 1996) and a fatality in an 18-year-old woman following self-injection (reported in Von Essen *et al.*, 2003).

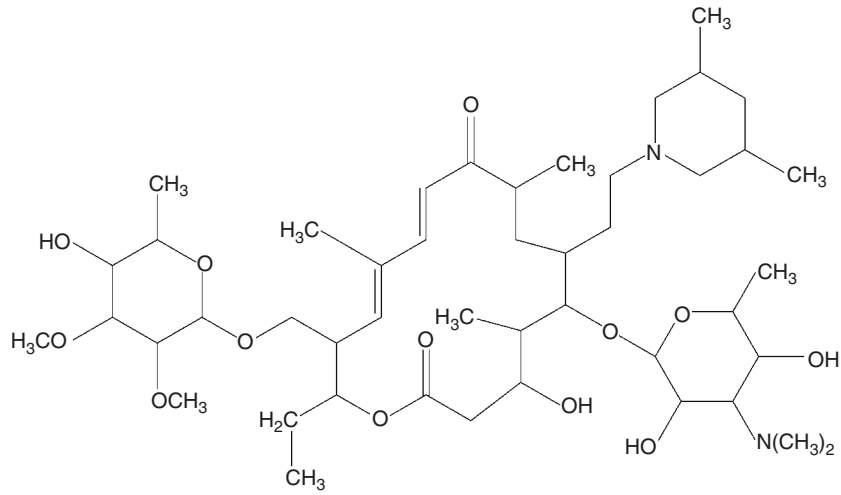

Fig. 20.3 Tilmicosin.

Similar toxicity has been noted with erythromycin, including torsades de pointes (Regan *et al.*, 1969; Nattel *et al.*, 1990; Farrar *et al.*, 1993; Brandriss *et al.*, 1994; Orban *et al.*, 1995). Studies in dogs show that a negative inotropic effect developed after intravenous administration of tilmicosin, with reductions in left ventricular systolic pressure and electrocardiographic abnormalities (Jordan *et al.*, 1993). Studies in conscious and anaesthetised dogs have shown abnormalities of cardiac function including:

- increase in heart rate;
- decrease in left ventricular function;
- decrease in aortic pulse;
- decrease in stroke volume, stroke work index and cardiac output;
- loss of ventricular contraction so that the aortic valve failed to open.

A study with isolated guinea-pig atria demonstrated a decrease in contraction force (JECFA, 1996).

These studies demonstrate that tilmicosin can pose an occupational risk when administered by injection, but the quantities required orally to exert cardiac effects are too great for residues to pose a risk. Nevertheless, they indicate the need for caution when using the drug by injection, and they underline the importance of protective measures to avoid self-injection, although the risks are low for induction of serious adverse effects – two for every million doses administered (Veenhuizen *et al.*, 2006).

In May 2004, the marketing authorisation for the product containing tilmicosin was temporarily suspended by the French regulatory authority because of another reported death in the USA in 2003 (Agence Nationale du Médicament Vétérinaire, 2004; Anonymous, 2004b; Department of Labor (Nebraska), 2004). This temporary suspension was lifted and the French authority is said to be satisfied with the safety of the product provided that veterinarians and those handling cattle are made more aware of the hazards of using the drug (Anonymous, 2004c).

Recommendations have been made to change the labelling to include a reminder that Micotil injection may be fatal in humans, that *extreme caution* should be observed when using the product, and that syringes should not be carried in the pockets. Similar advice has been issued by the European Commission (2006). Poor restraint of the animal being treated is recognised as one of the contributory factors in accidents involving the drug and there are now clear instructions on the use of the product in practice to minimise such incidents. These label changes are in a distinctive colour to give them prominence (Anonymous, 2004d, e; Lawrence, 2004, 2007). The drug is a prescription only medicine and so its distribution is only through veterinarians.

The lincosamide drugs clindamycin and lincomycin have caused contact dermatitis following human therapy (Fisher, 1983; Conde-Salazar *et al.*, 1985; Yokoyama *et al.*, 1991; Lammintausta *et al.*, 2002), but there have been no reports of similar effects following occupational exposure in veterinary practice.

The ionophore antimicrobial agent monensin resulted in the death of a 17-year-old male after ingestion of an undetermined amount of the drug. There was evidence of rhabdomyolsis with myoglobin deposits in the kidneys (Kouyoumdjian *et al.*, 2001).

Chlorhexidine, an antiseptic agent used widely in human medical and veterinary practice, is known to cause contact dermatitis (Lasthein *et al.*, 1985; Barbaud *et al.*, 2005; Aalto-Korte and Mäkinen-Kiljunen, 2006; Lim and Mam, 2008).

Tranquillisers and anaesthetic drugs

Xylazine

Xylazine (*Figure 20.4*) is a veterinary anaesthetic, analgesic and sedative closely related to clonidine. In humans, toxicity involves central nervous system depression, bradycardia and hypotension (Fyffe, 1994). Most cases of poisoning with xylazine, some involving farmers, result from intentional self-administration, and patients generally recover with supportive treatment (Carruthers *et al.*, 1979; Gallanosa *et al.*, 1981; Spoerke *et al.*, 1986; Hoffmann *et al.*, 2001). It has been implicated in both homicides and suicides (Mittleman *et al.*, 1998; Moore *et al.*, 2003). There has been a report of hypotension, bradycardia and coma in a veterinary nurse who accidentally injected himself with xylazine. He recovered with supportive measures (Samanta *et al.*, 1990). Xylazine abuse has resulted in syncope (Liu *et al.*, 2007). Systemic toxicity has been reported after ocular exposure to xylazine (Velez *et al.*, 2006).

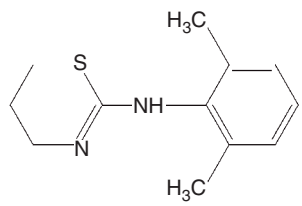

Fig. 20.4 Xylazine.

Chlorpromazine and related drugs

Chlorpromazine is a member of the phenothiazine class of neuroleptic drugs used in both human and veterinary medicine. This class can induce dystonia and dyskinesias in both animals and human patients (Cottom and Newman, 1966; Porsolt and Jalfre, 1981; Rupniak *et al.*, 1986; Messiha, 1991; Gross, 2001). These drugs can induce photosensitisation in human subjects (Epstein and Wintroub, 1985; Eberlein-Konig *et al.*, 1997; Moore, 2002) and there have been reports of contact dermatitis and photodermatitis in farmers as a result of exposure to chlorpromazine (Ertle, 1982; Schauder, 1985). The related phenothiazine drug acepromazine has been used in a suicide and in an attempted suicide (Stowell, 1998; Bryant and Mycyk, 2002).

Azaperone is butyrophenone neuroleptic agent closely related chemically to the antidyskinetic and antipsychotic human drug haloperidol (*Figure 20.5*). It is used as a tranquilliser in pigs. There has been a report of contact dermatitis in a pig breeder exposed to the drug (Brasch *et al.*, 1991).

Antidepressants

An attempted suicide has been reported with the tricyclic antidepressant drug amitryptyline prescribed for a dog (Bryant and Mycyk, 2002).

Anaesthetics

Halothane has been used for many years in veterinary surgery. This anaesthetic may cause mild liver damage in human patients, but around 1 in

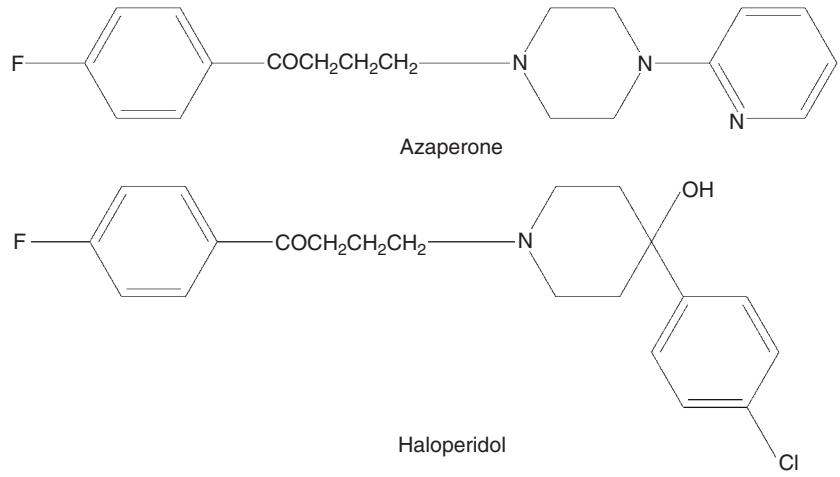

Fig. 20.5 Azaperone and haloperidol.

30,000 develops severe liver damage due to reactive metabolites combining with liver proteins which then elicit an autoimmune response (Neuberger and Williams, 1988; Bird and Williams, 1992; Kharasch, 2008). In human medical practice, occupational exposure to halothane and other medical gases has long been recognised as an occupational health issue and efforts have been made to monitor and reduce this (Linde and Bruce, 1969; Cohen *et al.*, 1975; Hunter, 1976; Whitcher and Piziali, 1977; Korttila *et al.*, 1978; Davenport *et al.*, 1980; Harrison, 1990; Kole, 1990; Henderson and Matthews, 2000; Sitarek *et al.*, 2000; Byhahn *et al.*, 2001; Stachnik, 2006). There have been reports of adverse effects including neurotoxicity, hepatotoxicity and spontaneous abortion among anaesthetists and other exposed medical workers (Belfrage *et al.*, 1966; Klatskin and Kimberg, 1969; Grimmeisen, 1973; Corbett *et al.*, 1974; Popova *et al.*, 1980; Duvaldestin *et al.*, 1981; Neuberger, *et al.*, 1981; Keiding *et al.*, 1984; Lings, 1988; Franco, 1989; Luchini *et al.*, 1996).

Concerns have been expressed over the safety of veterinary personnel working with gaseous anaesthetics, and recommendations made for ventilation and scavenging systems (Schuchman *et al.*, 1975; Milligan *et al.*, 1980; Dreesen *et al.*, 1981; Green, 1981; Wingfield *et al.*, 1981; Ward and Byland, 1982a, b; Potts and Craft, 1988; Burkhart and Stobbe, 1990; Gardner *et al.*, 1991; Stimpfel and Gershey, 1991; Moore *et al.*, 1993; Korczynski, 1999). There are no well-documented reports of liver or any other disease associated with halothane exposure in veterinarians, although there have been reports of hepatotoxicity in animal laboratory workers (Johnson and Mendelsohn, 1971; Sutherland and Smith, 1992).

The anaesthetic isoflurane has been reported to cause dermatitis, while propofol (2,6-di-isopropylphenol) has resulted in a fatality after self-administration (although the drug was not of veterinary origin) (Drummer, 1992; Caraffini *et al.*, 1998). Propofol has resulted in dependency in a 25-year-old male; the drug was obtained from various veterinarians, ostensibly for anaesthesia of tropical fish (Fritz and Niemczyk, 2002). In fact, this drug is being increasingly abused for recreational purposes, frequently leading to dependence (Roussin *et al.*, 2007). Telazol is an injectable anaesthetic containing zolazepam and tiletamine hydrochloride, a congener of phencyclidine and ketamine (Bransom, 2001). It has resulted in the death of a veterinarian and it may be subject to abuse (Cording *et al.*, 1999; Quail *et al.*, 2001). Local anaesthetics including benzocaine and tetracaine have been reported to cause contact dermatitis in veterinarians (Falk *et al.*, 1985).

Barbiturates available for veterinary purposes have been used in suicides and suicide attempts

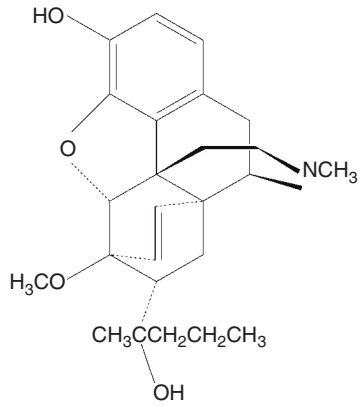

Fig. 20.6 Etorphine.

(Cordell *et al.*, 1986; Binder and Fredrickson, 1991; Résière *et al.*, 2001).

Etorphine

Etorphine (*Figure 20.6*) is a semi-synthetic opiate derivative of oripavine which induces catatonia and has been used for immobilising game animals as well as domestic species (Blane *et al.*, 1967; Wallach, 1969; Durrant, 1971; Still *et al.*, 1996; Bransom and Gross, 2001). Its action can be reversed by the antagonists naloxone, nalorphine and diprenorphine (Møller and Anderson, 1979; Yoxall, 1979). It is supplied as an aqueous solution which also contains acepromazine (Large Animal Immobilon) or methotrimeprazine (Small Animal Immobilon) along with the reversing agent containing the antagonist diprenorphine (Large and Small Animal Revivon) intended for reversing the action of etorphine in animals (Anonymous, 2003). Depending on the species, etorphine is 1,000 to 80,000 times more potent than morphine (Blane *et al.*, 1967) while the lethal dose for humans through accidental injection is estimated to be between 30 and 120 µg (Haigh and Haigh, 1980).

There have been reports of accidental self-injection with the product which were successfully treated with antagonists (Firn, 1973, 1974; Vaudrey, 1974; Summerhays, 1976; Goodrich, 1977). Following one incident with Immobilon which resulted in the death of a veterinarian from a 'wet needle', the UK's VPC suspended the licence for the product (Anonymous, 1976a). The suspension was ended some weeks later after the accompanying instruction booklet was revised to include:

- strengthening of warnings;
- revision of method of use;
- that naloxone or nalorphine should be used as reversing agents but if not available then the Revivon intended for use as the reversing agent in animals be used instead (Anonymous, 1976b).

An accident procedure was also placed on the reverse of the pack along with a paragraph pointing out that Immobilon is highly toxic to humans and providing advice on treatment (Volans and Whittle, 1976). Only Large Animal Immobilon now remains available in the UK. It carries the warning 'Etorphine can be life-threatening if absorbed by any route. Extreme care should be taken'. The product literature also carries advice on administration, recommending the use of two needles, one to fill the syringe, and the other, already placed in the patient, to administer the drug.

There have been a number of reports of adverse effects following skin contamination or presumed injection (Orr, 1977; Munro, 1978; Sheridan, 1981; Omersa, 1986). However, as etorphine is poorly absorbed through the skin, it seems other factors, including psychological ones, were also responsible (Bentley, 1987). There is a report of overdose with nalorphine following one etorphine self-injection where the patient eventually recovered (Summerhays, 1976). Although etorphine still has many practical uses, safer alternatives now exist. For example, a combination of butorphanol and azaperone has been successfully used as a method of chemical restraint for rhinoceros (Radcliffe *et al.*, 2000).

There has been some concern that etorphine could be the subject of drug abuse (Marcoux, 1996).

Tanax (T-61)

Tanax or T-61 is a product intended for the euthanasia of companion and laboratory animals. It has three components: embutramide, mebenzonium iodide and tetracaine hydrochloride (Hellebrekers *et al.*, 1990; Giorgi and Bertini, 2000). The combination is effective as embutramide induces deep anaesthesia, mebenzonium causes curariform paralysis of skeletal muscles, including those involved in breathing, while tetracaine hydrochloride reduces painful tissue reactions at the injection site as well as being toxic in its own right (Giorgi and Bertini, 2000). It has been used in suicide attempts, some successful, on several occasions (Cordell *et al.*, 1986; Smith and Lewis, 1989; Hantson *et al.*, 1996; Kintz *et al.*, 2002). Ingestion of the product, rather than injection, may result in severe hepatic failure (Nicolas *et al.*, 1990; Trevisani *et al.*, 1993). Other veterinary euthanasia agents, including barbiturates, have been used in suicide attempts (Cordell *et al.*, 1986).

Discussion

It is clear that veterinarians, farmers and others involved in looking after the welfare of livestock and other animals face a variety of hazards on a daily basis. Veterinary medicinal products are just one of these hazards and the risks involved appear to be low in comparison with the numbers of animals treated. However, at the individual level, these hazards and their associated risks cannot be discounted and veterinary medicinal products are labelled so that operator warnings are prominent on their labels and in the product literature, and advice on protective measures, including suitable clothing where appropriate, is provided.

Quite clearly, all due measures must be taken to avoid user contamination, especially with products containing overtly toxic materials such as organophosphorus compounds and potent pharmacologically active agents. High-pressure injection injuries, although they are potentially serious medically, are rare, and are usually associated with oil-based products. Again, oil-based pharmaceuticals and vaccines intended for injection carry prominent warnings and advice to medical practitioners as to what course of action to take when faced with a patient who has suffered one of these injuries. It is important to recognise that the danger with these products frequently lies with the high-pressure mode of administration and the associated high kinetic energy involved rather than with the oil-component itself, or indeed with the active ingredient or vaccine antigens. This is illustrated by the fact that injection site injuries can be caused by water under pressure. There is no similar hazard and risks associated with conventional injection in the circumstances where it is feasible to administer the products in this way. The danger usually arises from the high-pressure-induced tissue damage, and not from the product components, although these may serve to exacerbate the injury and cause systemic toxicity.

The European Commission guidance in the Notice to Applicants recommends that in cases of self injection, medical advice is sought immediately and surgical intervention is instigated (European Commission, 2002). This, however, may be overly cautious and should perhaps be reserved only for those instances where high-pressure delivery systems were involved or the symptoms suggest that medical intervention is indicated. Nevertheless, the guidance ensures that oil-based products are adequately labelled.

Many dusty formulations (e.g. those given in feed) of antimicrobial compounds have been associated with irritation and skin and pulmonary sensitisation. However, regulatory authorities now require such formulations to have minimal dusting potential. This is usually achieved by pelleting or by incorporation of edible or inert oil into the feed mix.

The use of antineoplastic drugs in veterinary medicine is growing in importance, but few if any products are authorised specifically for animal medicine. However, many antineoplastic drugs are genotoxic and carcinogenic in experi-

mental animals, and many are known to be carcinogenic in humans following therapeutic use. Nurses, physicians and others exposed to antineoplastic agents in the course of their work have been shown to have increased levels of chromosome damage including sister chromatid exchanges and micronuclei (Norppa *et al.*, 1980; Waksvik *et al.*, 1981; Nikula *et al.*, 1984; Pohlová *et al.*, 1986; Oestreicher *et al.*, 1990; Milković-Kraus and Horvat, 1991; Goloni-Bertollo *et al.*, 1992; Anwar *et al.*, 1994; Machado-Santelli *et al.*, 1994; Fucic *et al.*, 1998; Rubeš *et al.*, 1998; Pilger *et al.*, 2000; Jakab *et al.*, 2001; Burgaz *et al.*, 2002; Turci *et al.*, 2003; Cavallo *et al.*, 2005; Testa *et al.*, 2007). Hence there is a great need for careful use of these drugs in veterinary oncology to prevent or minimise worker exposure (Moore *et al.*, 1993; Thomann, 2003).

The potential for adverse effects of gaseous anaesthetics are now often mitigated and minimised by the use of efficient air-scavenging systems in veterinary operating theatres and treatment rooms. These systems are often imposed by local health and safety at work requirements and the need to comply with occupational exposure limits, and they assist in reducing atmospheric levels of airborne contaminants.

Many of the incidents and reports of adverse effects described here have arisen from accidental exposures. Careful attention to clear product literature and labelling should help to further reduce the numbers of adverse reactions in those occupationally exposed. However, it is unlikely that all accidents involving the work of veterinarians will ever be prevented. Where there is evidence that a particular medicine cannot be used safely, whatever precautions are taken, then it is inevitable that its use will be scrutinised, possibly restricted and in the worst cases prohibited, by the regulatory authorities.

Regardless, it must be borne in mind that exposure to human medicines, either intentional or unintentional, remains a major cause of reporting of adverse drug reactions (Papich, 1990; Chyka, 1999), and while needless human exposure to veterinary drugs is an important aspect of their safety, it needs to be viewed in perspective. Nonetheless, the adverse effects described here underline the importance of the appropriate assessment of user safety, and the availability of cogent and informative regulatory guidelines to ensure that this is carried out adequately, and that known hazards, associated risks and measures to reduce exposures and risks are conveyed to the end user.

This is emphasised by reference to the US Food and Drug Administration's CVM website (http://www.fda.gov/cvm) which lists the cumulative adverse reactions from 1987–2007. Many of the events discussed in this chapter are found there also, including the results of self-injection or needlestick injuries (*Table 20.3*). Many of the reactions described there arise from local contamination of eyes and skin, although more serious effects, including fatalities, also appear along with systemic effects and non-specific reactions. Again, these underline the need for care when using veterinary medicinal products.

Table 20.3 Cumulative human adverse reactions to veterinary medicinal products – FDA, CVM, 1987–2007.

Drug	*Exposure route*	*Number reported*	*Adverse effects*
Acepromazine	Oral	1	Stupor, unconsciousness
Albendazole	Oral	1	Haematochezia
	Topical	1	Inflammation at skin site, headache
Altrenogest	Oral	2	Diarrhoea, abdominal pain, headache, nausea, vomiting
	Topical	53	Abnormal menses, headache, abdominal pain, abnormal oestrous cycle

Table 20.3 *Continued*

Drug	*Exposure route*	*Number reported*	*Adverse effects*
Amitraz	Oral	4	Unconsciousness, apnoea, convulsions
	Topical	51	Rash, pruritus, skin congestion
Amoxicillin	Oral	1	Diarrhoea, abdominal pain
	Topical	1	Headache, rash
Atipamezole	Ophthalmic	2	Irritation, mydriasis
Betamethasone, gentamicin	Topical	2	Erythema, urticaria
	Ophthalmic	2	Eye irritation and pain
Butorphanol	Oral	1	Dizziness, nausea, abdominal pain
	Parenteral	1	Injection site phlebitis and anaesthesia
N-butyl chloride	Ophthalmic	1	Pain
	Oral	53	Nausea, vomiting, depression/lethargy, diarrhoea, dizziness, hypersalivation
	Topical	5	Pruritus, unconsciousness
	Ophthalmic	36	Eye irritation and pain
	Parenteral	4	Injection site pain and inflammation
Cefpodoxime	Topical	2	Rash
Ceftiofur	Topical	7	Rash, 'ill', eye irritation and congestion
	Parenteral	35	Injection site pain, swelling and inflammation, dizziness
Cephapirin sodium	Topical	3	Rash
	Parenteral	4	Injection site pain, swelling, diarrhoea
Chlortetracycline	Topical	1	Skin abnormality
Clindamycin	Oral	1	Abnormal taste
	Topical	1	Partial deafness, nausea
	Parenteral	1	Injection site pain
Clomipramine	Oral	165	Depression/lethargy, nausea, dizziness, vomiting, xerostomia, diarrhoea
	Topical	6	Respiratory disorders
Cyclosporine	Oral	6	Nausea, dizziness, dysphagia, oesophageal irritation
	Topical	1	Nasal congestion, headache
Deracoxib	Oral	7	Depression/lethargy, nausea, vomiting
Detomidine	Unknown	1	Abnormal breathing, unconsciousness
	Ophthalmic	1	Eye irritation
	Parenteral	4	Bradycardia, depression/lethargy, shock, somnolence
Dexamethasone	Oral	1	Behavioural disorder
Dexamethasone, neomycin, thiabendazole	Ophthalmic	7	Eye irritation
Diclofenac	Topical	3	Skin and eye irritation
Difloxacin	Oral	1	Nausea, abdominal pain
Dinoprost tromethamine	Oral	1	Abortion, diarrhoea, abdominal pain
	Topical	12	Abdominal pain, vaginal bleeding, nausea, pruritus, skin irritation
Doramectin	Oral	3	Nausea, abdominal pain, diarrhoea, dizziness
	Topical	31	Nausea, dizziness, headache, diarrhoea, skin and eye irritation, dyspnoea
	Ophthalmic	3	Eye irritation

Table 20.3 *Continued*

Drug	*Exposure route*	*Number reported*	*Adverse effects*
Enrofloxacin	Oral	5	Hyperesthesia
	Topical	4	Hyperesthesia
	Unknown	2	Injection site pain
	Parenteral	13	Injection site pain, swelling, inflammation, cellulites, dizziness
Eprinomectin	Topical	9	Headache, abdominal pain, rash, convulsions
Famphur	Topical	37	Eye irritation, nausea, vomiting, fever, headache, rash, skin congestion and irritation, skin disorders
	Inhalation	6	Irritation, cough, dyspnoea, headache
	Ophthalmic	10	Eye irritation, eye pain, conjunctivitis, epiphora
	Parenteral	1	Diarrhoea, dizziness, abdominal pain, sweating, vomiting
Fenbendazole	Oral	6	Diarrhoea, death
	Topical	7	Skin disorders, rash
Fenthion	Topical	3	Dizziness, hypoesthesia
	Inhalation	2	Dizziness, gastroenteritis
Firocoxib	Oral	3	Abdominal pain, depression/lethargy, fever, joint pain, vomiting
Florfenicol	Oral	1	Hot flush, joint pain
	Parenteral	21	Injection site pain, swelling, inflammation, hypoesthesia
Flunixin	Topical	2	Rash, skin irritation
	Parenteral	3	Dizziness, injection site swelling, sweating
Furazolidone	Topical	1	Skin inflammation
Gentamicin	Topical	2	Erythema, urticaria
	Ophthalmic	2	Eye irritation
Imidacloprid, moxidectin	Topical	2	Erythema, headache
Imidocarb	Parenteral	2	Injection site swelling, nausea
Isoflurane	Topical	1	Skin irritation
	Inhalation	4	Headache, death, dizziness
Ivermectin	Oral	1	Depression/lethargy, dizziness, sedation
	Topical	10	Nausea, depression/lethargy, anorexia, headache
	Parenteral	5	Skin congestion, depression/lethargy, skin rash (one suicide attempt)
Ivermectin, praziquantel	Topical	2	Headache, ocular blood, dizziness, epiphora, nausea, vision disorder, vomiting
	Ophthalmic	1	Eye irritation
Ivermectin, pyrantel	Oral	1	Diarrhoea, dizziness, bloody vomiting
Ketamine	Topical	1	Pain, vision disorder
Levamisole	Topical	12	Diarrhoea, arrhythmia, depression/lethargy, headache
Lufenuron	Oral	1	Nausea
	Ophthalmic	1	Eye irritation
	Parenteral	3	Injection site pain and inflammation, diarrhoea, nausea

Table 20.3 *Continued*

Drug	*Exposure route*	*Number reported*	*Adverse effects*
Mebendazole	Oral	1	Depression/lethargy, gastritis
Medetomidine	Oral	1	Dizziness
	Unknown	5	Bradycardia, ataxia, depression/lethargy, headache
	Parenteral	3	Hypoesthesia, anaphylaxis, cough, injection site reactions
Melarsomine	Ophthalmic	7	Eye irritation
Meloxicam	Oral	3	Hypoesthesia, abdominal pain, vision disorder
S-methoprene	Oral	3	Constipation, diarrhoea, flatulence, nausea
Methoxyflurane	Inhalation	5	Nasal discharge, dizziness, headache
Milbemycin	Oral	60	Nausea, diarrhoea, headache, vomiting, dizziness, abdominal pain
Milbemycin, lufenuron	Oral	17	Nausea, vomiting, abdominal pain, apprehension
Monensin	Topical	5	Ocular discharge, rash, ocular swelling, urticaria
Moxidectin	Topical	25	Diarrhoea, skin inflammation, hypoesthesia, abdominal pain, pain in face/head, anorexia, headache, hyperesthesia, nausea, paraesthesia, vomiting
	Unknown	5	Dizziness, abdominal pain, anaemia, kidney failure
	Ophthalmic	2	Eye irritation
	Parenteral	3	Ecchymoses, skin congestion, skin inflammation
Nitanoxanide	Topical	3	Skin abnormality, depression/lethargy, anorexia, dizziness
Orbifloxacin	Oral	1	Dizziness, nausea
Ormetoprim, sulfadimethoxine	Oral	1	Diarrhoea, nausea
Oxytetracycline	Oral	2	Abnormal-coloured urine, skin congestion, gastritis, headache, hepatitis
	Topical	1	Skin congestion, irritation
	Parenteral	5	Injection site swelling, fever, injection site bleeding, inflammation, injection site mass
Oxytetracycline, Polimixin B	Ophthalmic	1	Diarrhoea, oedema of head/face, vomiting
Pentobarbital, phenytoin	Topical	4	Temporary blindness, depression/lethargy, dizziness, eye disorder, headache
	Ophthalmic	22	Eye irritation, eye pain, dizziness
	Parenteral	7	Injection site pain, hypoesthesia, injection site swelling, death
Praziquantel	Oral	1	Insomnia, abdominal pain
	Ophthalmic	2	Eye irritation/congestion
	Parenteral	1	Hypoesthesia
Prednisolone, trimeprazine	Oral	2	Depression/lethargy, somnolence
Progesterone	Topical	2	Fever, insomnia, abnormal menses
Pyrantel	Oral	2	Arrhythmia, diarrhoea
Ractopamine	Unknown	4	Pain, anaphylaxis, anaemia
	Various	9	Tachycardia, arrhythmia, dizziness, abnormal ECG
	Inhalation	2	Epistaxis, fever, hypoesthesia

Table 20.3 *Continued*

Drug	*Exposure route*	*Number reported*	*Adverse effects*
Roxarsone	Various	13	Immune disorder, neoplasm, death
Selamectin	Oral	18	Nausea, taste abnormalities, diarrhoea, dizziness, mouth/lip irritation
	Topical	568	Rash, pruritus, skin irritation and congestion, hyperesthesia, taste abnormalities, hypoesthesia, skin inflammation, diarrhoea, eye irritation, dizziness, nausea, eye swelling, swelling of mouth/lips, vomiting, anaphylaxis, dyspnoea, pain, cough, paraesthesia
	Unknown	114	Rash, urticaria, pruritus, skin irritation and congestion, swelling of eyes
	Ophthalmic	17	Irritation, pain, eye congestion, nervousness
Selegiline	Oral	4	Diarrhoea, eye disorders, headache, hypoesthesia, nausea, nervousness
Semiduramycin	Topical	3	Pruritus, rash
Sometribove	Parenteral	44	Injection site pain and swelling, inflammation
Tiletamine, zolazepam	Unknown	4	Death, hypoesthesia, nausea
	Parenteral	5	Injection site swelling, convulsions, delirium, depression/lethargy
Tilmicosin	Oral	154	Taste abnormalities, headache, dizziness, nausea, pain, vomiting, hypoesthesia, fever, death (suicide)
	Topical	169	Taste abnormalities, dizziness, nausea, application site pain, weakness, hypoesthesia, headache, application site erythema, apprehension, tachycardia, 'ill', dyspnoea, fever
	Unknown	22	Death, injection site swelling, cardiac arrest, dizziness, hypoesthesia, injection site pain
	Various	191	Taste abnormality, hypoesthesia, nausea, headache, depression/lethargy, eye pain, tachycardia, dizziness, pain, hypoesthesia
	Ophthalmic	29	Eye pain, eye irritation, vision disorders, eye congestion, eye swelling, taste abnormality
Trenbolone	Parenteral	1	Myositis
Triamcinolone	Topical	2	Pruritus, pain, taste abnormality
Tulathromycin	Oral	1	Taste abnormality
	Unknown	7	Injection site pain, inflammation
	Ophthalmic	3	Eye irritation
	Parenteral	24	Injection site pain, inflammation, swelling, hypoesthesia
Tylosin	Oral	4	Diarrhoea
	Topical	6	Rash, skin inflammation, pruritus
	Inhalation	1	Vomiting
	Ophthalmic	1	Eye irritation
	Parenteral	15	Injection site pain, bleeding, oedema, inflammation, swelling, taste abnormality
Virginiamycin	Topical	2	Skin abnormality
Xylazine	Oral	2	Mouth irritation, somnolence
	Unknown	1	Bradycardia
	Parenteral	1	Hypoesthesia

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21 Medicines used to control and treat external parasites of sheep – toxicology and the phenomenon of reported adverse human responses to organophosphorus sheep dips

T.C. Marrs and P. Edwards

Introduction

Sheep are affected by a number of external parasites (ectoparasites). The most unpleasant of these are green and blue bottles (*Lucilia sericata*, *Phormia terrae-novae* and *Calliphora erythrocepala*), which cause flystrike. Sheep with flystrike are usually restless and may bite or kick at the affected area, while those with severe flystrike may appear systemically very ill. The larvae cause skin liquefaction and, later, liquefaction of underlying tissues and secondary bacterial infection develops. Unless promptly treated, tissue liquefaction and maggot secretions result in toxaemia and death. Sheep scab (sometimes called ovine psoroptic mange) is caused by *Psoroptes ovis*, a parasitic mite, which lives on the skin of sheep. The mite causes irritation and distress, while stunting, loss of the fleece and even death may occur.

Sheep keds, *Melophagus ovinus*, are wingless flies, found in the wool, where they cause irritation: they also damage the underlying skin. Another ovine ectoparasite that lives in wool is the biting louse, *Bovicola ovis* (Beesley, 1994).

The prevalence of sheep ectoparasites varies from year to year and there are geographical variations within the UK (Bisdorff *et al.*, 2006; Broughan and Wall, 2007). There is some evidence that outbreaks of lice, like scab, have increased since the cessation of compulsory dipping.

Treatment for sheep ectoparasites

Treatments and prophylaxes for these conditions involve the use of ectoparasiticidal formulations containing insecticides. In the EU and some other countries, these products are regarded as veterinary medicines whereas elsewhere they are regulated as pesticides. There are several methods of applying these insecticides:

- plunge dipping (immersion);
- shower dipping;
- jetting races;
- pour-ons (sprays);
- injection.

Plunge dips

Products available for use in sheep dips contain ectoparasiticidal solutions to kill the larvae of green and blue bottle flies responsible for flystrike and to control sheep scab and keds. In Roman times, olive oil dregs, wine lees and

lupins were used (Henderson, 1991). In the early nineteenth century, arsenical dips were used. Subsequently copper sulphate, glyceryl biborate, tar wash, rotenone from derris[1] and sulphur were used.

Dipping was compulsory from 1906, using lime, sulphur, arsenicals or phenolic compounds (Beesley, 1994). In the mid-twentieth century organochlorine compounds (OCs) were introduced, and in 1948, the Ministry of Agriculture, Fisheries and Food recommended the use of lindane, the γ isomer of hexachlorocyclohexane. By 1952, sheep scab had been eliminated but reappeared in 1972, probably from Ireland (Beesley, 1994). Other OCs, namely dieldrin, aldrin, chlordane, toxaphene, heptachlor and endosulfan were also used, as was DDT.

Up until 1985, lindane was the main substance used in sheep dips, and it is probably the best sheep dip active from both the sheep welfare and human toxicity point of view. Lindane is also less persistent in soil than other OCs, but, however severe the sheep welfare problems, there is no possibility of the reintroduction of lindane in the foreseeable future, lindane having been banned throughout the European Union, largely on environmental grounds. Since 1985 the majority of dips used in the UK have contained organophosphorus compounds (OPs) as the active ingredient, in particular propetamphos, diazinon and chlorfenvinphos. More recently, dips containing synthetic pyrethroids (flumethrin and cypermethrin) have become more widely used (see below). The neonicotinoid insecticide, imidacloprid, has shown some promise against certain sheep ectoparasites (Mehlhorn *et al*., 2001).

Non-dip treatments

As alternatives to traditional sheep dipping (plunge dipping), other means of topical application such as shower dipping and jetting races using similar chemicals have become available in some countries (Bates *et al*., 2005), but are not authorised in the UK. Pour-ons (actually sprays in common parlance) containing pyrethroids are available and control a range of ectoparasites but not *Psoroptes ovis*. Cyromazine pour-ons are widely used against fly-strike and are claimed to be effective against the larvae for 8–10 weeks after treatment. This compound is not effective against established flystrike and has to be used early in the season (DEFRA, 2007). Unlike OPs, pyrethroids and OCs, cyromazine does not affect the mature insect nervous system. In fact, cyromazine is an insect growth regulator, which interferes with pupation and moulting. Cyromazine does not stop the female fly laying eggs, but it does stop the larvae from developing fully (DEFRA, 2007). Dicyclanil is another insect growth regulator that can be used in the same way as cyromazine: it gives protection for longer than cyromazine (DEFRA, 2007). None of the pour-on products provides protection against the range of ectoparasites that is achieved by plunge dipping.

There are a number of injectable, systemic treatments for the control of ectoparasites of sheep; all contain avermectins such as ivermectin or doramectin, or the milbemycin, moxidectin. They are effective in the control of a range of sheep ectoparasites, including *Psoroptes ovis* but are ineffective against flystrike. As with the OPs and synthetic pyrethroids, they act via the insect nervous system.

Toxicology of sheep ectoparasiticides

Organophosphates (OPs)

The OPs that are used in veterinary medicine as ectoparasiticides, including sheep dips, are cholinesterase inhibitors. The active ingredients that have been used in veterinary medicines are frequently the same as those used in OP pesticides. OPs are also used as parasiticides in human

[1] Derris is a popular garden insecticide derived from two Asiatic plants, *Derris eliptica* and *Derris mallaccensis*. The plants contain rotenone, which interrupts electron transport in mitochondria in a variety of organisms, including insects and mammals.

Table 21.1 Sources of information on human health effects of OP anticholinesterases.

Use of OP	*Cause of exposure*	*Exposed subject*	*Example references*
Insecticides/ acaricides (used as pesticide or veterinary ectoparasiticides)	Accidental	User General public	Numerous case reports in scientific literature; some summarised by JMPR* and JECFA**, also government publications such as the annual reports of the Veterinary Products Committee from 2005 and the Veterinary Appraisal Panel before 2005†
	Deliberate self-harm	Suicide	
Contaminants	Accidental	Consumers of contaminated foods/ drinks	Ferrer and Cabral (1989, 1991, 1995); Jurewicz *et al*. (2006)
Human pharmaceuticals	Deliberate medicinal use	Patients	Kale (1982); CSM (2001)
Nerve agents	Deliberate aggressive use	Targets of aggression (armed forces in wartime and civilians in terrorist use)	Okumura *et al*. (2007)
	Experimental	Volunteers	Sidell (2007)

*Toxicological monographs: Joint Meeting of Experts of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and WHO Expert Group on Pesticide Residues (series). World Health Organization, Geneva. Available at http://www.inchem.org/pages/jmpr.html.
**Toxicological monographs: Joint Food and Agricultural Organization/World Health Organization Expert Committee on Food Additives (JECFA) monographs. Food Additive Series. World Health Organization, Geneva. Available at http://www.who.int/ipcs/publications/jecfa/monographs/en/index.html.
†Available at http://www.vpc.gov.uk/Public/reportsAR.html.

medicine; notably malathion to treat head-lice and metrifonate/trichlorfon[2] in tropical medicine (Cioli, 1998). Other important OP anticholinesterases include the chemical warfare nerve agents (Marrs, 2007) and a natural compound, anatoxin-As (Dittmann and Wiegand, 2005).

Because of the widespread use of OPs for a range of purposes that involve deliberate or inadvertent exposure of humans, there is a wealth of information on the human health effects of OPs (see *Table 21.1*).

The acute effects of cholinesterase poisoning from all the classes of OPs in *Table 21.1* are as follows:

- tightening of the chest;
- constricted pupils;
- abdominal cramps;
- muscle tremors and fasciculation;
- confusion;
- convulsions.

There are also chronic syndromes associated with severe acute poisoning episodes. Some OPs also give rise to a delayed polyneuropathy, which is not characteristic of the whole class of compounds and is unrelated to cholinesterase inhibition. Three OP active ingredients have been widely used as sheep dips in the UK: propetamphos, diazinon and chlorfenvinphos. None of these OPs is known to produce delayed polyneuropathy. At the present time (2008) diazinon is the only OP authorised for use in sheep dips in the UK.

[2] Metrifonate is the international non-proprietary name (INN) for use as a pharmaceutical and trichlorfon the ISO (International Organisation for Standardisation) pesticide common name for dimethyl (RS)-2,2,2-trichloro-1-hydroxyethylphosphonate.

Synthetic pyrethroids

The synthetic pyrethroids are compounds that are structurally similar to the pyrethrins, natural insecticides produced from *inter alia* pyrethrum, a plant of the *Asteraceae* (*Compositae*) (daisy) family. They are generally of low acute oral toxicity to mammals as they hydrolyse easily, but they are very toxic to aquatic organisms (Zitco *et al*., 1979). When they are administered to mammals parenterally, the synthetic pyrethroids are neurotoxic by virtue of their action upon voltage-dependent sodium channels (Vijverberg and van den Bercken, 1990; Vijverberg, 1994) and they can be separated into two classes on the basis of the central neurotoxic syndrome that they produce (Vijverberg, 1994):

- Type I synthetic pyrethroids, which include permethrin and resmethrin as well as the components of natural pyrethrum, lack an α-cyano group and give rise to the T-syndrome (characterised by tremor).
- Type II compounds, which include flumethrin and cypermethrin, have an α-cyano group and give rise to the CS-syndrome (choreoathetosis and salivation) (Aldridge, 1990; Joy, 1994).

These syndromes, while of considerable interest to the mechanistic toxicologist, are of no clinical significance in relation to ingested or topically administered material. In humans, the most prominent effect of the pyrethroids is paraesthesia mainly in the face and there is little evidence of any permanent effects in man.

Insect growth regulators

Cyromazine is of low acute toxicity. In short-term studies in rats and dogs and in long-term studies in mice and rats, effects on body weight were seen. Red blood cell counts and haemoglobin levels were reduced in dogs at high dietary concentrations. In a rat multigeneration study, cyromazine did not affect fertility, but there was increased perinatal pup mortality and reduced pup weight, at maternally toxic doses. The Joint FAO/WHO Meeting on Pesticide Residues (JMPR) did not consider cyromazine to be teratogenic, nor was it genotoxic (FAO/WHO, 1991). It seems unlikely that cyromazine would give rise to appreciable toxicity in humans. Dicyclanil is another insect growth regulator and is moderately hazardous when given as a single oral dose to rats: the LD_{50} values were 560 mg/kg bw (males) and approximately 500 mg/kg bw (females). Neurotoxicity, liver toxicity and changes in red blood cells were seen in animal studies (see review by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (2000)).

Avermectins

Ivermectin, doramectin and moxidectin are all complex macrocyclic sugars structurally related to the avermectins derived from the soil bacterium *Streptomyces avermitilis*. All are active against parasites and toxic to mammals by virtue of the opening of GABA-gated chloride channels. Toxicity in mammals is seen as tremor, ataxia, anorexia and similar signs, depending on the agent, test species and the dose. Sensitivity in mammals is inversely related in part to the activity of the P-glycoprotein transporter, which exports avermectins from the brain, and CF-1 mice, which are deficient in this transporter, are highly sensitive. The avermectins are also reproductive toxins at, or close to, maternally toxic doses. The acceptable daily intakes (ADIs) range from 0.012 mg/60-kg person for doramectin and ivermectin to 0.048 for moxidectin (CVMP MRL Summary reports, available at http://www.emea.europa.eu/htms/vet/mrls/i.htm).

Legal situation

Sheep dips are veterinary medicines and in the early 1990s were licensed in the UK under the Medicines Act (1968). Agriculture and Health

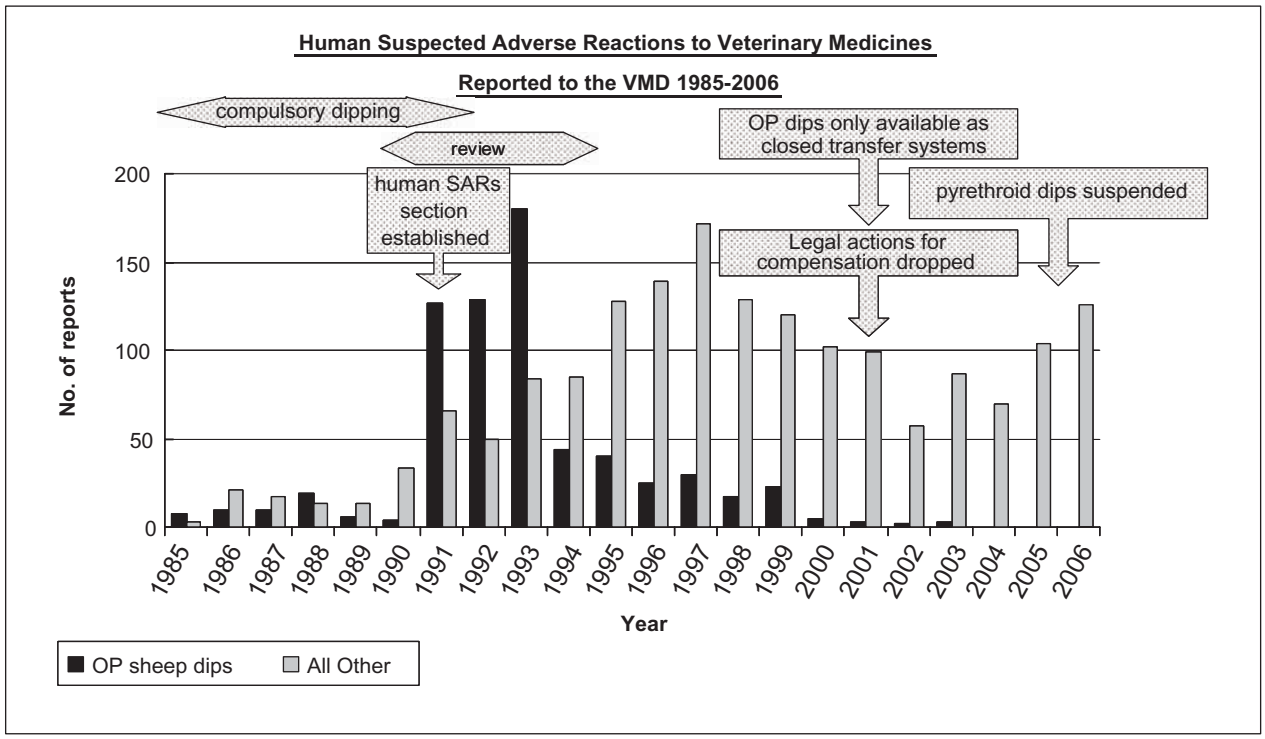

Fig. 21.1 Human suspected adverse reactions to veterinary medicines reported to the VMD 1985–2006.

Ministers constituted the Licensing Authority and acted upon advice from the Veterinary Products Committee (VPC), a body of independent experts, mostly academics. The licensing system was administered by the Veterinary Medicines Directorate (VMD), an agency of the Ministry of Agriculture, Fisheries and Food (MAFF), now the Department for the Environment, Food and Rural Affairs (DEFRA). Authorisation of sheep dip as veterinary medicinal products is now implemented by the same bodies initially under the European Directive 81/851/EEC (European Council, 1981), as amended, and subsequently under Directive 2001/82/EC as amended by Directive 2004/28/EC. Dipping of sheep has not been compulsory in the UK since 1992, but many farmers are continuing to dip, partly because of continuing outbreaks of scab or flystrike.

Adverse reactions

Stories first began to appear in the early 1990s that suggested that sheep farmers' and shepherds' health was being adversely affected by exposure to sheep dips (Swanston and Shaw, 1990), particularly to OP-containing dips. The symptoms can broadly be divided into two groups:

- Acute – consisting of a transient influenza-like illness.
- Long term – the symptoms and clinical signs of the latter were more varied but might include poor memory, depression, headache, pyrexia and sometimes signs referable to the peripheral nervous system (see review by Jamal, 1995).

These symptoms are not characteristic of either acute cholinergic effects or the delayed polyneuropathy known to be a problem with certain OPs (but not those used in sheep dips).

There was a large increase in the number of reports of adverse reactions to all veterinary medicines in 1992, in part due to the establishment of a separate scheme for the reporting of adverse reactions in humans (as opposed to reactions in treated animals) and extensive publicity and media coverage of the scheme (*Figure 21.1*).

Suspected adverse reactions to veterinary medicines are examined by the Appraisal Panel for Human Suspected Adverse Reactions to Veterinary Medicines (AP)[3]. This meant that many of the reactions reported during the first years following 1991 were related to exposures to veterinary medicines in earlier years. However, it is noteworthy that the number of reactions to OP sheep dips was almost equal to the number of reactions reported to all other veterinary medicines. The increase in reports of adverse reactions to OP sheep dips coincided with a study to investigate such effects by the National Poisons Information Service (NPIS) (London Centre). The number of human adverse reactions to OP sheep dips reported to the VMD adverse reactions scheme peaked at 180 reports in the year 1993. Consideration of these reports was the major activity of the AP for 9 of the 10 years from 1991 until 2001. During this period, only 31 reports of adverse reactions to synthetic pyrethroids, also used as sheep dips, were received.

Since the peak in 1993, reports of adverse reactions to OP sheep dips have declined steadily and none has been received in the years 2004–2006. There may be many reasons for the decline, but it is not predominantly the result of decreased use of OP dip products. Most of the exposure of concern occurred during the period when dipping sheep to prevent sheep scab was legally compulsory. This obligation was introduced in an unsuccessful attempt to eradicate sheep scab in the UK but was abandoned in July 1992. The compulsory element may have coloured attitudes to OP dips.

The VPC review (see below) of the OP dips resulted in changes, over several years, to the products available. The most recent modification to improve the safety of OP dip products was the replacement, in 2001, of the old containers, with packaging that is intended to avoid contact of operators with sheep dip concentrates. The review also stimulated various initiatives to increase awareness of the need for care in handling the products. The importance attached to improving knowledge was exemplified by the introduction of a compulsory certification scheme for competence in sheep dipping, first introduced in 1994 and modified in 2007 (see below). The failure of various attempts to show a clear causal association between using OP sheep dips and ill health and the collapse of pending legal action may also have contributed to the decline in reports.

The NPIS carried out an analysis of reports related to organophosphorus insecticides in 2003 (www.hpa.org.uk/chemicals/npis_reports.htm). Of a total of 137,458 enquiries, 175 concerned suspected exposure to OPs. Of these only seven occurred at an agricultural workplace, but it is not reported whether any were related to sheep dip use. This is in line with the VMD reporting scheme which indicated, by 2003, that adverse reactions to OP sheep dip were not a significant problem. Adverse reactions to other sheep ectoparasiticides are rare, even for the cypermethrin products used in sheep dipping.

VPC review

The European Directive 81/851/EEC (European Council, 1981) required member states to carry out a review of all veterinary pharmaceutical products licensed before 1983 to ensure that they met with the EU standards applicable at the time. The VMD commenced this work, involving the call-up of 2,700 products, in 1991. Many of the OP dip products had by then been licensed for a long time and the sponsor companies did not have the information required to meet the new standards of quality, efficacy and safety. Many products were withdrawn or reformulated to meet these requirements.

The review was largely completed by 1994, but changes in the products occurred throughout the period until the final change to the containers in 2001. Therefore the dip products available on the market today are not comparable with those that were in use in the 1980s. Because of concern about adverse human reactions to OP sheep dips, the VPC considered the VMD reviews of OP sheep dips twice during 1993, and advised that, because

of evidence that adequate precautions were not being observed during dipping, a Certificate of Competence should be held by those wishing to use OP sheep dips.

Regulations putting that into force were enacted in 1994, as the Medicines (Veterinary Drugs) (Pharmacy and Merchants' List) (Amendment) Order 1994. These regulations came into force on 1 April 1994 and had the effect of requiring that a certificate of enrolment for the certificate of competence would be necessary for OP sheep dip to be sold or supplied by a registered agricultural merchant. The same regulations required the sheep dipper to have the certificate of competence (as opposed to just having enrolled) from April 1995. These certificates are issued in England, Wales and Northern Ireland by the National Proficiency Tests Council (NPTC), and in Scotland by the NPTC or the Scottish Skills Testing Service. From April 2007, dipping could only be carried out under the supervision of a person holding a certificate of competence.

Because of concern that farmers and shepherds might not appreciate the inherent toxicity of the OPs used in sheep dips, a poster (VMD, 1993) and a leaflet (HSE/VMD, 1994) were produced, both directed at sheep farmers. The latter, has periodically been updated and is available in Welsh (HSE, 2007a) as well as English (HSE, 2007b).

Data on sheep dippers

Typically a sheep farmer will dip sheep on several consecutive days (depending on the size of the flock) and then be exposed to little or no OP for the rest of the year except possibly by handling the sheep. Thus the exposure pattern is of exposure for a few consecutive days once or twice a year rather than long-term low dose exposure. Some sheep dipping is done by contract dippers; these individuals may well be exposed for longer periods.

Few studies have attempted to measure operator exposure to OPs in sheep dips, but where this has been done, there is little biochemical evidence of exposure that would be sufficient to produce symptoms of poisoning (e.g. Rees, 1996). A number of studies have been undertaken on sheep dippers, but many have limitations: thus studies of self-selected groups, e.g. Ross *et al*. (2007), can do little to establish a causative relationship between exposure and symptoms and clinical signs.

Stephens *et al*. (1995) undertook a cross-sectional study of sheep farmers and shepherds. They compared neuropsychological performance in 146 sheep farmers who were exposed to organophosphates in the course of sheep dipping with 143 non-exposed workers (quarry workers). The farmers performed significantly worse than the non-exposed group in tests to measure sustained attention and speed of information processing. These effects remained even after adjustment for covariates. In addition, the exposed individuals showed greater vulnerability to psychiatric disorder than the quarry workers as measured by a general health questionnaire. There were no observed effects on short-term memory and learning. The authors concluded that repeated exposure to OP-based pesticides caused subtle changes in the nervous system. The main criticism of this study was the poor response rate.

Sub-groups of the exposed and non-exposed groups from the above study were further investigated by Beach *et al*. (1996). From a questionnaire given immediately after sheep dipping, the 10 most symptomatic and 10 least symptomatic sheep farmers and shepherds were chosen for study. Several months afterwards these two groups, along with 10 of the unexposed (quarry) workers, were investigated using a standardised neurological examination. Of the endpoints examined, two-point discrimination on the dorsum of the hand and the dorsum of the foot and mean calf circumference showed intergroup differences. Two-point discrimination in both sites was worst in the symptomatic farmers and best in the quarry workers. Calf circumference was least in the symptomatic farmers and greatest in the quarry workers.

This study is just one of many that were considered by the Committee on Toxicity of

Chemicals in Food, Consumer Products and the Environment (COT) in its review (see below).

The COT review

The Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT), a UK committee of independent experts, carried out a review, which was initiated because of reports from sheep dippers. However, the Committee endeavoured to look at all the available data on patterns of exposure to OPs likely to be relevant to agricultural workers. In the UK, much of the available data was from studies of sheep dippers. However in other countries, there was a considerable corpus of data on other patterns of agricultural exposure. The report of the COT, published in 1999 (COT, 1999), concluded that there was no clear evidence indicating that repeated low dose exposure to OPs, insufficient to cause acute effects, resulted in long-term damage to peripheral or central nervous system function in humans. However, the report identified a number of outstanding issues, and included recommendations for further research aimed at resolving these issues. In particular, the report recognised that the data available to date could not exclude the possibility that OPs cause disabling neurologic or neuropsychiatric disease in a small subgroup of exposed sheep dippers.

In September 2007, the COT considered the initial results of research commissioned by the UK Government in response to the recommendations of the 1999 report. The research had not provided clear evidence of a susceptible subgroup, but several of the studies were still incomplete and a further review is planned once the projects have been completed and can be considered in detail in the context of other recent work on OPs.

Other reviews and symposia

A number of symposia were organised by interested parties, such as that held in Plymouth in 1994 (Rosén *et al*., 1994) and in London (NFU, 1995).

The long-term effects of previous high dose exposure, the long-term effects of low dose exposure and patterns in between have been extensively reviewed (e.g. Eyer, 1995; Steenland, 1996). The UK Department of Health asked the Institute for Environment and Health (IEH) to review the chronic neurotoxic effects of OPs (IEH, 1998). At the same time, the Chief Medical Officer for England asked the Royal College of Physicians of London and the Royal College of Psychiatrists to look at the management of those claiming to be affected by sheep dip. The Royal Colleges established a working group, which reported in 1998 (RCP/RCPsych, 1998). Because of anecdotal reports about the amount of depression and suicide in sheep farmers, the UK Department of Health commissioned a study of suicide amongst farmers. This concluded that there was no evidence of an association of suicide with any particular farming activity (Hawton *et al*., 1998). The effects of low-level exposure to organophosphorus pesticides on fetal and childhood health have also been reviewed (IEH, 2002).

Steenland (1996) concluded that studies had shown chronic sub-clinical effects in the central and peripheral nervous system in individuals previously poisoned by OPs but that the outcome after long-term low-level exposure was less consistent, although some studies had shown effects. This conclusion is broadly consistent with that of the COT's findings (1999).

Government-funded research

In 1997, the incoming Labour government established a 'high-level' group of officials to report on OP products to ministers, to monitor the processes whereby information is shared between government departments, to draw together scientific evidence relevant to policy issues and to examine licensing procedures. This group comprised officials from the Ministry of Agriculture, Fisheries and Food, the Department of Health,

the Department of the Environment, Transport and the Regions, the Ministry of Defence, the Scottish Office, the Department of Agriculture for Northern Ireland, the Welsh Office, the Cabinet Office, the Veterinary Medicines Directorate, the Pesticides Safety Directorate, the (then) Medicines Control Agency and the Health and Safety Executive (OGOP, 1998). This group gave rise to a further group to coordinate research on OPs and to enable exchange of information about research being carried out in government laboratories and, under contract, in university departments.

Three government departments/agencies, the Health and Safety Executive, the Department of Health and the (then) Ministry of Agriculture, established a research programme to look at sheep dips. An important study of sheep dippers was undertaken by the Institute of Occupational Medicine in Edinburgh, in collaboration with the University of Glasgow (Pilkington *et al*., 1999, 2001; Sewell *et al*., 1999). This study was a cross-sectional study of 612 sheep dippers with two non-exposed groups (53 farmers who did not dip sheep and 107 ceramics workers). Neurological symptoms were recorded and thermal and vibration sensory thresholds were studied. There was a weak positive association between exposure to OPs and neurological symptoms, but there was no association between exposure to OPs and either thermal or vibration sensory thresholds. The prevalence of symptoms was higher in sheep dippers who handled the OP concentrate. There was also some evidence that thermal and vibration sensory thresholds were greater among those handling concentrate.

Conclusions

The sheep dip imbroglio was largely a phenomenon of the early 1990s and there are now few adverse reports regarding OP sheep dips. Clear evidence of exposure sufficient to cause classical cholinergic toxicity has not been found, while epidemiology studies of sheep dippers have proved difficult to perform because of poor response rates and difficulties in finding appropriate non-exposed referent groups. One conclusion of the 1999 COT review was that the data available to date could not exclude the possibility that OPs cause disabling neurologic or neuropsychiatric disease in a small subgroup of exposed sheep dippers. This has proved to be very difficult to refute or confirm, and may remain permanently unresolved. Some uncertainty about potential subtle effects therefore remains and the COT will carry out a second in-depth review of the evidence that exposure to OPs from a variety of causes may harm human health.

In the meantime, whether as a result of the changes to product content and presentation or many other potential explanations, OP dips are now being used without apparent adverse human health effects. These products provide one important component in the armoury of treatments available for the control of ectoparasite infestations in sheep.

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22 User safety assessment of veterinary medicinal products

K.N. Woodward

Introduction

The assessment of user safety is an important part of the regulatory pre-authorisation considerations for veterinary medicinal products under many regulatory schemes across the world. User safety is an important part of safety assessment for veterinary medicinal products in the European Union (EU) (Woodward, 2004) and, as described in Chapter 22, it is a legislative requirement within the European Union (EU).

The Committee for Medicinal Products for Veterinary Use (CVMP) has developed, through its Safety Working Party, a user safety guideline for pharmaceutical products, and through its Immunologicals Working Party, a user safety guideline for immunological products. These are available through the European Medicines Agency (EMEA 2003, 2007).

The guidelines are intended to provide guidance on the development of user safety assessments, which generally take the form of an expert report, which are included in dossiers submitted to support marketing authorisation applications in the EU. Their intention is to address the hazards and associated risks of a veterinary medicinal product, and to recommend risk management and risk communication measures which should ensure safe use, minimise risks and hence reduce the numbers of adverse reaction reports relating to human exposure (Woodward, 2005). Hence, they play a critical role in veterinary pharmacovigilance activities. These guidelines, or more specifically the pharmaceutical user safety guideline, are not without shortcomings (Woodward, 2007, 2008). Nevertheless, they are useful templates for authors of user safety assessments, and they provide prompts for the various considerations that the author should make. The pharmaceutical guideline will be used here to examine some of the more important points that arise.

It is important to recognise that the user safety assessment of veterinary medicinal products takes into account the actual formulation. While this means that the physical, chemical and biologicals hazards of the individual components, active ingredients, antigens and excipients cannot be ignored, it is the properties of the formulation, associated with specific presentations, that must be taken into account. In this chapter, the properties of a single potential component of a veterinary medicinal product, propylene glycol, will be examined to exemplify the process.

Propylene glycol (1,2-propanediol), an aliphatic diol, is a ubiquitous chemical used as a solvent and humectant in a wide variety of products including coolants, cosmetics, shampoos, sun protection products, agrochemicals, foodstuffs and tobacco (Fisher, 1980a; Motoyoshi *et al*., 1984; Andersen, 1994; LaKind *et al*., 1999; Kibbe, 2000; Cavender and Sowinski, 2001; Carmichael, 2005). In the European Union it is an approved food additive (E 1520). It has been favourably reviewed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1974).

The substance is widely used in human and veterinary medicines as a solvent for oral and parenteral products and as a ceruminolytic (Andersen, 1994; Mottu *et al*., 2000; Boothe, 2001). In veterinary medicinal products, propylene glycol is frequently used as a dust suppressant in medicated feeds and premixes and as a solvent in oral and parenteral preparations. Hence, it is a useful substance to consider in the context of user safety assessment.

The Guideline

The Guideline comprises a number of sections, the first three of which are of an introductory nature. Of these three sections, the first is the introduction itself, and the second, a description of the scope of the Guideline. The third section describes the basic principles of user safety assessment. It asserts that the following steps are involved in user safety assessment:

Exposure assessment > hazard identification and characterisation > risk characterisation > risk management

This may not be the most efficient way to proceed, as hazard identification and assessment might be seen as a better starting point, while risk communication, particularly for medicinal products (of any type), is certainly the most appropriate end-point (Woodward, 2008). Nevertheless, for the purposes of this chapter, and as the Guideline is currently in this form, the format shown above will be adopted.

Exposure assessment

Clearly, this will depend on the nature of the product. For an oral preparation, exposure to propylene glycol is likely to be dermal through spillage, while an injectable product may also involve dermal exposure, but needlestick injuries and self-injection must also be taken into account. Under most circumstances, self-injection of any appreciable amount of substance is only likely to occur with automatic administration equipment. Self-injection with manual equipment is only likely to occur under bizarre circumstances (intentional or accidental, i.e. falling onto the syringe or impact with the syringe, for example as a result of crushing by large animals during administration). With dust-suppressed medicated feeds, exposure is likely to be through the dermal routes with the hands being the major target of exposure (during mixing/admixing or feeding).

The above factors are self-determining. What is not self-determining, and indeed is difficult to quantify, is the extent of exposure:

- How much of an oral solution will users spill on their hands (and elsewhere)?
- What fraction of an injectable dose will be administered in a self-injection incident?
- What is the extent of exposure to propylene glycol during handling of a dust-suppressed medicated feed?

These are problematic areas on which the Guideline offers little assistance. Inevitably, a pragmatic approach is required. For example, if the product is supplied as an oral presentation in a 10-ml bottle, then spilling the entire contents over the hands during administration might seem to offer a reasonable worst case. However, if the product is supplied in a litre bottle, then contamination with the entire contents might appear unreasonable.

It should be borne in mind that the Guideline itself makes it clear that any exposure assessment should represent worst case but realistic exposure scenarios. It does not cover exposure during manufacture. Those considered to be exposed

include veterinarians, farmers, breeders, pet-owners, millers, animal beauticians, sheep shearers and bystanders (although it may be difficult to define this latter category in all circumstances).

For dermal exposures, methods and models are available which may be used to estimate exposure (see, for example, Schneider *et al*., 1999; Marquart *et al*., 2003; Oppl *et al*., 2003; Jakasa and Kezic, 2008; Kezic, 2008), while for self-injection, some basic and practical, albeit groundless (on an evidential basis), assumptions must be made. For example, for a manual syringe, it might be considered realistic, although highly unlikely, that between 10 and 50% of the intended dose is delivered through self-injection or that a needlestick injury delivers 0.1 ml of solution (Woodward, 2008). On the other hand, automatic injection may be assumed to deliver a 100% intended dose from the possibly hundreds of doses available in the reservoir. However, it then needs to be recognised that with automatic equipment, the nature of the adverse reaction is likely to be physical (trauma) rather than biological (pharmacological or toxicological).

The purpose of this entire part of the exercise is to predict the type and degree of exposure, and from this to extrapolate the dose. From this information, and from other data available, e.g. pharmacokinetic and physicochemical data, it should then be possible to estimate the systemic dose. Nevertheless, less obvious routes of exposure should also be considered, including hand to mouth oral exposure as a result of food consumption or smoking. Furthermore, the likely frequency and duration of exposure should be factored into the assessment.

Hazard identification and assessment

Data may be available from a variety of sources including from the published literature, from sponsor-conducted or sponsor-financed studies and from monographs or summaries generated for regulatory purposes. The data needed to assess user safety include (but are not limited to):

- physico-chemical: flammability, explosivity, vapour pressure, octanol-water partition co-efficient;
- pharmacological: degree and extent of absorption, rate of excretion, degree of biotransformation, metabolic conversion to innocuous metabolites;
- toxicological: acute toxicity, repeat dose, effects on reproduction, genotoxicity, carcinogenicity, local effects.

With propylene glycol, there is a considerable body of data available. However, much of this data may at best be regarded as mature (generated in the 1960s and 1970s), while some is downright elderly (1940s and 1950s). Such considerations are important but should not be used to condemn older information. Each publication should be judged on its own merits and on its criticality to the assessment. New data may have to be generated if it is critical and highly relevant to the assessment.

The biological data available for propylene glycol can now be examined.

Metabolism

A study using ligated loops of jejunum from rats, cats and rabbits suggested that propylene glycol is well absorbed from the mammalian gastrointestinal tract. In in vivo studies in rats, around a third of the administered dose was excreted in the urine over a 7-day period (van Winkle, 1941). After oral administration to rats, propylene glycol appeared in the blood, with maximum concentrations at around 2 hours post-dose, and was excreted in the urine. Propylene glycol concentrations in the urine of rats pre-treated with the alcohol dehydrogenase inhibitor pyrazole were higher than in control animals given propylene glycol only. The rate of metabolism was high at 8.3 mmol/kg/hour (Morshed *et al*., 1988).

In rabbits given intravenous infusion doses of propylene glycol, the main route of clearance was metabolism. Renal excretion accounted for only 14% of the administered dose (Yu and Sawchuk, 1987). After oral doses of propylene glycol to rabbits, metabolism was rapid. Concentrations of lactate in blood were elevated (Morshed *et al*., 1994).

In human patients given the antineoplastic drug mitoquidone in propylene glycol by intravenous infusion over a 4-hour period, the plasma half-life was around 2.3 hours. There was no significant increase in plasma lactate concentrations and no evidence of adverse effects related to propylene glycol (Speth *et al*., 1987).

The major pathway of metabolism of propylene glycol appears to be through methyl glyoxal to lactaldehyde and lactate, and pyruvate which then may enter gluconeogenesis (Ruddick, 1972; Christopher *et al*., 1990).

Toxicity

Acute toxicity

Propylene glycol has low acute oral toxicity to rats, mice, rabbits and guinea-pigs, with LD_{50} values being in the order of, or in excess of, 20 g/kg bw (Bost and Ruckebusch, 1962; Ruddick, 1972; Bartsch *et al*., 1976; Clark *et al*., 1979). The lowest lethal dose was 20.9 g/kg bw (Clark *et al*., 1979).

Single oral doses of 730 or 2,940 mg/kg bw propylene glycol to rats resulted in decreases in haemoglobin, packed cell volume and red cell counts. These rapidly returned to normal. Osmotic fragility of erythrocytes was unaffected (Saini *et al*., 1996).

Following intraperitoneal administration, LD_{50} values were in the order of 11 g/kg bw in mice and 13 g/kg in rats (Bartsch *et al*., 1976; Budden *et al*., 1979), while intravenous LD_{50} values were in the range 6–8 g/kg bw for mice and 6 g/kg bw for rats. The intramuscular LD_{50} was 20 g/kg bw in rats and the subcutaneous LD_{50} was 18.5 g/kg in the mouse (Andersen, 1994). An intravenous dose of 25 g/kg bw was lethal in the dog (Spector, 1956).

Repeat dose toxicity

When rats were given up to 3.1 g/kg bw propylene glycol for 3 days by gavage some animals had hyperaemia of the gastrointestinal tract. This was more severe in animals where the propylene glycol had not been diluted with water. Similar findings were made in dogs given propylene glycol orally at doses of up to 3.1 g/kg bw (Staples *et al*., 1967). Cats given a diet containing 12% propylene glycol (1.6 g/kg bw/day) for 5 weeks showed no ill effects although plasma lactate concentrations were slightly elevated. Animals in a separate group given propylene glycol in feed at 41% (8 g/kg bw/day) for 22 days showed significant rises in plasma lactate concentrations. Animals given the high feed concentrations showed polyuria and polydipsia, decreased activity, depression and a degree of ataxia (Hanzlik *et al*., 1939).

No compound related effects were noted in rats given diets containing 50,000 ppm propylene glycol for 15 weeks. Haematological examination revealed no abnormalities (Gaunt *et al*., 1972). Serum and urine analyses were normal while organ weights were unaffected by compound administration.

Rats given intravenous infusions of 5.2 g/kg bw/day propylene glycol in aqueous ethanol for 2 weeks developed red-coloured urine. One male and one female dog similarly administered 4.1 g/kg bw/day, again for 2 weeks, also developed red urine. Following this 2-week treatment, decreases in haemoglobin and erythrocyte counts with reductions in packed cell volumes were noted in rats and dogs. Splenomegaly and renal haemosiderin were found at necropsy (Fort *et al*., 1984).

When 0.2 ml (approximately 200 mg) was administered subcutaneously three times a week for 2 weeks to hairless mice, cytological changes were seen in the bladder epithelium. The major

effect noted was an increase in the number of diploid cells and a slight decrease in the number of tetraploid cells. Some of the bladder epithelial cells were necrotic (Farsund, 1974, 1978).

In an inhalation toxicity study, groups of rats were exposed to propylene glycol at atmospheric concentrations of 0, 41, 650 or 1,800 mg/m^3, 6 hours per day, 5 days per week, for 2 weeks with a total of nine exposures. At the end of the exposure period, half of the animals were subject to an 18-day recovery period. Haematology, serum chemistry, gross pathology, histopathology and organ weights were similar in treated animals to those in untreated controls and the highest concentration employed was the no-effect concentration (Scott *et al*., 2005).

Effects on reproduction

Reproductive effects

In a three generation study in rats, animals were fed diets containing 0–30% w/w propylene glycol as an isocalorific replacement for cornstarch in the feed. Two females were housed with one male and examined for pregnancy at days 70–80. There appeared to be no major effects on reproductive performance at 2.5 or 5.0% w/w propylene glycol, but at 7.5% and above the average number of pups per dam was reduced and the average number of pups per litter was decreased. At 30% w/w propylene glycol the number of females with litters was markedly reduced and the animals did not produce a third generation (Guerrant *et al*., 1947).

A more recent multigeneration study has been conducted by the US National Toxicology Program (NTP). In this study, mice were exposed to propylene glycol in drinking water at concentrations of 0, 0.5, 1.0, 2.0, 5.0 and 10.0% w/v, equivalent to 1.82, 4.8 and 10.1 g/kg bw/day for 14 days. Dams continued to be exposed through the drinking water in the F_1 generation. The fifth litters of F_1 animals were allowed to mate to produce the F_2 generation.

Propylene glycol had no adverse effects on reproductive performance in this study; there were no deleterious effects on body weights, water consumption, mating index, litter size, number of live pups, sex ratios or weights of pups. Sperm morphology and vaginal evaluations were normal. There were no significant differences in average weights of the seminal vesicles, right cauda, prostates, right testes and right epididymis in males. Sperm counts, motility and numbers of abnormal sperms were similar in treated animals to control values. Treated females had similar oestrous cycle patterns to untreated controls. Consequently, in this study, propylene glycol had no adverse effects on reproductive performance in rats at doses of up to approximately 10 g/kg bw/day (National Toxicology Program (NTP), 1985; Morrissey *et al*., 1989; Lamb *et al*., 1997).

Propylene glycol had no significant effects on differential ovarian follicle counts in mice when given at 5% in the drinking water. Other substances known to affect reproductive performance, including ethylene glycol monomethyl ether, produced significant reductions in follicle counts (Bolon *et al*., 1997).

Teratogenicity including embryotoxicity

In an in vitro study, zygotes derived from fertilisation of mouse oocytes were incubated for 20 minutes with 1.5, 3.0 or 6.0 M propylene glycol in phosphate buffered saline solution. Each of these and control zygotes were then incubated with 0, 0.1 and 0.25 M sucrose solution. The zygotes were then incubated under 5% carbon dioxide and the numbers that developed into two-celled embryos were identified.

Propylene glycol at 1.5 M had no effects on zygote cleavage. However, cleavage was reduced at 3.0 M and completely inhibited at 6.0 M. Further work has suggested that propylene glycol affects intracellular pH, but the effects on mouse zygotes may be as a direct result of effects on the cell membrane (Damien *et al*., 1989, 1990).

Pregnant CD-1 mice were given gavage doses of 0.5, 5.2 or 10.4 g/kg bw/day propylene glycol on days 6–15 of gestation. Animals were sacrificed on day 18 of gestation and the uterine contents were examined.

There were no adverse effects on maternal animals with respect to pregnancy rates, food consumption, body weights, the number of corpora lutea, resorptions, dead fetuses or sex ratio of pups, or on necropsy findings. However, water consumption was significantly increased in treated animals. There were no adverse developmental effects in pups from treated mice when compared to historical and concurrent control values (Driscoll *et al*., 1993).

Pregnant CD-1 mice were given oral gavage doses of 10 g/kg bw/day on days 8–12 of gestation. Propylene glycol had no adverse effects on maternal animals or on the incidence of resorptions. There were no adverse effects on the outcome of pregnancy in treated animals when compared with control values (Kavlock *et al*., 1987).

Taken together, these studies, along with other more experimental protocols (NTP, 2004), suggest that propylene glycol has no major effects on the outcome of pregnancy in mammalian species.

US FDA-sponsored studies were conducted in mice, rats, hamsters and rabbits. Mice and rats were given propylene glycol at doses of 0, 16, 74, 345 or 1,600 mg/kg bw/day on days 6–15 of gestation, hamsters were given 0, 15.5, 72, 334.5 or 1,550 mg/kg bw/day on days 6–10 of gestation and rabbits were dosed with 0, 12.3, 57.1, 267 and 1,230 mg/kg bw/day on days 6–18 of gestation. In all cases, administration was by gavage. Propylene glycol had no significant effects on the outcome of pregnancy in these animals (Food and Drug Research Laboratories, 1973).

Chronic toxicity

Rats given dietary propylene glycol for 2 years showed no evidence of profound adverse effects (see Carcinogenicity, below).

Similarly, no signs of toxicity were noted in dogs given 2 or 5 g/kg bw/day propylene glycol for 2 years except for an elevated production of urine in animals given the highest dose. There were no abnormalities seen in any organ on microscopic examination (Weil *et al*., 1971). Dogs given 5% propylene glycol for up to 9 months showed no signs of toxicity. There were no adverse effects on liver or kidney function (Van Winkle and Newman, 1941).

Genotoxicity

Propylene glycol has been evaluated in a number of in vitro and in vivo tests examining a number of genotoxicity end-points.

In vitro tests

Propylene glycol has been tested in bacterial reverse mutation (Ames) tests with *Salmonella typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100 and concentrations up to 1–10,000 µg/plate with and without metabolic activation in one study, and strains TA 1535, TA 1537, TA 92, TA 94 and TA 98 at 10 mg/plate with metabolic activation in another (Clark *et al*., 1979; Ishidate *et al*., 1984). Negative results were obtained in these two studies.

The material was also tested in an assay for DNA damage using alkaline elution where Chinese hamster lung V79 fibroblasts were incubated with the solvent, with and without metabolic activation for 1–4 hours and a concentration of 10 mM. There was no evidence of DNA damage in this assay (Swenberg *et al*., 1976).

Propylene glycol has given positive results in studies of sister chromatid exchange (SCE) and a test for chromosomal aberrations in Chinese hamster fibroblasts (Sasaki *et al*., 1980; Ishidate *et al*., 1984). Interestingly, these tests were conducted at high concentrations (up to 23 mg/ml in the SCE study and up to 32 mg/ml in the chromosome aberration study) and it is likely that these results reflect the effects of hyperosmolality rather than any genotoxic potential of the substance.

Negative effects were obtained in other studies, including mitotic recombination in yeast and base pair substitution in *S. typhimurium* strains G-46 and TA 1530 (Green, 1977).

In vivo test

Propylene glycol was tested in a mouse micronucleus using single intraperitoneal doses of 2,500–15,000 mg/kg bw. Polychromatic erythrocytes were harvested from each mouse. The substance was not genotoxic under the conditions of this test (Hayashi *et al*., 1988).

Carcinogenicity

Groups of 30 male and 30 female Charles River CD rats were given diets containing 0, 6,250, 12,500, 25,000 or 50,000 ppm (this latter dietary level being equivalent to 2.5 g/kg bw/day) propylene glycol for 2 years. There were no effects on body weight gain, food consumption, haematology or organ weights. Mortality was unaffected by propylene glycol intake. There was no increased incidence of any tumour type (Gaunt *et al*., 1972).

Irritant potential

Propylene glycol produced only mild skin irritation when applied undiluted to the intact and abraded skin of rabbits using a modified Draize test (Draize *et al*., 1944; Phillips *et al*., 1972). When applied to the skin surfaces of rabbits or guinea-pigs using 48-hour occlusive dressings or to miniature pigs using 48-hour or 21-day open or occlusive dressings, propylene glycol had no notable irritant effects (Motoyoshi *et al*., 1984).

The substance had negligible skin and eye irritant potential when tested in rabbits as part of a study of inter-laboratory variation in irritancy testing (Weil and Scala, 1971; Clark *et al*., 1979; Wahlberg and Nilsson, 1984; Guillot *et al*., 2002). An experimental method using the release of histamine from rat peritoneal cells concluded that propylene glycol had low irritant potential (Jacaruso *et al*., 1985).

Other aspects of toxicity and pharmacology

Propylene glycol induces Heinz body formation and anaemia in cats fed diets containing up to 1.6 g/kg bw propylene glycol for 5 weeks, a concentration that may be found in some commercial cat diets, and at 8 g/kg bw (Bauer *et al*., 1992a, b). In the low dose group, 28% of cats were affected, while in the high dose group, 92% were affected (Christopher *et al*., 1989a, b). There may be moderate lactic acidosis (Christopher *et al*., 1989b, c). Similar haematological effects were seen in kittens given diets containing 6 or 12% propylene glycol, and red blood cell survival was significantly reduced.

Dogs given propylene glycol developed vomiting and diarrhoea but showed no signs of the haematological adverse effects noted in cats (Ruble *et al*., 2006). However, haematological abnormalities were reported in two dogs anaesthetised with etomidate formulated in propylene glycol and given by infusion (Moon, 1994).

Propylene glycol poisoning, sometimes with fatal consequences, has occurred (rarely) in horses given large oral doses (8–17 g/kg bw) of the substance (McClanahan *et al*., 1998; Van den Wollenberg *et al*., 2000; Deprez *et al*., 2002; Dalefield, 2004). Propylene glycol toxicosis, with acidosis, has been reported in a llama (Ivany and Andersen, 2001). Cattle are often treated for bovine ketosis with propylene glycol. At lactation, there is a major increase in energy requirements produced by the mammary glands in order to supply milk production. This demand can be partly met by increasing feed intake and by mobilising fat reserves. However, excessive fat mobilisation can result in imbalances in liver carbohydrate and fat metabolism leading to ketosis, usually 2–7 weeks after calving.

Propylene glycol has been used in the treatment of ketosis for several decades. It is absorbed from the rumen and a proportion of the dose is

converted to lactate through methyl glyoxal. In turn the lactate is converted to pyruvate which then enters gluconeogenesis which offsets imbalances in hepatic carbohydrate and fat metabolism (Miettinen, 1993; Cozzi *et al*., 1996; Bobe *et al*., 2004; de Oliveira *et al*., 2004; Nielsen and Ingvartsen, 2004; Kupczyński *et al*., 2005; Rukkwamsuk *et al*., 2005; Mulligan and Doherty, 2008; Rizos *et al*., 2008). Similar activity is also apparent in sheep (Chiofalo *et al*., 2005). For these and possibly other reasons, propylene glycol may be less toxic in ruminants than it appears to be in cats and dogs, although even in these species, toxic effects are only generally seen with very large doses. However, the effects on gluconeogenesis are not restricted to ruminants, and hyperglycaemia, probably involving similar mechanisms, has been reported in rats and rabbits following propylene glycol treatments (Giri *et al*., 1970; Vaille *et al*., 1971).

There have been reports of ototoxicity following administration to the ears of guinea-pigs (Morizono and Johnstone, 1975; Vernon *et al*., 1978; Morizono *et al*., 1980).

Pharmacological studies have shown that propylene glycol may exert some CNS effects in various animal models (Singh *et al*., 1982). Some of these may be due to its alcohol-like effects.

Effects in humans

Systemic effects

In humans, accidental or intentional ingestion of relatively large quantities of propylene glycol are associated with hyperosmolality and metabolic acidosis. It may also induce central nervous system toxicity. The acidosis is probably due to conversion of propylene glycol to lactic acid and pyruvic acid resulting in lactic acidosis, whereas the CNS toxicity is more likely a result of excessive propylene glycol and its alcohol-like effects. Some cases of propylene glycol toxicity in humans have resulted in fatalities (Martin and Finberg, 1970; Arulanantham and Genel, 1978; LaKind *et al*., 1999; Brooks and Wallace, 2002; Guillot *et al*., 2002; deRoux *et al*., 2005; Zar *et al*., 2007a, b).

Some cases of propylene glycol toxicity have resulted from the use of the substance as a solvent in medicinal products given orally, topically or intravenously. Administration of lorazepam, diazepam and etomidate has been associated with toxicity, occasionally severe or even life-threatening (De Wiele *et al*., 1995; Levy *et al*., 1995; Van de Wiele *et al*., 1995; McConnell *et al*., 1996; Woycik and Walker, 1997; Varon and Marik, 1998; Arbour, 1999, 2003; Wilson *et al*., 2000, 2007; Al-Khafaji *et al*., 2002; Parker *et al*., 2002; Yaucher *et al*., 2003; Zar *et al*., 2007a, b). Topical administration of silver sulfadiazine in propylene glycol to an 8-month-old infant suffering from severe burns led to hyperosmolality and may have precipitated cardiac arrest (Fligner *et al*., 1985). Administration of high doses of intravenous pentobarbital and phenobarbital in propylene glycol vehicle for the treatment of refractory status epilepticus resulted in propylene glycol toxicity (Bledsoe and Kramer, 2008).

The dose or blood concentrations required for severe toxicity and lethality are unknown. Blood concentrations of over 3,000 mg/l have been reported in some cases of severe toxicity and fatalities (deRoux *et al*., 2005). However, oral doses of 2–15 ml were well tolerated by patients given the substance as part of an investigation into effects on skin (Hannuksela and Förström, 1978). The majority of an administered dose of propylene glycol is excreted through the kidneys and so renal disease is likely to exacerbate toxicity (deRoux *et al*., 2005). Renal toxicity has been observed in patients given lorazepam infusions, acute tubular necrosis has been reported in a patient given medications containing propylene glycol, and the material is cytotoxic in vitro; cytotoxicity has been noted in cultured human proximal tubule cells (Mochida and Gomyoda, 1987; Morshed *et al*., 1988, 1994, 1998; Hayman *et al*., 2003; Yaucher *et al*., 2003; Kraut and Kurtz, 2008). Hence, nephrotoxicity could also exacerbate its other toxic effects.

Local effects

Under certain conditions, propylene glycol is a primary skin irritant and usually with occlusion and prolonged exposure to the undiluted solvent or to preparations containing high concentrations (Warshaw and Herrmann, 1952; Marzulli and Maibach, 1974; Hannuksela *et al*., 1975; Nater *et al*., 1977; Goldsmith, 1978; Fisher, 1980b, 1996; Trancik and Maibach, 1982; Commens, 1990; Funk and Maibach, 1994).

There have been isolated reports of dermal sensitisation and dermatitis, largely in patients given topical preparations or procedures where exposure to propylene glycol occurs (Hannuksela *et al*., 1975; Pevny and Uhlich, 1975; Fisher, 1979, 1980a, b, 1996; Angelini and Menegghini, 1981; Angelini *et al*., 1985; Adams and Maibach, 1985; Cantanzaro and Smith, 1991; Claverie *et al*., 1997; Eun and Kim, 1989; Frosch *et al*., 1990; Kim and Kim, 1994; Gonzalo *et al*., 1999; Uter and Schwanitz, 1996). However, this is uncommon and the higher concentrations often used in patch tests may have induced irritation (De Groot, 1997). An analysis of 45,138 patients patch tested with 20% propylene glycol between 1992 and 2002 suggested that most skin reactions were irritant in nature and that the substance posed a very low risk for dermal sensitisation on uncompromised skin (Lessmann *et al*., 2005).

Exposure to high concentration propylene glycol mists may induce ocular and upper airway irritation, and occasionally cough (Wieslander *et al*., 2001).

Assessment of data

The data reviewed above vary in terms of quality and breadth, and some, as discussed earlier, are rather old. Nevertheless, they fill the majority of the regulatory toxicological boxes and it *is* possible to make a hazard assessment. Thus, propylene glycol has low mammalian toxicity in conventional laboratory toxicity studies. It has no adverse reproductive effects and is not teratogenic. There was no evidence of chronic toxicity and it was not carcinogenic in rats. There was no convincing evidence of genotoxicity and the molecule has no structural alerts for either carcinogenic or genotoxic potential. It had no notable dermal or ocular irritant potential in animal studies.

The substance is metabolised to lactate and pyruvate. In high doses this can lead to metabolic acidosis while propylene glycol itself can produce alcohol-like effects. Hyperglycaemic effects may occur. It also leads to hyperosmolality. These effects can produce evidence of toxicity in animals and humans, but usually only after large oral or intravenous doses and occasionally after topical administration. The toxicity thus produced can be severe and occasionally fatal, but again it is important to emphasise that high doses are required.

In humans, prolonged exposure to the undiluted material or to high concentration solutions can produce skin irritation. There is no evidence to suggest that propylene glycol is a potent skin sensitiser and recent data suggest that many instances of so-called sensitisation may in fact be due to skin irritation.

Taken together, the available animal and human data suggest that low oral doses of propylene glycol are unlikely to pose a significant human health issue, and occupational exposure to veterinary medicinal products during their normal use will pose minimal risks. Treated animals may be at risk from large oral or parenteral doses of propylene glycol, but these are unlikely to be a problem with the majority of veterinary medicinal products as the doses will be low in normal treatments. Moreover, it is likely that any potential adverse effects would be detected in routine target animal safety testing, and formulations modified to prevent such problems occurring in clinical situations. However, the data suggest that propylene glycol may not be an entirely suitable excipient for inclusion in otic preparations because of the possibility of ototoxicity. Pharmacovigilance activities post-marketing should lead to the detection or confirmation of rarer effects.

Risk assessment

In the context of user safety, risk assessment asks the question 'Is there a probability that the hazards revealed (in the forgoing section) will be expressed during use?' Or, and perhaps seen another way, 'What is the probability of any seen in the hazard assessment being expressed during use of the product under realistic exposure scenarios during normal use?' It is generally almost impossible to answer these types of questions. The probabilistic issues can rarely be underpinned by sound data, so quantitative risk assessment, at least in its strictest sense, is not possible. An alternative question to ask is then 'What is likely to happen if exposure occurs?', and especially if it happens in the circumstances predicted in the exposure assessment. Put another way, is it likely that the toxicity profile, or particular parts of it, which has been revealed in the hazard assessment will be expressed under the exposure scenarios described?

Clearly, for propylene glycol, this is not a major issue and, arguably, some skin irritation following extensive exposure is the only effect likely. Any other aspects of the toxicity noted are extremely unlikely to be expressed under *realistic exposure scenarios*. Equally clearly, significant toxicity could be predicted under extreme conditions, e.g. intentional consumption of a large quantity of a formulation as part of a suicide attempt. Such abuses fall outside the scope of the guideline. However, it is conceivable that a young child might be at risk following the ingestion of a large oral dose of the substance, and this aspect, being relevant, must be discussed in the user safety assessment.

For systemic toxicity and, more importantly, for dose-related effects, it is normal to look at no-observed effect levels from toxicity (and pharmacology) studies and compare these with the predicted delivered doses from the exposure assessment. Thus, margins of exposure (MOE) can be established for particular effects. For non-dose-related effects, such as skin or eye irritation and dermal sensitisation, it is usually practical (if uncomfortable) to assume that exposure equals effect. Occasionally, useful information may be derived from modelling. Physiologically based pharmacokinetic (PBPK) models can sometime have a useful role to play in risk assessment (Chiu *et al*., 2007).

Risk management

Risk management, perhaps better referred to as risk reduction, sets out the measures necessary to reduce or mitigate risks through exposure avoidance or reduction, generally by way of personal protective equipment and simple hygiene measures. This might amount to no more than a pair of impervious gloves and washing hands after use. However, it might extend to respirators, boots and other impervious clothing, depending on the nature of the product in question. For propylene glycol, gloves and a warning to wash hands after use would be appropriate.

Risk communication

The major purpose of risk communication is to convey not only information about the risks, but also information associated with the hazards and the measures needed to reduce or mitigate the risks, to the end user (e.g. the veterinarian) and to others (farmers and pet owners). These normally take the form of phrases and warnings in the summary of product characteristics and, subsequently, on the product label. Such phrases might include:

- May cause skin sensitisation.
- Avoid contact with skin.
- Wear protective gloves when using this product.
- In case of skin contact, wash affected parts with copious amounts of water.
- If you know you are sensitive to (ingredient) do not use this product.

And even:

- In case of skin contamination and severe skin reaction, seek medical advice and show this label to your physician.

Little of this would be relevant to propylene glycol. In the case of a veterinary medicinal product containing the substance, the following would be adequate:

- May cause skin irritation.
- Avoid contact with skin.
- Wash hands after use.
- Keep away from children.

Discussion

Much of the foregoing is subjective, and others carrying out this assessment, including those in regulatory authorities, might reach other, but hopefully similar, conclusions. With medicines, including veterinary medicines, there are two major reasons for the extensive testing required, and for the data generated:

- Is it safe, of adequate quality and does it work? Hence, should it be authorised/approved?
- If it is authorisable/approvable, what labelling, advice, etc. should go into the product literature?

The labelling and advice are not restricted to warnings and contraindications. They also cover indications and other practical aspects such as methods of administration. The user safety assessment is a codified method of translating the outcomes of safety studies, including toxicology studies, combining these with exposure assessments, and finally leading to useful and meaningful advice, warnings and recommendations for the user.

The example chosen here was intentionally simplistic and generic. It was, however, real. For an actual veterinary medicinal product with propylene glycol as a solvent to dust suppressant, the whole process would be repeated using data on the active ingredient, and indeed on any other excipients and constituents.

Moreover, as indicated earlier, other information related to user safety would have to be considered (is it volatile and hence inhalable, is it flammable, could it explode?).

The ingredients used in veterinary medicinal products are generally safe, especially if used as recommended. However, that does not mean that these ingredients, or the formulated products, have absolute safety – few things have. The user safety report is an amalgam of hazard and risk assessment on the product with *all* of its ingredients, active or otherwise, and the subsequent risk management outcomes and risk communication options. Of course, some active ingredients used in veterinary medicinal products are more toxic than others. These include:

- diazinon and other organophosphorus compounds used as ectoparasiticides;
- euthanasia agents;
- volatile anaesthetics;
- pyrethroids, especially synthetic pyrethroids.

The purpose of the user safety assessment, through the end-results of risk management and risk communication options, is to ensure that these products, and indeed all veterinary medicinal products, including vaccines and other biological products, can be used safely when used as recommended. In doing so, this makes a huge contribution, hopefully a quelling of adverse reaction reports in humans, in veterinary pharmacovigilance.

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23 Maximum residue limits

K.N. Woodward

Introduction

In accordance with Directive 2001/82/EC as amended by Directive 2004/28/EC, pharmacologically substances intended for use in food animals must have maximum residue limits (MRLs) or an MRL not be required on public health grounds, before marketing authorisations can be used in the European Union (EU). To be more specific, pharmacologically active substances must be entered into one of the Annexes I–III of Council Regulation (EEC) No. 2377/90, the so-called MRL Regulation (Woodward, 1996, 1997, 2000; Dixon, 2001).

In fact, the purpose of this legislation is to ensure that substances intended for use in food animals are adequately assessed for their harmful potential and that consumers of food of animal origin are adequately protected. As we shall see, these assessments take into account factors other than toxicity. As noted elsewhere in this book, with the example of clenbuterol, pharmacological properties that may be desirable for treated animals may not be at all desirable if they occur in the consumer who has eaten animal products. Not only does this sentiment apply to pharmacodynamic effects of drugs expressed in the animal (e.g. β-adrenergic effects, various hormonal effects, anaesthesia, analgesia), but also it is true of more indirect effects.

For example, it is evidently desirable that the antimicrobial effects of antibiotics are seen in the treated animal, i.e. that the drug exerts its bacteriostatic or bactericidal effects on the pathogenic bacteria causing the disease. However, it is not desirable that active residues of such drugs adversely affect the normal gastrointestinal flora of consumers eating meat containing antimicrobially active residues. This issue, although not essentially a problem of toxicity (although it is related to the toxicity of the drug to bacteria), will be discussed later as it is relevant to safety assessment and needs to be considered along with pharmacological and toxic properties of the drug in question.

Finally, the presence of a particular drug in an edible product is not in itself problematic. What is critical is how much of the drug (and its metabolites) is present, and how long it persists. Veterinary drug residues may be composed of the original substance, the parent drug and, frequently, various metabolites. Some of these may be present as bound residues, i.e. residues that are covalently bound to macromolecules such as proteins or nucleic acids (Baer *et al.*, 1977; Thorgeirsson and Wirth, 1977; Farber, 1980;

Weber, 1990). These residues are subject to various metabolic processes including eventual conversion to non-toxic metabolic products including eventually water and carbon dioxide and other physiological substances, and excretion in the urine, expired air or bile. In other words, they will eventually decrease in concentration as time passes, as a result of the animal's metabolism. This is known as residues depletion or depuration.

Consequently, the risks posed by residues of a veterinary drug depend not only on its toxic, pharmacological and microbiological activities, and those of its metabolites, but also on its rate of disappearance from the animal. It is clear from this that another critical factor therefore is the analyst's ability to measure the concentration of the drug and its metabolites, which in turn is dependent on having an adequate analytical method. All of these factors are important in the elaboration of MRLs.

Regulation of residues in the EU

In the EU, MRLs are established by the Committee for Medicinal Products for Veterinary Use (CVMP), a part of the structure of the European Agency for the Evaluation Agency of Medicinal Products (EMEA). Specifically, the CVMP issues an opinion after consideration of the available toxicological and residues depletion data and the information on the proposed analytical method, provided by the drug sponsor. This opinion is usually for entry into one of the four Annexes of Regulation (EEC) No. 2377/90. The actual decision, in legal terms, is taken by the European Commission, and the Annex entries are published in the *Official Journal of the European Union*. The nature of the Annexes is shown below:

- Annex I: full MRLs; the data supplied are adequate to address safety and residues concerns.
- Annex II: on public health grounds, MRLs are not necessary. These entries include those for simple salts, innocuous substances and compounds that are rapidly converted in the animal to non-toxic metabolites.
- Annex III: provisional MRLs. The majority of data in the supporting dossiers are satisfactory, but some relatively minor points need addressing. Satisfactory resolution leads to Annex I (or possibly Annex II) entry.
- Annex IV: substances are not considered safe on public health grounds. Annex IV entries include nitrofurans, nitroimidazoles, chloramphenicol and dapsone.

Companies wishing to market a veterinary medicinal product for use in food-producing animals must therefore supply sufficient data to satisfy the CVMP that the drug is safe for consumers and that MRLs can therefore be established. It will come as no surprise therefore from what has been said above to find that the main components of these data are toxicological, pharmacological and microbiological, along with data on residues depletion and analytical methodologies. In fact, the two major components of an MRL application are termed the safety file and the residues file, and the outline contents of these are shown in *Tables 23.1* and *23.2*.

From the studies outlined in the safety file, the critical areas of toxicology, microbiology and pharmacology can be identified and a toxicological profile, or perhaps more appropriately a biological profile, can be constructed. Equally important, no-observed effect levels (NOELs) can be identified, and from the point of view of hazard assessment, the lowest NOEL is usually chosen unless there is good reason to discount it (e.g. because the toxicity noted is irrelevant to human risk assessment, usually because it is species-specific to the animal used in the test system or discountable on mechanistic or dose-response considerations).

The NOEL forms the basis of the MRL because it forms the basis of the calculation of the acceptable daily intake or ADI. The ADI concept was developed in 1957 by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (JECFA, 1957) and its use is described by the World Health Organisation's *Environmental Health Criteria 70*

Table 23.1 Major contents of the safety file.

- Safety expert report
- Characterisation (e.g. name, structure, impurities, molecular weight)
- Physico-chemical properties (e.g. melting and boiling points, vapour pressure, solubility in water and organic solvents, pH, density)
- Pharmacology
  - Pharmacodynamics
  - Pharmacokinetics
- Toxicological studies
  - Single dose (acute toxicity)
  - Repeat dose (at least 90 days' duration)
  - Reproductive toxicity
    - Study of effects on reproduction
    - Embryotoxicity/teratology
  - Genotoxicity
  - Carcinogenicity
- Microbiological effects on human gut flora
- Pharmacological, microbiological and toxicological observations in humans (where available)

Table 23.2 Major contents of the residues file.

- Residue expert report
- Characterisation (e.g. name, structure, impurities, molecular weight)
- Physico-chemical properties (e.g. melting and boiling points, vapour pressure, solubility in water and organic solvents, pH, density)
- Pharmacokinetics in target animals (sheep, pigs, cattle, fish, etc.)
- Residues studies
  - Residues depletion studies in each target species
    - Studies with radiolabelled drug
    - Studies with unlabelled drug
- Elaboration of MRLs
- Routine analytical methods
  - Description of the method
  - Validation of the method (e.g. precision, accuracy, limit of detection, limit of quantification, susceptibility and interference, practicability and applicability)

(WHO, 1987). This concept was largely based on the ideas of René Truhaut (Poulsen, 1995; Benson, 2000).

In the ADI calculation, the NOEL is divided by a suitable safety factor, usually 100, to give the ADI value. The 100-fold safety factor concept is empirical and arises from the contention that there is a 10-fold human variability in susceptibility, and a 10-fold animal–human variability, giving the overall safety factor of 100. It follows from this that in those (few) examples where the ADI is based on human-derived data, the safety factor is usually 10 (Herrman and Younes, 1999; Woodward 1991, 1997, 2004a, b; Harding, 2003). However, higher safety factors may also be used, for instance where there are minor flaws in the data package or because of the nature of the toxicity observed. As an example, irreversible effects such as teratogenicity may sometimes attract a higher safety factor. As the NOEL is usually expressed in milligrammes of substance per kilogramme of body weight per day, the ADI is based on the same units:

$$\text{ADI} = \frac{\text{NOEL mg/kg body weight}}{100}$$

Note that as the term 'daily' is built into the ADI, the value is not expressed in terms of 'per day'. It is often considered useful to factor in the average human body weight, taken by most regulatory authorities including the EU as 60 kg, to give the ADI in terms of milligrammes per person:

$$\text{ADI} = \frac{\text{NOEL} \times 60\,\text{mg per person}}{100}$$

The ADI has received critical attention over the years, not least because of the arbitrary nature of the safety factor and the lack of scientific justification for its 10×10-fold nature. It has been suggested that increased scientific knowledge of pharmacokinetics and pharmacodynamics for specific molecules could be used to determine safety factors that are more scientifically sound than the 100 factor usually employed. Thus, rather than a factor of 10 for species differences, and a further factor of 10 for human differences, there would be sub-factors for species

differences in kinetics and dynamics, and human differences in kinetics and dynamics for specific substances (Renwick, 1991), and so differences in absorption, first pass metabolism, renal plasma flow and plasma half-life could be taken into account (Renwick, 1993).

However, the major drawback to such an approach is the lack of relevant data, particularly from human exposure that would leave part of the safety factor incomplete, and would require more animal data to contribute to other aspects of the calculation. There are few examples where all the necessary data are available (Kroes *et al*., 1993). Other approaches, including graphical representation of data (Dourson *et al*., 1985) and the fitting of dose response models to toxicological data (Crump, 1984) suffer from other drawbacks, but as with the pharmacokinetic and pharmacodynamic approach, require more data than are currently provided by routine laboratory testing.

Although the ADI concept and the magnitude of the safety factor used to derive it have been addressed and refined by Renwick and others in recent years (Rubery *et al*., 1990 Renwick, 1991, 2005; International Programme on Chemical Safety, 2005; Boobis *et al*., 2008; Galli *et al*., 2008), the considerations have yet to be extended to ADI calculations for veterinary drugs.

The ADI is defined as the quantity of a substance or, in the context of this chapter, residues of a veterinary drug that can be ingested by humans over the course of a life-time without causing adverse effects. Clearly this definition too presents some problems, although these could be considered semantic in most cases. Consider a drug that is otherwise non-toxic, but causes some degree of fetotoxicity. The NOEL is established on the basis of fetotoxicity, and the ADI calculated accordingly. It is likely that this ADI is applicable to only a limited part of the population, namely pregnant women, and probably only for a limited period of gestation (the sensitive stage of organogenesis). As it is the lowest NOEL that has been employed, then it can be argued that the entire population is protected. However, it does call into question the ADI definition and its concept of life-time exposure.

Similar criticisms could be made when the ADI concept is applied to substances where the major toxic or pharmacological effects are acute rather than subchronic or chronic. Of particular importance is the question, 'Does the current ADI concept protect groups who might be more sensitive to the toxic effects of a substance such as the elderly, pregnant women and the very young?' (Somogyi, 1979). While this is probably addressed by the current very large safety factors used in the ADI calculation, it cannot be answered with certainty.

Microbiological safety of residues, although not a toxicological issue, must also be considered. The concerns here arise from several areas (Corpet and Lumeau, 1989; Corpet, 1992, 1993; Boisseau, 1993; Gorbach, 1993; Kidd, 1994) as residues of microbiologically active drugs such as antibiotics could conceivably:

- perturb the bacterial ecology of the gastrointestinal tract, particularly that of the colon;
- weaken the barrier effect of the gastrointestinal flora, allowing the ingress and growth of pathogens;
- as a result, thus increase the susceptibility and vulnerability of the consumer to pathogenic bacteria, and significantly to bacteria pathogenic to the gastrointestinal tract;
- provide conditions that could lead to the colonisation of the gastrointestinal tract by other organisms, although not necessarily pathogens, including bacteria and fungi;
- provide conditions that could be conducive to the development of antimicrobial resistance.

Many of these concerns arise from the use of antimicrobial drugs in humans, as therapeutic doses may lead to some of these effects. Indeed, sometimes the perturbations in colonic flora can be dramatic following the therapeutic use of antibiotics in humans. However, there is no firm evidence that residues present in food of animal origin can have such effects in humans and as the concentrations of residues in food to which humans are exposed are extremely low, it seems highly unlikely that major adverse effects would occur. Nevertheless, it is considered prudent to

investigate the potential of residues of antimicrobial drugs to adversely affect the human gastrointestinal flora.

Unfortunately, there are no well-validated or even widely accepted experimental models for this, but several approaches are available:

- *Studies in humans*: these involve human volunteers given doses of the test compound. The faeces are then examined for population changes in species of bacteria.

- *Studies in gnotobiotic animals*: gnotobiotic animals are animals whose own gut flora is absent. They are then implanted with human gut flora and treated with antibiotic drugs to determine whether there are any adverse effects on the adopted bacteria. These studies are notoriously difficult to interpret, not least because the effects of the host animal on the implanted gut flora may be greater than those of the administered drug. Nevertheless, a recent study with germ-free mice investigated the effects of ciprofloxacin on the implanted human gut flora. The drug significantly decreased the populations of anaerobic bacteria, and notably the population of *Enterobacteriacae.* In mice challenged with a strain of *Salmonella*, the bacteria were found in the faeces, suggesting a breakdown of the barrier effect. The NOEL in this study was found to be less than 0.125 mg/kg bw, the lowest dose used (Perrin-Guyomard *et al*., 2005). The study demonstrates the utility of this type of experiment in investigating the effects of antimicrobial substances on the human gut flora.

- *In vitro studies*: these may examine a number of end-points, including the development of antimicrobial drug resistance (Rumney and Rowland, 1992; Woodward, 1998; Cerniglia and Kotarski, 1999). They generally involve determination of the so-called minimum inhibitory concentrations (MIC_{50} values) or some similar measurement, either through serial dilution or using continuous culture methodologies that aim to model microflora interactions, the ecology of the human colon and the effects of pH and anaerobiosis. It seems likely that a more systematic approach, using both in vitro and in vivo models, is likely to be employed in the future (Cerniglia and Kotarski, 1999, 2005) along with harmonised guidelines and approaches to hazard assessment (Silley, 2007).

Not surprisingly, many antimicrobial drugs have the capacity to disrupt fermentation due to toxic effects on the microorganisms involved. This is important if the drug is intended for use in lactating animals where the milk may be employed to produce cheese or yoghurt. Under these circumstances it is necessary to conduct studies with dairy starter cultures to determine the likely inhibitive effect of the antimicrobial in question, and to identify the inhibitive concentration. As these tests are very sensitive, this value usually plays a leading role in establishing the MRL and often takes precedence over the ADI value.

Occasionally, the main biological effects of a drug may be pharmacological rather than toxicological, and again may be noted in animal studies or in investigations in humans. Such effects may be more significant with some substances such as anaesthetics, analgesics and β-agonists, as noted earlier with clenbuterol, than classical toxicological effects, and in those circumstances the NOEL, and the subsequent ADI, may be based on the pharmacological properties (van Leeuwen, 1991).

Regardless, the important issue is to identify the residue of toxicological concern (or where relevant of microbiological or pharmacological concern) and to understand their pharmacokinetic and biological behaviours in vivo (Fitzpatrick, 1995; MacDonald, 1995; Mulligan, 1995).

The major requirements for EU MRLs are set out in a number of Guidelines issued by the CVMP through the EMEA, as well as in the *Rules Governing Medicinal Products in the European Union, Volume 8*. Together, these provide a major source of advice on all aspects relating to MRLs in the EU including such aspects as minor species, injection site residues and acceptable daily intakes. They are shown in *Table 23.3*.

Table 23.3 EU Guidelines relevant to the establishment of MRLs.

Guideline	*Content*
Rules Governing Medicinal Products in the European Union, Volume 8. Notice to Applicants and Note for Guidance. Establishment of maximum residue limits (MRLs) for residues of veterinary medicinal products in foodstuffs of animal origin	Covers all requirements for contents of the safety file and residues file, and provides advice on studies, methodology and legal requirements
EMEA/CVMP/SWP/66781/2005 Safety and residues data requirements for veterinary medicinal products intended for minor uses or species	Provides extensive advice on approach to be taken when developing data to support MRLs intended for minor veterinary use or in a minor species
EMEA/CVMP/153a/97-FINAL Note for guidance on the establishment of maximum residue limits for minor animal species	General guidance on the approach to minor species and MRLs
EMEA/CVMP/153b/97-FINAL Note for guidance on the establishment of MRLs for Salmonidae and other fin fish	Establishes criteria and procedures for determining MRLs for fish, notably for salmon
EMEA/CVMP/SWP/139646/2005-CONSULTATION Concept paper on guidance on the approach to demonstrate whether a substance is capable of pharmacological activity	Sets out ideas for developing a guideline to demonstrate pharmacological activity (or lack of it). Especially intended for use where sponsor attempts to demonstrate lack of pharmacological activity and hence exemption from MRL requirements
EMEA/CVMP/542/03-FINAL Guideline on injection site residues	Provides advice on scientific, procedural and regulatory aspects of injection site residues, including how to address the injection site from the sampling and analytical chemistry view points
EMEA/CVMP/SWP/122154/2005-CONSULTATION Concept paper on a guideline on the assessment of pharmacological/pharmacodynamic data to establish a pharmacological ADI	Establishes ideas to determine where appropriate pharmacological ADI on the basis of pharmacodynamic data
EMEA/CVMP/276/99-FINAL Note for guidance for the assessment of the effect of antimicrobial substances on dairy starter cultures	Provides guidance for the conduct and interpretation of studies designed to investigate inhibitory effects of antimicrobials, e.g. on yogurt and cheese starter cultures
EMEA/CVMP/187/00-FINAL Note for guidance on risk analysis approach for residues of veterinary medicinal products in food of animal origin	Discusses extrapolation of MRLs from major to minor species or from several species to 'all food species' based on risk analysis approach (see also EMEA/CVMP/069/02, Implementation of note for guidance on risk analysis approach for residues of veterinary medicinal products in food of animal origin)

Table 23.4 Daily food intake factors (grams) used in the EU in the elaboration of MRLs.

Large animals		*Poultry*		*Fish/bees*	
Muscle	300	Muscle	300	Muscle + skin	300
Liver	100	Liver	100	Honey	20
Kidney	50	Kidney	10		
Fat	50	Fat + skin	90		
Milk	1500	Eggs	100		

MRLs

Elaboration of MRLs is far more problematic in many ways than the calculation of ADI values. There is no simple equation that can be applied and the approach is much more iterative. This is because a number of factors have to be taken into account. Fundamentally, the magnitude of the MRLs has to be such that the ADI is not exceeded by consumers of food of animal origin. In addition to this, the MRL values established for different tissues have to be practicable; there is little point in setting the MRL for muscle at an order of magnitude higher than that for liver for a particular species if pharmacokinetics and residues depletion data show that in reality the values are likely to be the other way around. Consequently, patterns of residues depletion across a limited range of tissues must also be considered.

Some information on the distribution and metabolism of a specific drug in a particular animal species is provided by pharmacokinetic studies in that animal. However, the main information is provided by determination of specific residues depletion profiles. Groups of the intended target species – cattle, sheep, pigs or fish, for example – are given the drug at the therapeutic dose, in the intended market formulation, and groups of animals are then serially slaughtered (or milk collected at sequential time points) and tissues (or milk) collected for chemical or radiochemical analysis. In practice, the major tissues designated for analysis are muscle, liver, kidney and fat, except for pigs, fish and poultry where skin, which is also eaten, is additionally analysed.

The amount of residue consumed by humans depends not only on how much is present in tissues and organs, but also on how much food containing the residue is eaten. Consequently, a 'market basket' approach to food intake has been adopted as the pragmatic solution. This makes use of food intakes that are certainly in excess of what might be considered normal, but, in doing so, it does take into account individuals who might be considered to be extreme consumers. The values used in the EU are given in *Table 23.4*. This approach could be improved by a more accurate knowledge of actual dietary intake (Tomerlin *et al*., 1997; Kroes *et al*., 2002) and better information on dietary food and food commodity consumption (Tennant, 2001).

Thus, MRLs are elaborated (rather than calculated) by considering the practical aspects of pharmacokinetic factors and residues time-depletion profiles, particularly the depletion of the marker residue (International Programme on Chemical Safety (IPCS), 1990, 1999; Woodward, 1997, 2000), while bearing in mind the ADI, and ensuring that in considering the magnitude of the MRLs, the ADI values will not be exceeded. Under the requirements of Regulation (EEC) No. 2377/90, MRLs must be practicable, and that is taken to mean, in part, that there is an adequate analytical method with which to determine the drug or its metabolites. Indeed, there is a direct requirement for the provision of an analytical method (*Table 23.2*).

Similar requirements for toxicity and residues depletion data exist under legislation in the United States (Guest, 1990; Teske, 1992; Miller and Flynn, 2000; Woodward, 2000; Frank and

Schafer, 2001; Sundlof, 2001). Not surprisingly, many of the issues surrounding the calculations of ADI values, the types of toxicity and residues studies to be conducted, the use of microbiological safety studies, to name but a few, apply here also (Perez, 1977; Kobylka, 1982; Friedlander *et al*., 1999; Paige *et al*., 1999a). In the United States, there is no separate MRL legislation as such, and in fact the approach to determining safety limits is subtly different from that of the EU. Having calculated an ADI, the next step is to calculate a safe concentration for a particular tissue (Brynes, 2005), for example, for liver. Using an ADI value of 0.1 μg/kg/day, the safe concentration calculation (SC) is:

$$SC = \frac{ADI \times \text{human weight}}{\text{Daily Tissue Intake}}$$

$$SC = \frac{0.1\mu g/kg/day \times 60\,kg}{0.1\,kg/day} = 60\,\mu g/kg = 60\,ppb$$

Using this value, and data from total residues depletion studies, a tolerance for liver can be established for the drug. The same process can then be conducted for other tissues and for milk (Friedlander *et al*., 1999; Frank and Schafer, 2001). Food consumption values used in the United States are essentially similar to those used in the EU and are shown in *Table 23.4*. The tolerance is essentially equivalent to the MRL, although the use of simple arithmetic to derive it makes it somewhat easier to understand.

A different approach is used for carcinogenic veterinary drugs. The Federal Food, Drug and Cosmetic Act prohibits the use of carcinogenic drugs in food animals unless it can be shown that no residues are present as a result of drug treatment. Clearly, this is almost impossible as modern methods of analysis are capable of detecting minute amounts of compound. To ensure food safety, a model is used to estimate an upper limit of low-dose risk based on a lifetime risk of one per million as an 'insignificant risk' for cancer. Due to uncertainties, including the uncertainties of animal to human extrapolation and those concerned with the magnitude of the risk, the model has numerous conservative elements in-built, thus ensuring consumer safety (Gaylor *et al*., 1997).

The MRL and tolerance values are employed to derive withdrawal periods (see Chapter 24) for marketed veterinary medicines. The withdrawal period is the time from administration of the medicine, or last administration in a multi-dose regime, to the point where residues have depleted to below the MRL or tolerance. This is done by conducting studies where animals are treated with the medicine in question, as the formulation to be marketed, and then are slaughtered at intervals and the key tissues of muscle, fat, liver and kidney are analysed. Similar studies are conducted with dairy cattle for milk. A withdrawal period is then derived by examining the time-dependent tissue depletion (or depletion in milk) against the MRL or tolerance values. In practice, use is made of various statistical models in calculating the withdrawal period.

The withdrawal period, or milk withhold time, then becomes part of the terms of the marketing authorisation, and appears as such in the product literature and on the product label (Friedlander *et al*., 1999; Woodward, 1999). Farmers are then required to observe these withdrawal times after their animals have been treated with veterinary medicines to ensure that any residues present are below the relevant MRL or tolerance values.

The EU and the United States have in place extensive systems for residues surveillance so that residues can be monitored and violations of statutory limits such as MRLs can be detected (Van Dresser and Wilcke, 1989; Paige *et al*., 1997, 1999b; Woodward, 1997, 1999; Sundlof *et al*., 2000). This not only provides significant confidence for consumers but also allows offenders who have permitted violations to occur to be prosecuted. The results of residues monitoring are published in many countries including the US and the UK. These results demonstrate that residues of veterinary medicines are indeed generally very low in food of animal origin, and that MRL and tolerance violations are extremely rare

(Pullen, 1990; Paige *et al*., 1999b; Sundlof *et al*., 2000; Veterinary Residues Committee, 2002).

The MRL process in the EU was retrospective; it applied not only to new pharmacological substances, but also to existing ones used in food animal products. From 1990 onwards, the CVMP undertook a major programme of work reviewing these older substances while at the same time dealing with applications for new chemical entities. Perhaps inevitably, some of these fell by the wayside and found their way into Annex IV for safety reasons. Others were withdrawn by the sponsor either because of the costs of providing data packages, often for off-patent materials, or because the CVMP was unable to reach a conclusion on safety on the basis of the available data. The consequences for all of these materials are exactly the same – they cannot be used in veterinary medicinal products intended for food animals. They are listed in *Table 23.5*.

Despite these losses, over the period 1992–2008, a whole range of therapeutic substances has been entered into one of the Annexes I–IV of the Council Regulation, as shown in *Figure 23.1* (European Medicines Agency, 2007), through a series of amending regulations (*Table 23.6*).

The majority of the substances contained in Annex I are antimicrobial drugs and antiparasitics including ectoparasiticides and endectocides (*Figure 23.2*). Similarly, a range of substances, mainly excipients, has been entered into Annex II. These include salts, vitamins, medical gases, solvents and polymers which are classified into the arbitrary categories of inorganic, organic, generally recognised as safe (GRAS), homeopathic materials, E numbers and substances of vegetable origin (*Figure 23.3*). A range of substances are authorised under EU legislation as permitted additives for human foodstuffs, and the E numbers are also included in Annex II (*Figures 23.3* and *23.4*). The full list of amending regulations is available at http://ec.europa.eu/enterprise/pharmaceuticals/mrl/regindex.htm, while a consolidated list of MRLs to November 2006 is located at http://ec.europa.eu/enterprise/pharmaceuticals/eudralex/home5.htm.

Table 23.5 Substances in Annex IV of Regulation (EEC) No. 2377/90, substances withdrawn from the EU MRL procedure or substances for which the CVMP could not make a recommendation.

Annex IV	
Aristolochia spp. and preparations	Dapsone
Chloramphenicol	Dimetridazole
Chloroform	Metronidazole
Chlorpromazine	Nitrofurans (including furazolidone)
Colchicine	Ronidazole
No recommendation	
2-ethyl-1,3-hexanediol	Populeum ointment
Phenylbutazone	*Chelidonii herba*
Suxibutazone	Benzylidenacetone
Ramifenazone	Metesculetol sodium
Withdrawn	
Decoquinate	Testosterone
Niclosamide	Fenprostalene
Bromopropylate	Methylprednisolone
Heptenophos	Benzonaphthol
Camylofine	Clanobutin
Narcobarbital	Haloquinol
Thiopental sodium	Benzonicotinate
Propionylpromazine	Copper naphthenate
Dextrometorphan hydrobromide	Cuproxoline
Ammonium phthalamate	Glycofurol
Pentetrazol	Polyethylene terephthalates

In addition to the formal entries into Annexes I–III, there is also an informal Out of Scope list. This lists substances not considered to be subject to the scope of the MRL Regulation. It includes:

- natural products such as olive and sesame seed oils
- normal constituents of foodstuffs including cereals, carbohydrates, honey and peptides and proteins found in the normal human diet
- chemically unidentified substances of natural origin such as organ autosylates and probiotic components
- oxygen.

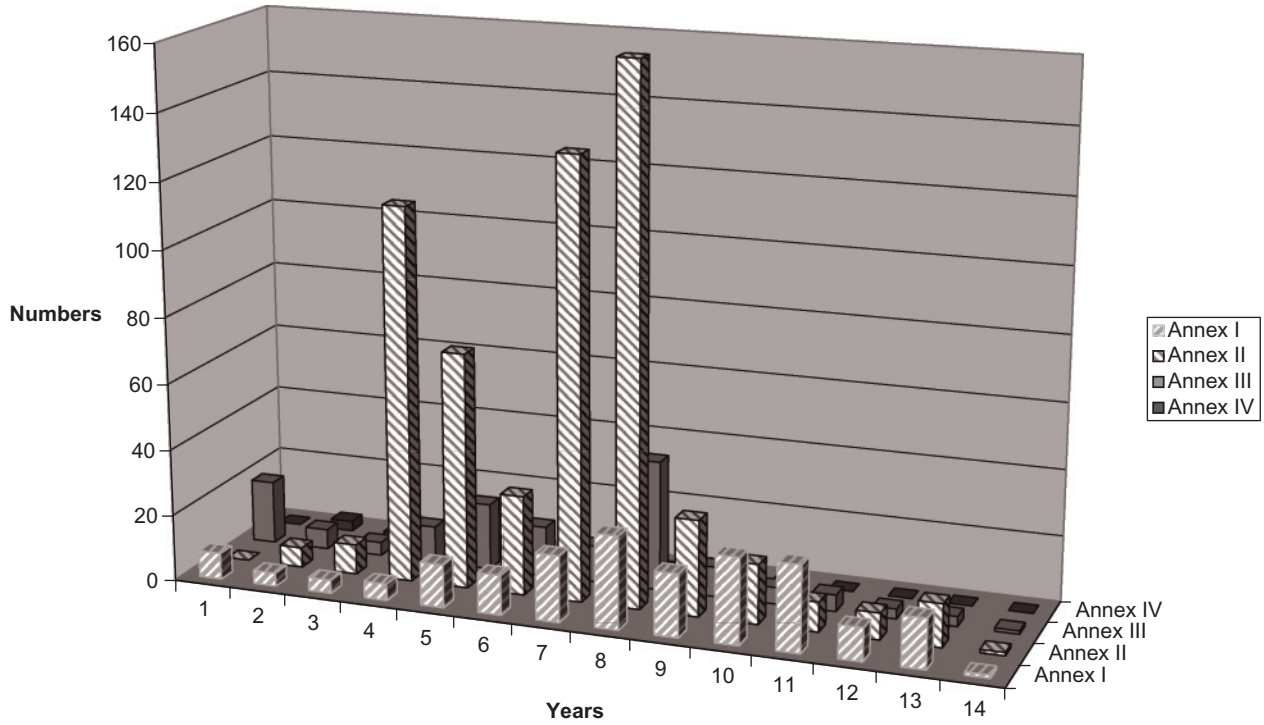

Fig. 23.1 Amendments to Regulation (EEC) No. 2377/90 over a 14-year period, 1992–2005.

Table 23.6 Amending regulations to Regulation No. (EEC) 2377/90 adding substances to Annexes I–IV.

Year	*Amending regulations*
1992	2
1993	4
1994	4
1995	7
1996	12
1997	11
1998	16
1999	15
2000	7
2001	13
2002	8
2003	9
2004	9
2005	8
2006	8
2007	5
2008 (to November)	3

It also includes substances shown not to have pharmacological activity, as this precludes them from the scope of the Regulation. Chlorobutanol, meglumine, diethanolamine and ethoxyquin fall into this latter group. For these substances to be included in this list it was necessary for the sponsor to show that they lacked pharmacological activity 'at the dose given to the target species', in the words of the Regulation. To achieve this, sponsors generally conduct a battery of pharmacological tests. These might include gut transit time and effects on barbiturate-induced sleeping time in rodents and effects on body temperature. A study on cardiovascular and respiratory effects in dogs is often required. However, the CVMP has no formal guidance on this subject at the present time, although it is the subject of a concept paper with future guidance in mind (see *Table 23.3*).

Sponsors have the option to consider conducting pharmacological safety studies intended to define pharmacological activity for human studies set out in the document agreed through

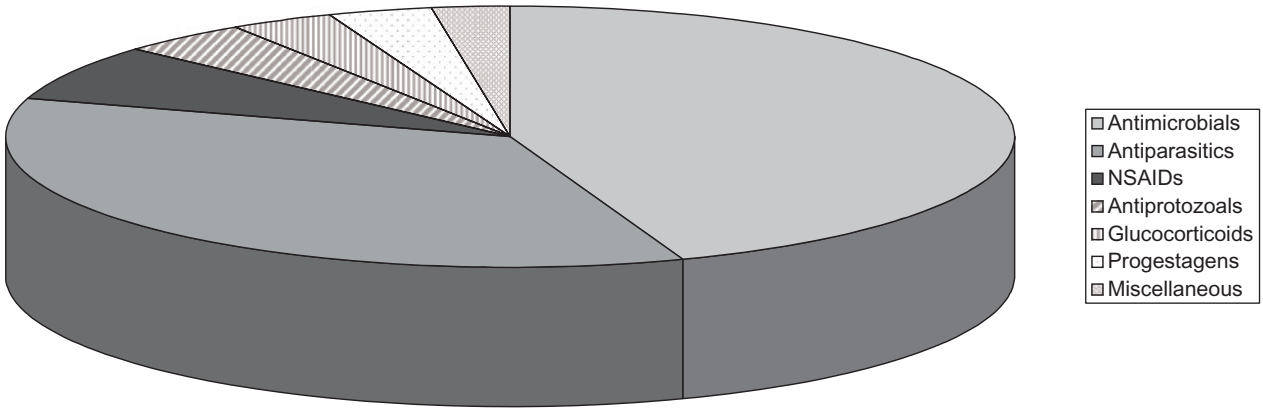

Fig. 23.2 Categories of substances included in Annex I of Regulation (EEC) No. 2377/90.

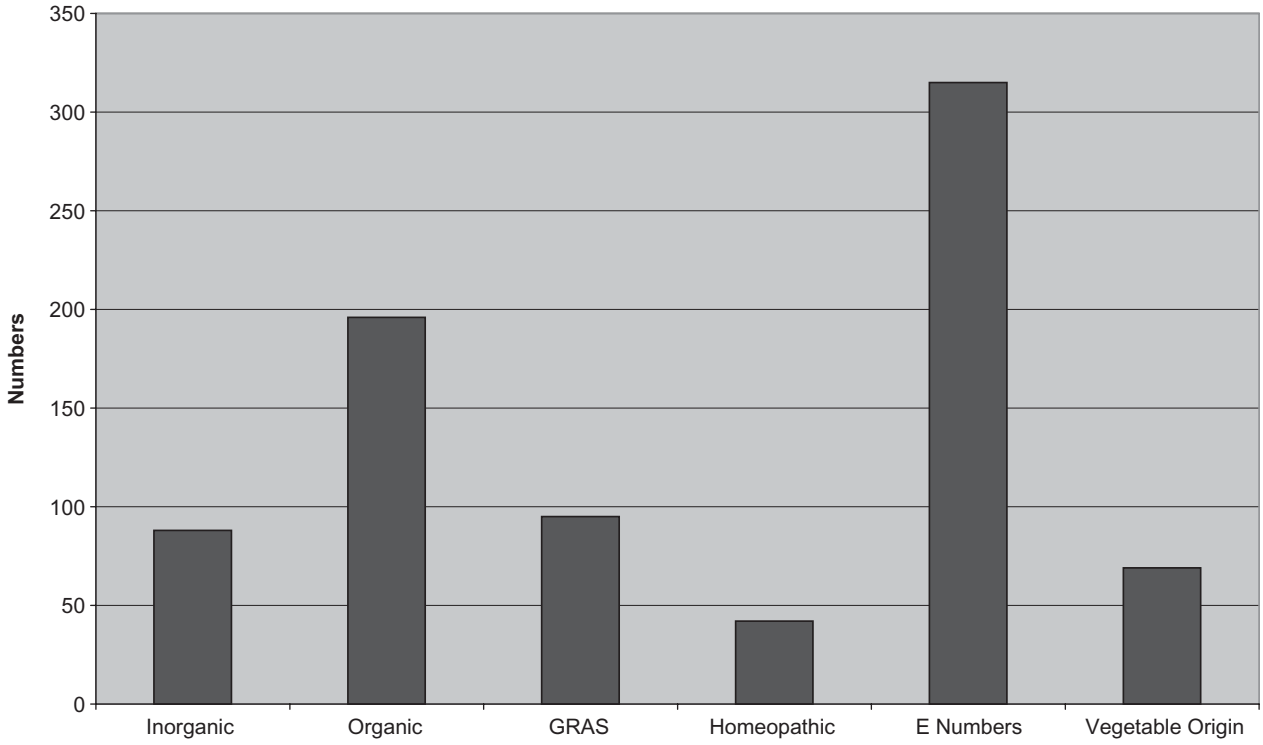

Fig. 23.3 Categories of substances included in Annex II of Regulation (EEC) No. 2377/90.

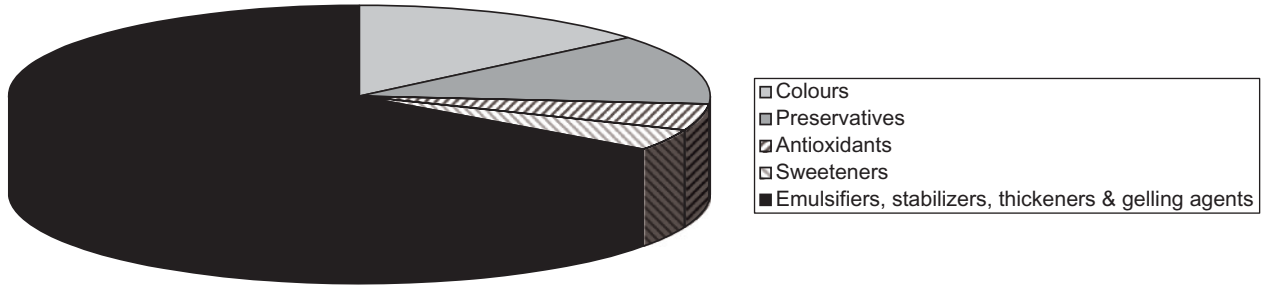

Fig. 23.4 Categories of E numbers (315) included in Annex II of Regulation (EEC) No. 2377/90.

Table 23.7 Veterinary drugs evaluated by JECFA.

Chloramphenicol	Ractopamine hydrochloride	Danofloxacin
Thiamphenicol	Isometamidium	Diclazuril
Trenbolone acetate	Enrofloxacin	Imidocarb
Doramectin	Dihydrostreptomycin	Azaperone
Ivermectin	Streptomycin	Cyhalothrin
Eprinomectin	Progesterone	Dicyclanil
Moxidectin	Porcine somatotrophins	Trichlorfon
Thiabendazole	Colistin	Carazolol
Flubendazole	Flumequine	Spiramycin
Triclabendazole	Lincomycin	Tylosin
Febantel	Ceftiofur	Chlorpromazine
Fenbendazole	Procaine penicillin	Propionylpromazine
Oxfendazole	Chlortetracycline	Dexamethasone
Cefuroxime	Tetracycline	Cyfluthrin
Clenbuterol	Oxytetracycline	Fluazuron
Xylazine	Nicarbazin	Phoxim
Neomycin	Closantel	Oestradiol-17β
Gentamicin	Ronidazole	Testosterone
Tilmicosin	Sulphadimidine	Melengesterol acetate
Cypermethrin	Spectinomycin	Erythromycin
α-Cypermethrin	Olaquindox	Deltamethrin
Furazolidone	Carbadox	Pirlimycin
Nitrofurazone	Levamisole	Oxolinic acid
Bovine somatotrophins	Sarafloxacin	

the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) (2000), but for ingredients used in veterinary medicinal products, the intention would be to demonstrate *lack* of any activity. Clearly, this approach would not be considered for substances used to achieve pharmacodynamic effects, i.e. the active ingredients used in veterinary medicinal products.

The role of JECFA

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) began evaluating toxicity and residues data on veterinary drugs in the mid-1980s, with a view to establishing MRL values (Food and Agriculture Organisation, 1985). The MRLs developed are taken into the Codex Alimentarius system, which like JECFA is a joint FAO and WHO body, as part of its food standards programme, through the Codex Committee on Residues of Veterinary Drugs in Food (Crawford and Kugler, 1986; Herrman, 1993; Herrman and Younes, 1999; Berg, 2001; Chen, 2001; Luetzow, 2003). In practice this means that veterinary drug assessments and MRL values are available to developing countries that might not have the means to do this for themselves, and that scientific monographs on toxicity and residues characteristics are readily available in the public domain. It also means that the deliberations and decisions of the JECFA are transparent as these are published in a separate report series as are the toxicology (by WHO) and residues (by FAO) monographs. Some of the drugs evaluated by JECFA are shown in *Table 23.7*.

Occasionally, the MRLs set by JECFA are different from those set by the EU or from US tolerances. Or JECFA might set an MRL whereas other bodies felt unable to do so. For example, the EU has not published an MRL for the anabolic steroid trenbolone acetate, whereas JECFA established an MRL (van Leeuwen, 1991). This raises the spectre of trade disputes between the EU and

countries that adopt the JECFA MRL, or at least its scientific approach, or those that develop and use their own national standards.

Scientific opinions can differ for a number of reasons, including scientific approaches, attitudes to risk assessment and different benefit:risk conclusions (Illing, 1991, 1999, 2001; Nilsson *et al*., 1993). However, some of the variations in MRLs which arise from various national, multinational (e.g. the EU) and international bodies (e.g. JECFA and Codex) arise not because of differences in the interpretation of toxicity data, but because different food intakes values are used in their elaboration. Approaches to resolve this problem, which could lead to disputes between various trading blocks, would either be to harmonise food intake values across regulatory authorities and international bodies, or to determine the equivalence of MRLs to reveal whether or not the ADI values in each country are being exceeded (Fitzpatrick *et al*., 1995, 1996). However, the development of international food standards should not only help to protect consumers at the global level, but also eventually prevent the erection of barriers to trade and ensuing international trade disputes (Woodward, 1991, 1993; Woodward and Shearer, 1995) in the same way that EU MRLs facilitate intercommunity trade.

On this final point, because of the definition of the ADI, and because of the magnitude of the safety factors involved, it has to be appreciated that occasional intakes of a specific residue in excess of the ADI do not necessarily mean that human health is compromised. Similarly, although MRL violations are undesirable from a legal viewpoint, because of the uncertainty factor built into these, and the safety factors built into the ADI which underpins them, residues concentrations above the MRL values do not in themselves constitute a threat to public health (McEvoy, 2001).

The risks involved in exceeding the ADI are dependent on the biological properties of individual drugs. The nature and magnitude of these risks can only be evaluated through knowing the duration of human consumer exposure and the dose response of the drug in the studies from which the NOEL (and hence the ADI value) were derived (Renwick and Walker, 1993). As violative residues form a part of veterinary pharmacovigilance in the EU, it is important that not only is there adequate residues surveillance, but also any ensuing risks are seen in perspective.

The MRL has a number of in-built conservatisms, including the safety factors used in the calculation of the ADI and the magnitude of the food intake values. Exceeding the MRL by no means suggests that the ADI will be exceeded, and if it is, individual scientific analysis is required to determine if this presents a consumer safety issue. This may have specific implications if the concept of hormesis, adverse effects induced by very low levels of potentially toxic agents, is shown to have foundation (Calabrese, 2001; Calabrese and Baldwin, 2001, 2003; Rozman and Doull, 2003; Stebbing, 2003).

Purposes of MRLs

MRLs have several practical purposes, most notably to protect the consumer by ensuring that residues of veterinary drugs consumed in food of animal origin do not exceed the ADI. As previously mentioned, to achieve this, the withdrawal period concept is employed. Here, studies are performed whereby groups of target animals are treated with a drug using the commercial formulation and then slaughtered at intervals for residues analysis. The withdrawal period, the period between treatment or last treatment in a multidose regimen and when the animal may be slaughtered for human consumption, is derived from the point when residues deplete to below the MRL in all target tissues in all the animals in a group. Similar concepts apply for milk and eggs, although here of course residues do not deplete and the commodity has to be discarded until residues fall below the milk or egg MRLs (Dixon, 2001; Sanquer *et al*., 2006b).

Honey often presents a particular problem as bees, which are treated on a hive basis, often need medication during the period of maximum honey flow. If this results in residues of honey above the MRL, it will mean that the supply of honey is unusable as residues do not deplete. Consequently, drugs for the treatment of diseases in

bees need to be formulated so that MRLs for honey are not exceeded in the first instance.

Fish are poikilothermic animals but possess extensive drug-metabolising capacities (Kleinhow and Lech, 1988;, Kleinhow *et* al., 1990; Droy *et al*., 1990; Nichols *et al*., 1990; Segner and Cravedi, 2001). Their rates of metabolism and indeed the nature of their metabolic processes can vary with the temperature, depending on the species of fish, as well as season, sex and prior exposure to inducers of cytochrome P-450 (Guarino, 1991; Binder *et al*., 1984; Lech and Vodicnik, 1984; Allen and Hunn, 1986; Guarino and Lech, 1986; James, 1986; Barron *et al*., 1987; Kleinhow *et al*., 1987, 1992; Niimi, 1987; Droy *et al*., 1989; Cravedi, 2002; Woodward, 1996; Livingstone, 1998; Sarasquete and Segner, 2000). Hence, whereas withdrawal periods for mammals and avian species are quoted in days, those for fish are quoted in degree days to take account of the dual effects of time and temperature (Woodward, 1996).

Withdrawal periods are legal requirements in the EU and are established during the authorisation process. The withdrawal period, even if it is zero, must appear in the product literature and on the label for veterinary medicines intended for food-producing animals. However, it is futile imposing withdrawal periods if these are not observed on the farm. Withdrawal periods and MRLs must be monitored and enforced through surveillance for residues of veterinary drugs in food of animal origin.

Problems arising from MRLs

A major area that can cause problems is the persistence of residues at the intramuscular or subcutaneous injection site (Nouws, 1990; Nouws *et al*., 1990; Banting and Baggot, 1996; Gaylor and Monro, 1996; Mawhinney *et al*., 1996; Brown, 2000; Beechinor *et al*., 2001; Beechinor and Bloomfield, 2001; Sanquer *et al*., 2006a, b). This is particularly noticeable in the case of irritant drugs which cause inflammation, necrosis, fibrosis and encapsulation of the injection site, leading to enhanced drug persistence. It is particularly significant as some products are designed to act in this way to provide a convenient depot effect. These can lead to long withdrawal periods which experience suggests are more likely to be ignored, and to violative residues as a consequence. There is now growing regulatory opposition in some parts of the EU and elsewhere to the authorisation of such formulations.

Injection site residues are usually taken into account by basing the withdrawal period on depuration of residues at that injection site which is treated as normal muscle. This generally results in long withdrawal times which not only may result in the affected product being regarded as less commercially attractive, but also may mean that the withdrawal period is ignored, with the consequence of violative residues occurring. One solution is to discount the injection site either in the establishment of MRLs or in the setting of withdrawal periods. This would mean that residues at the injection site were evaluated toxicologically to ensure consumer safety without having a formal MRL value in place. These issues need to be resolved, not only to assure consumer safety, but also to prevent disruption of international trade in meat and meat products (Reeves, 2007). In the EU, the CVMP has developed a guideline on this issue (see *Table 23.3*).

Problems can arise when drugs are used off-label (Damian *et al*., 1987; Payne *et al*., 2006). The MRL is based on the residues depletion and hence pharmacokinetic behaviour in the target animal. If used in another species, residues problems could occur, although this is probably unlikely. One way around this problem is to have very long withdrawal periods. This approach is used in the EU where standard withdrawal periods are employed. These are greatly in excess of any withdrawal period that is likely to have been arrived at through residue depletion studies. Another approach is used in the United States through establishing safe concentrations for off-label use. Other proposals employ provisional acceptable intakes to assess safety and establish withdrawal periods and risk-based approaches (Baynes *et al*., 1999; Gehring *et al*., 2006).

As already alluded to, generating the safety and residues data to support MRL applications is extremely expensive. Not surprisingly, manufacturers prefer not to make this investment for either minor therapeutic uses (e.g. rare diseases) or minor species (e.g. rabbits, goats, deer, reindeer, ducks, turkeys and fish). Of course, safety data might be available to establish MRLs for major species but that still leaves a significant cost to generate residues depletion and pharmacokinetic data in the minor species, to develop a validated analytical assay, and to then generate depletion data post-MRL to determine withdrawal periods.

In view of this, the CVMP has drawn up guidance and advice for establishing MRLs for minor species. Historically, MRLs have been established on a species-specific basis, but the CVMP has used a risk-based approach to extrapolate MRLs from major species to minor or from major species to 'all food species' or 'all ruminant species', depending on the available data. This has served to make MRLs 'available' to food species that would otherwise have been left without and consequently deprived of appropriate medications.

However, even with this provision, the costs of generating species-specific data for post-MRL withdrawal period depletion studies can be significant. This often means that sponsors are deterred from investments in minor species products. This is particularly important with fish for although it might be economic to generate data for a major fish species such as Atlantic salmon, it might prove less attractive to go on further and generate data packages for other species, even related ones like rainbow trout. Faced with a range of chemotherapeutic products for use in aquaculture, and a range of species (Brown, 1989; Roth *et al*., 1993; Bell, 1995; Burka *et al*., 1997; Stone *et al*., 1999), this obviously raises major issues for treatment and animal welfare.

This has led to the concept of crop grouping, where a surrogate species represents a number of species or even many species. In addition to water temperature, a number of factors affect drug metabolism, distribution and excretion in fish, including gill ventilation volumes and rate, gill anatomy, intestinal anatomy and motility and cardiac output and oxygen consumption rate. Taking these factors into account along with phylogenetic considerations and typical habitat temperatures, it should be possible to group types or species of fish together and generate regulatory data in one to satisfy requirements for all (Hayton, 1995; Gingerich *et al*., 1998). The US authorities have expressed an interest in this approach, providing the concept of crop grouping stands up to scientific scrutiny (Greenlees and Bell, 1998). However, there currently appears to be no enthusiasm for this approach the outside of the United States.

Discussion

Violation of MRLs in the European Union and elsewhere constitutes an area of veterinary pharmacovigilance. These violations usually occur because animals have been overdosed with a drug or because the withdrawal period has not been observed. As MRLs are intended to protect consumer health from any potential harmful effects of residues in food of animal origin, then clearly violation of MRLs may constitute a public health risk. However, the consumer is only likely to be at risk if the ADI value is exceeded, and even then, there are a number of conservatisms built into the ADI and the MRL to ensure that in most cases there will be no significant health risk.

Nevertheless, policing of levels of residues of authorised drugs, and indeed policing of residues of illegal or prohibited drugs, is of importance to prevent veterinary drug misuse and abuse and to ensure sound public health practices are maintained. This is true whether or not national laws regard violation of MRLs or similar designations as an aspect of veterinary pharmacovigilance. It is clearly in the interests of international trade to ensure that MRLs are harmonised, and that food commodities are not the subject of violative residues. This is in fact an opportune time for a degree of harmonisation.

The European Commission has noted that the establishment of MRLs is not without problems.

In a document published in 2003, the Commission noted that the availability of veterinary medicines had been reduced by the MRL exercise, because manufacturers either had declined to support some products or had only supported their uses in major species. It recognised *inter alia* that the legal framework was too inflexible and, moreover, some drugs that had no MRLs or Annex II entries in the EU were legally available and present in foods imported from third countries (European Commission, 2003).

As a result of its deliberations, the Commission has come forward with proposals for new legislation which it believes will provide the missing flexibility (Commission of the European Communities, 2007). This exercise attempts to introduce a number of improvements including achieving a balance between medicine availability and consumer protection, dealing with third country imports of food of animal origin, and reorganising the ways that MRLs are presented (Clayton, 2008). However, in view of the internal negotiations and debates involving the European Parliament and the Council of Ministers, and external consultation with other stakeholders, it is unlikely that there will be any changes to the legislation prior to late 2008 or early 2009, although many of the proposals, including those intended to simplify the existing legislation, have been adopted by the European Parliament and are now further subject to adoption by the co-decision procedure between the European Parliament and the Council of Ministers (Anonymous, 2008; Clayton, 2008).

Regardless of the approach or approaches taken, the MRL concept is a more practical approach to the evaluation of the safety of veterinary drug residues in food of animal origin than any of the possible contenders, including zero tolerance and widespread application of the precautionary principle (Heberer *et al*., 2007; Jostmann, 2007), and they are likely to be around, in one form or another, for some time to come.

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24 Determination of withdrawal periods for pharmaceutical products used in food animals

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Introduction

A variety of animal species, including cattle, sheep, goats, pigs, horses, fish, birds and bees, are kept for the purpose of providing food for the human population. In order to maintain their wellbeing, it is sometimes necessary to treat these animals with pharmaceutical products and such treatments can result in residues of the active ingredients, or their metabolites, entering the human food chain.

It has been explained in Chapter 23 how maximum residue limits (MRLs) are established to define safe residue concentrations that will protect the consumer from ingesting harmful residues. Depending on the intended use pattern of a pharmaceutical product, MRLs are set for the appropriate produce. Produce includes edible tissues, namely liver, kidney, muscle and fat (or skin with muscle or fat in natural proportions where the skin is intended to be eaten), as well as milk, eggs and honey.

The MRL established for each type of food is the maximum concentration of a residue that is permitted to be in that food at the time it is collected for human consumption. However, it is quite possible that residues could be above the relevant MRL shortly after administration of the pharmaceutical product and only deplete to below the MRL some time afterwards. The interval between treatment of animals and the time when the produce from the animals contains residues below the MRL is referred to as the withdrawal period, or in the case of milk, sometimes as the withholding period. Depending on the product, and how it is used, the withdrawal period can be anything from a few hours to several weeks and the farmer is responsible for ensuring that the withdrawal period is complied with and that produce from treated animals is not used for human consumption until completion of the withdrawal period.

This chapter is concerned with the methods used by pharmaceutical companies and regulatory authorities to establish appropriate withdrawal periods to protect the consumer. The focus of the chapter is primarily on the methods used in the European Union, but some reference is also made to procedures followed in other parts of the world.

Residue depletion studies

To establish a withdrawal period it is first necessary to undertake experiments to determine the

rate of depletion of residues from each item of food produce that is of interest. As part of the investigations to elaborate MRLs, a considerable amount of information will already be known about residue depletion. In particular, experiments will have been performed using radiolabelled drugs to measure the total residue (i.e. the parent drug plus all its metabolites) at various time points after dosing. These same experiments will also have identified a marker residue, which is usually a major component of the total residue, and will have established a relationship between the concentrations of this marker residue and those of the total residue at various times after dosing. This marker residue will be defined in the MRL expression and it is the concentrations of this marker residue that will have to be measured in subsequent residue depletion studies.

If so much information has been generated during the elaboration of MRLs it may be wondered why further residue depletion investigations are necessary to determine withdrawal periods. The reason is that the rate of depletion of residues can depend on the nature of the formulation, and two different formulations can quite possibly result in different pharmacokinetics which, in turn, may lead to different withdrawal periods. In the MRL experiments, emphasis would have been placed on using the proposed dose route and dose rate, with less emphasis on using the exact formulation. In fact, with radiolabelled studies it is usually very difficult to mimic the pharmacokinetics of a commercial (non-labelled) formulation except in the case of very simple solutions. With solid dosage forms, and with suspensions, any differences in particle size, or in the distribution of particle sizes, between the radiolabelled and non-labelled products may be reflected in alterations to the pharmacokinetics. Additionally, because of the costs and environmental concerns over the use of radiolabelled drugs, it is likely that not enough animals will have been used in MRL experiments to set withdrawal periods with the required degree of statistical significance.

Consequently, despite the considerable amount of information on residues gained during the establishment of MRLs, it is still very likely that further studies, using the intended commercial formulations, will need to be performed with the specific purpose of setting withdrawal periods. In the EU, there are written guidelines issued by the Committee for Medicinal Products for Veterinary Use (CVMP) that recommend how a withdrawal period study is undertaken (EMEA CVMP, 1996, 2000). Related guidelines have been issued by regulatory authorities in other parts of the world. These guidelines are subject to change at any time and prospective applicants are always advised to contact the relevant agency for the latest advice before initiating studies.

Despite differences in current guidelines issued by different agencies, there is a general agreement that it is essential that the study is carried out using the intended commercial product, administered by the proposed therapeutic route and at the maximum intended dose rate and for the maximum intended number of doses or duration of dosing. The objective is for dose rate, dose route and number of doses to be as close to the worst-case real farm situation as possible. Using the wrong formulation, or a different route of dosing, or a lower dose rate or fewer doses could result in a withdrawal period that is shorter than required in practice, which might lead to violative residues in food intended for human consumption being found during residues surveillance. The finding of residues above the MRL constitutes a reportable event under EU pharmacovigilance requirements, so it is essential that the withdrawal period is correctly determined.

Nevertheless, limited extrapolation is sometimes permitted when undertaking studies to set withdrawal periods. For example, if an authorisation is being sought for both the intramuscular and intravenous routes of administration of a veterinary product, then it may be possible to undertake an experiment using the intramuscular route alone knowing that the residues from intravenous administration are likely to deplete faster. Furthermore, the intravenous route will not give residues at the site of injection, which will be found after intramuscular treatment. Thus

a withdrawal period established for the intramuscular route will usually encompass that for the intravenous route.

Also, if two formulations are very similar, and have been shown to be bioequivalent in terms of plasma pharmacokinetics, then it may be possible to undertake a residue depletion study using just one of the formulations but to apply the resulting withdrawal period to both products. An exception to this could be for formulations administered by intramuscular or subcutaneous injections where injection site residues could be markedly different, despite apparent bioequivalence of the plasma pharmacokinetics. For example, consider a hypothetical case of two formulations administered by intramuscular injection. Assume that with one formulation 99.9% of the drug was released from the site of injection whereas with the other formulation, over the same time period, only 99.0% of the drug left the site. These two formulations would have indistinguishable plasma pharmacokinetics yet the residues remaining at the injection site from the second formulation would be 10 times higher than those from the first product, which would probably require a longer withdrawal period to avoid MRL violations.

In the withdrawal period studies it is only necessary to measure the residues of the marker residue. After all, the MRLs are defined in terms of the marker residue, so monitoring the depletion of the marker residue allows a withdrawal period to be established that ensures that the total drug-derived residues will be below MRL. Nevertheless, it should be noted that sometimes the marker residue is the sum of more than one component. For example, the EU marker residue for gentamicin is the sum of gentamicin C1, gentamicin C1a, gentamicin C2 and gentamicin C2a, each of these being a component of gentamicin (EMEA CVMP, 2001), while the marker residue for oxfendazole is expressed as the sum of extractable residues which may be oxidised to oxfendazole sulphone (EMEA CVMP, 2003) and the marker residue for florfenicol in tissues is defined as the sum of florfenicol and its metabolites measured as florfenicol amine (EMEA CVMP, 2002).

Animals used for residue depletion studies should be similar in age and breed to those for which the veterinary product is intended, although it is recognised that most products will be used in a wide selection of animals of different breeds and probably different ages. All the same, it is inadvisable to use exotic breeds (e.g. miniature breeds or rare breeds) that are not representative of the main target population. Animals used for milk studies should reflect the likelihood that the product may be used in animals at different stages of lactation and producing a range of milk yields because with some drugs a correlation is seen between milk yield and residue concentrations. With lipophilic drugs, milk residues may correlate with fat content so that higher residues may be seen in cows producing richer milk, or differences may occur in residue concentrations between morning and evening milk, reflecting diurnal variations in fat content.

Particular care needs to be taken with products that are administered by intramuscular or subcutaneous injection because injection site residues can rise disproportionately with injection volume on account of the relationship between surface area and volume. Assuming, for the sake of argument, that an injected volume of drug assumes the shape of a sphere, then the surface area increases in proportion to the square of the radius whereas volume increases in proportion to the cube of the radius. Consequently, for a small volume the surface area to volume ratio is greater than for a large volume and this can affect the rate of absorption of the injected drug. With large animals it may be necessary to divide the total injection volume between two or more sites to promote absorption and to minimise residues. However, an applicant should appreciate that the maximum volume injected into any one site during the course of a residue depletion experiment is likely to be the maximum volume that is permitted for use by the authorities post-authorisation. Anyone who subsequently injects a larger volume takes the risk that injection site residues may still be present, above the MRL, at the withdrawal period, thus leading to a reportable event under EU pharmacovigilance requirements.

Although any gender differences in the target species are likely to have been identified during pre-MRL studies, it is still recommended that animals of both sexes are included in tissue residue studies, except in cases where the target population will be exclusively of one gender. An example of this could be a tissue residue study with an intramammary product or with a drug used to affect sexual reproduction; in these cases, the depletion studies would (and in the case of intramammary products, could) only be undertaken using the relevant gender.

In terms of animal numbers to include in residue depletion studies, regulatory guidelines contain recommendations, although applicants should judge each case on its merits with consideration being given to the statistical implications of calculating the withdrawal period. In the EU, for tissue studies undertaken with cattle, sheep and pigs it is often acceptable to use just four animals per time point. For milk residue studies with cattle and sheep, 19 or more animals are needed. With poultry, 6 birds per time point are suggested while for fish it is 10 per harvest interval. For products intended for treatment of bees, five honey samples from each of five hives are recommended.

The requirements in terms of animal numbers in other parts of the world may differ from the EU. For example, currently the FDA requires groups of five animals, rather than four, for tissue residue studies, while Australia specifies a minimum of only three animals in a group. Where feasible, companies usually try to undertake single studies that meet regulatory requirements in all countries where approval is sought, although this is not always possible. For example, with ectoparasiticides there are climate considerations that need to be taken into account when designing a study.

Provision should also be made for obtaining control samples from untreated animals. These are imperative for giving confidence in interpreting the findings from incurred samples. In the case of tissue studies, untreated animals should be kept close to, but separated from, the treated animals, and should experience the same husbandry in terms of bedding, food, water and handling. For milk studies, each animal can serve as its own control by providing pre-treatment milk for comparison with post-treatment milk. Although a placebo product can be administered to control animals, this serves little purpose and is not normally required, at least not in the EU.

The EU recommends that three to five time points are used to get an understanding of the depletion kinetics from tissues. When selecting time points for obtaining tissue samples it is important, where possible, that early intervals are included where residue concentrations are substantially above the MRL. First, this demonstrates exposure, but second, and more crucially, it can allow the rate of depletion of the residues to be observed which results in a more accurate assessment of the withdrawal period. The other time points need to be spaced so that residues are below the MRL in all tissues by the final slaughter time because significant extrapolation of the data beyond the final sampling time to establish a withdrawal period is undesirable. Where possible, a depletion curve established for other produce, such as milk, should also show a decline in concentrations from above the MRL to below the MRL for the same reasons.

In theory, and if the ratio of total residues between edible tissues has been taken into account when MRLs were elaborated, then the marker residue concentration in all tissues should deplete to below MRL at approximately the same time point for each tissue. In reality, this is often not the case and it is not unusual to find that residues in muscle and fat deplete to sub-MRL concentrations very quickly after treatment, while the concentrations in liver and kidney, as well as injection sites, may take much longer. This should be considered when designing residue depletion studies, and a spread of times is needed so that residues are found in all tissues at the earliest time points but are below the MRL in all tissues by the final time point. Usually, the radiolabelled studies undertaken earlier for the purpose of establishing MRLs provide useful information to aid the design of the definitive residue depletion studies.

When formulations are administered by intramuscular or subcutaneous injection then the injection sites must be excised for analysis. However, it must be appreciated that the method of sampling can affect the magnitude of residues because the distribution of residues at an injection site is usually heterogeneous, and contained within a relatively small area or volume. If a very large amount of muscle is dissected out, then this ensures that none of the drug residue is missed, but when the site is homogenised for analysis the residues are diluted in a large mass of muscle which lowers their concentration. On the other hand, if only a small amount of muscle is taken, then the dilution factor is lower and the reported residue concentration is higher; however, there is a risk when taking a small sample that some or all of the injection site will be missed.

With these potential problems in mind the CVMP has published an injection site guideline that contains advice on sampling for studies intended for EU registration submissions (EMEA CVMP, 2004). It is noted that it is essential that the site of injection is clearly identified so that it can be unequivocally located at slaughter. This can be accomplished in several ways, including shaving the area of the site prior to injection and/or using tattoos. After slaughter, the Committee's recommendation is that where possible a cylindrical inner core sample of muscle should be excised for homogenisation. This cylinder should be centred on the point of injection and for intramuscular injections should have an approximate diameter of 10 cm and an approximate depth of 6 cm, while for subcutaneous injections it should have a diameter of about 15 cm and a depth of about 2.5 cm. Cylinders of these dimensions should yield a mass of muscle weighing between 400 and 600 g which is homogenised and then subsequently analysed for drug residues. When removing the inner core, particular care must be taken to ensure that the needle track, the area of drug release and any area of tissue reaction are included.

Besides this inner core sample, where possible a second sample of muscle should be cut out which consists of approximately 300 g of muscle forming a concentric outer ring surrounding the inner core sample. This sample is homogenised and analysed to confirm that the majority of the residue is contained in the inner core: in other words, that the injection site has been properly sampled. If the residue in the outer ring exceeds that in the inner core, then the CVMP maintains that serious consideration should be given to excluding that sample from the withdrawal period assessment.

The CVMP recognises that the target weights of 500 ± 100 g for the inner core and 300 g for the outer ring are not always achievable, particularly in small animals or, in the case of the outer rings, when injections are made into the neck. These cases have to be individually examined to ensure that representative samples of the injection sites are taken. Where the weights of excised muscle are lower than the target weights no adjustment of the reported residue is permitted, which means that the residue concentration will be exaggerated compared with what would have been reported had the target weights been taken. Where animals are treated over several days, the sampling should include sites from the last day's treatment. In addition, where a particular day's treatment has to be injected into more than one location, because of dose volume considerations, the sampled sites should include those that received the maximum volume of formulation that will be recommended for administration on the product label.

With milk residue studies it is customary to take samples from every milking for at least a week after treatment. However, if there is any doubt that the residues may not have depleted to below MRL by that time then samples should be collected for longer, since this can easily be done without increasing the number of subjects. Animals are usually machine-milked following standard farm practices and milk samples should be sampled for analysis before cream separation occurs. Milk from individual animals should not be pooled but should be kept separate because, at least in the EU, withdrawal periods are calculated on an individual animal basis and not on a bulk tank basis. It is also recommended that

individual animal milk yields are recorded because with some veterinary products there is a correlation between milk yield and residue concentrations.

There are special requirements for fish residue studies. Fish are poikilothermic (i.e. cold-blooded) and the rate at which they metabolise and excrete drugs depends on the prevailing water temperature. In other words, the colder the water temperature, the slower the rate of depletion of residues is, and the longer the withdrawal period will be. This is illustrated in a study undertaken by Roy *et al*. (2006) where emamectin was administered to rainbow trout in their diet. Concentrations of the marker residue, emamectin B1a, were measured in samples of muscle with skin in natural proportion. At 6 hours after treatment, mean residues in fish maintained in water at 15°C were 80.5 ± 62.8 ng/g, while those from fish kept at 6°C were 68.0 ± 35.5 ng/g. However, the residues depleted faster in the fish kept in the warmer water and, by 21 days after treatment, the residues in samples from the 15°C fish had depleted to 10.9 ± 11.7 ng/g, whereas those in the 6°C fish had only depleted to 40.2 ± 38.2 ng/g. Similarly, Stehly *et al*. (1998), after investigating the pharmacokinetics of benzocaine in rainbow trout at 6, 12 and 18°C, reported that uptake clearance and metabolic clearance increased at higher temperatures.

Consequently, where possible it is advisable to undertake fish residue studies at the lowest temperatures at which the product will be used so as to represent a worst-case scenario. However, when undertaking studies that involve administration of medicated feed, a factor to bear in mind when selecting the temperature is that fish may not eat if the water temperature is very low. Therefore in such cases a compromise may be needed between a low temperature to represent the worst case in terms of residues, yet at the same time a temperature that is not so low that the fish refuse to feed.

Besides choosing a low temperature, it is also quite common to investigate a higher temperature as well, so as to discover how the rate of depletion of residues varies with temperature. While it is possible to undertake residue studies at commercial fish farms, using outdoor ponds or nets in sea lochs, this approach has the disadvantage that there is no control over temperature, other than to make use of seasonal variations. Furthermore, in studies that have a long in-life phase, the temperature may vary during the course of the investigation. Therefore, a better method is to use indoors tanks where the temperature can be artificially controlled and maintained. In addition, the use of tanks better allows the investigators to monitor the fish, and check for health, stress or feeding problems. Naturally, tanks have to be large enough to accommodate the fish and expert advice should be sought before embarking on such studies.

Where residue studies involve the administration of medicated feed to fish it is necessary to feed the fish collectively because dosing fish on a one-by-one basis is unrealistic and can cause stress to the animals. When feeding fish by the tank, an estimate can be made of feed intake (and hence drug intake) by the whole population but not by individual fish. Many species of fish live in hierarchical groups where the bigger and more dominant fish bully the smaller and less dominant fish. This results in the dominant fish consuming more feed and therefore having higher residues. On the other hand, the bullied fish may eat almost nothing and thus have negligible residues. This large inter-fish variability needs to be taken into account when establishing withdrawal periods, otherwise there is a danger of residues above the MRL being found in some fish after the completion of the withdrawal time.

The animal produce in which residues must be determined for the setting of withdrawal periods depends on species and, of course, on the intended use of the veterinary medicine. For mammals, the edible tissues in which residues must be measured are muscle, liver, kidney and fat, or, in the case of pigs and poultry, fat and skin in natural proportions rather than fat alone. For fish, the edible product is considered to be muscle, with skin in natural proportions. As already noted, milk, eggs and honey are also edible produce in which residue concentrations must be

established if withdrawal periods are needed for these commodities, although sometimes for commercial reasons it may be specified on the label that a veterinary pharmaceutical must not be used in animals producing such foodstuffs for human consumption.

Analytical methodology

An essential prerequisite for conducting a residue depletion study is to have available a fully validated analytical method for determining concentrations of the marker residue. Such a method would have been developed and validated by the sponsor as part of the MRL dossier submitted to the authorities for evaluation. Nevertheless, there are circumstances, for example with off-patent generic drugs, where another company may wish to determine a withdrawal period for its formulation but not have access to the MRL method. Whatever the reason, the choice of method to be used for determining residues is the decision of the company that is applying for the marketing authorisation, but this method must meet defined criteria.

For example, the procedure must be specific for the marker residue. Non-specific methods, such as microbiological methods used to detect antibacterial residues, are not suitable for measuring residues for the purpose of establishing withdrawal periods. However, such methods can be useful on dairy farms where they can be employed as cow-side tests to help in the detection of residues that might lead to bulk tank failures and possible financial penalties from dairies and milk processors (Hillerton *et al*., 1999).

However, for defining withdrawal periods, residues should be determined using chromatographic procedures, preferably incorporating mass spectrometry, for example high performance liquid chromatography combined with mass spectrometry or tandem mass spectrometry (LC–MS and LC–MS/MS, respectively). These procedures use chromatography to separate the marker residues from other co-extractives and then employ mass spectrometry to identify the marker residue by its molecular weight. Such a combination allows excellent measurement of the marker residue, with minimal interference from endogenous co-extractives or from substances closely related to the marker residue such as metabolites.

Besides being specific, the selected method must also be accurate and precise. Accuracy is defined in the EU as:

> 'the closeness of agreement between the true value and the mean result, which would be obtained by applying the experimental procedure a very large number of times' (European Commission, 2005).

Factors affecting accuracy are random errors, such as occasional mis-extractions, and systematic errors. The CVMP requires that accuracy should be within –30% and +10% of the true value for residue concentrations exceeding 1 μg/kg. At concentrations below 1 μg/kg, a wider range is acceptable, namely –50% to +20% of the true value.

The EU definition of precision is:

> 'the closeness of agreement between mutually independent test results' (European Commission, 2005).

The term covers both repeatability and within-laboratory reproducibility, where the former is defined as:

> 'the closeness of agreement between mutually independent test results obtained under repeatability conditions, i.e., with the same method on identical test material in the same laboratory by the same operator using the same equipment within short intervals of time (one batch analysis)'

and the latter as:

> 'the distribution of measurement results obtained under in-house reproducibility conditions, i.e., at the same laboratory and with the same method, specified test materials, preferably different operators, different

environmental conditions (multiple batch analysis)'.

In the EU, the limits for repeatability are defined in terms of the coefficient of variation (CV) of the mean and acceptable limits are 35, 30, 20 and 15% over the respective ranges of <1, >1 to <10, >10 to <100 and >100 μg/kg. For within-laboratory reproducibility, the acceptable CV is determined using Horwitz's equation:

$$CV = 2^{(1-0.5\log C)},$$

where C represents the concentration of the analyte expressed as a decimal fraction (thus 1 μg/kg becomes 10^{-9}).

For the purposes of obtaining MRLs, it is only necessary that accuracy and precision are determined at concentrations of half-MRL, MRL and twice MRL, but when establishing a residue depletion curve it is usually required that the validation is extended to higher concentrations so as to encompass the MRLs likely to be encountered in the study. It is particularly important to have an accurate knowledge of all concentrations in cases where a linear regression method is to be used to calculate a withdrawal period. If necessary, samples containing extremely high residues might be diluted prior to or during analysis so as to bring the measured concentrations within a more reasonable range. Care must also be taken to ensure that samples containing very high residues do not cause contamination that affects the integrity of the results from the low concentration samples. It is also essential that the method is able to quantify residues at concentrations below the MRL. If the limit of quantitation (LoQ) of the method is only equal to the MRL then it cannot be confirmed with confidence that residues have depleted to below the MRL.

Stability of residues is another essential factor that should be taken into account if reliable results are to be obtained. Although it is conventional to store samples frozen before analysis, this is no guarantee of stability and experiments should be undertaken to evaluate losses over the period that samples are stored prior to processing. These tests may be carried out using either fortified samples or incurred samples. The former technique has the advantage that the starting concentration is accurately defined, which makes it easier to measure losses, but the disadvantage that the possible conversion of metabolites to marker residue during storage is not necessarily evaluated. On the other hand, when incurred samples are used the initial concentration is harder to quantify but the overall scenario is more realistic because, whereas fortification usually results in the residues being extracellular, naturally incurred residues may be both extra- and intracellular.

It also needs to be appreciated that residues may also degrade during repeated freeze–thawing of samples, possibly as the result of bacterial contamination and growth. This should be investigated, but it is always advisable to keep the number of freeze–thawing cycles to a minimum by freezing sub-samples of the main bulk samples. Losses may also occur in final extracts of samples prior to quantitation of residues and this also needs to be assessed. Although some of these areas may have been examined during validation of the method prior to obtaining MRLs, it is possible that storage conditions and duration of storage may be different for samples collected from a residue depletion study.

Even after a method has been thoroughly and successfully validated, it is still possible that its performance will change over time. Therefore, it is essential that quality control (QC) samples, consisting of fortified and unfortified control samples along with matrix blanks, are included in each batch of incurred samples to monitor performance. Only by such careful attention to detail can it be ensured that valid results are being generated. Without such results there is a risk that residues may be underestimated, leading to an inaccurate withdrawal period being established, with consequent pharmacovigilance problems.

In the EU, there is no absolute requirement that the same method is used for the depletion study as was included in the MRL dossier and, indeed, as noted earlier, this is difficult to achieve in the case of off-patent drugs where the generic manufacturer may not have access to the pioneer

company's MRL method. Nevertheless, whenever possible it is advisable that any deviations from the original method are kept to the minimum, particularly with regards to the initial extraction steps. This is because it is likely that the original method will have been tested on samples containing incurred radiolabelled residues where the efficiency of the method to extract residues could be easily measured. In contrast, attempting to validate a method using only extraction of fortified control samples provides no information on how good that method would be at extracting incurred residues. Therefore, significant changes to the MRL analytical method must be approached with considerable caution and can require additional validation, otherwise the modified method may lead to the setting of a withdrawal period that is too short, again leading to violative residues being found.

As residue depletion studies, including the validation of analytical methods, are concerned with aspects of human safety, they must, under the EU's veterinary legislation, be undertaken in compliance with Good Laboratory Practice (GLP) regulations (OECD, 1998). Compliance with GLP ensures the quality and integrity of the data and makes certain that the report provides an accurate reflection of the data generated during the investigation. This allows confidence to be placed in the withdrawal periods, which is important in terms of pharmacovigilance.

Calculation of withdrawal periods

Having undertaken residue depletion studies, using the proposed commercial formulation, administered at the proposed dose rate, and by the proposed dose route, to the target species, it is then necessary to analyse the residue concentrations and establish withdrawal periods. In general, statistical methods are favoured for the setting of withdrawal periods. Various methods have been proposed in the literature, sometimes with the intention of obtaining a worldwide consensus which would lead to international harmonisation of withdrawal periods (Concordet and Toutain, 1997a, b; Martinez *et al*., 2000). However, no international agreement has been reached and, at the time of writing, the defining of withdrawal periods is not a topic listed for discussion by the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Products (VICH), whose remit is to harmonise regulatory requirements in the EU, Japan and the USA.

Consequently, various methods of establishing withdrawal periods are used in different parts of the world, although all EU member states have now adopted a consistent approach to setting withdrawal periods. The EU technique for tissues is described in a guideline issued by the CVMP and is based on the assumption that the terminal elimination of drugs from tissues follows first-order kinetics (EMEA CVMP, 1996). Thus a plot of the logarithmically transformed residue concentration data against time will be a straight line and a linear regression analysis of this line can be used to calculate a withdrawal period. Normally, in the EU, the tissue withdrawal period is calculated using residue concentrations that have been corrected for recovery (accuracy) of the analytical method, whereas in the USA, for example, no correction is usually made.

The EU linear regression model relies on several assumptions being valid (EMEA CVMP, 1996):

1. The regression analysis requires that residue concentrations are independent of one another. This is usually true for tissue data because the samples that were analysed came from different animals. A possible exception to this assumption might be in the analysis of injection site data where it could be that several injection sites were obtained from each animal.
2. There is an assumption that there is homogeneity of variances of the logarithmically transformed data at each slaughter time. This assumption can be checked for the data set using one of several statistical tests. The test preferred by the CVMP is Cochran's whereas the FDA recommends Bartlett's test in its

regression model. The latter is considered to be the more powerful test but is more sensitive than Cochran's test to deviations from normality. It should only be used with animal group sizes of five or more, whereas group sizes of four are generally used in the EU.

3. It is assumed that the logarithmically transformed residue concentrations are linear with time, and thus that the depletion kinetics are first order. To some extent, this may be tested by visually examining the residues depletion line for deviations from linearity, but statistical confirmation can be obtained using a lack of fit test.
4. It is assumed that there is normality of residuals and this may be assessed using the Shapiro-Wilk test.
5. It is assumed that the residue concentration in each tissue sample was determined the same number of times in the laboratory; this could be single analysis, or duplicate or triplicate analysis. Analysing different numbers of replicates of different samples could affect the linear regression by biasing the slope and intercept of the line. Where residues are determined in duplicate or triplicate then the mean concentration should be used in the statistical analysis.

Providing that these assumptions are met, then linear regression can be used to estimate the tissue withdrawal period. In the EU, the withdrawal period is defined as the time when 95% of animals, with 95% confidence, will have residues below the MRL, with this time being rounded up to the next whole day if necessary.

The FDA has adopted a similar approach but favours using the 99th percentile of the population with 95% confidence. This leads to longer withdrawal periods, and a concern by the EU regulators, expressed in their guideline, is that it can also require extrapolation of the data set beyond the last time point used in the residue depletion study, which can lead to inadequately derived withdrawal periods (EMEA CVMP, 1996). The view in the EU is that while a small degree of extrapolation is permissible, and possibly inevitable given that the LoQ of the method is probably only half-MRL, this has to be approached with care. Naturally, interpolation carries no such problems.

A problem that can arise with the statistical method concerns residue concentrations that are below either the LoQ or the limit of detection (LoD) of the method. The general advice given in the EU is that such data should be assumed to be one-half of the LoQ or LoD, as appropriate (EMEA CVMP, 1996). In contrast, according to the CVMP, the FDA recommends excluding below LoD data from the analysis (EMEA CVMP, 1996). Even in the EU, exclusion of the entire time point is recommended when all or most of the reported data are below these analytical limits. If this results in fewer than three slaughter times remaining in the depletion data then it is not possible to use the statistical method and an alternative approach is necessary, which is discussed below.

The EMEA provides free software that may be used by regulators and pharmaceutical companies for calculating tissue withdrawal periods for EU authorisations. This program also tests the assumptions referred to above relating to homogeneity of variances, linearity and normality of residuals and allows the user to see if any of these assumptions have been violated.

As a result of the need for residue concentrations to be derived from at least three time points after treatment of the animals, plus the requirement that the statistical pre-conditions be met, it is not uncommon to find that the data are not suitable for statistical analysis, or else that the outcome of the analysis needs to be treated with some caution. In these events, alternative approaches to estimating a tissue withdrawal period are permitted in the EU and mentioned in the CVMP guideline (EMEA CVMP, 1996).

One alternative method, which is frequently used, is to establish the withdrawal period on the basis of the time point at which residue concentrations in all tissues are below the relevant MRL. To this time is added a safety margin which is intended to compensate for inter-animal variability and is aimed at ensuring that at the proposed withdrawal period there is little likelihood of

finding incurred residues that exceed MRL in a larger population of animals. The magnitude of the safety margin, sometimes referred to as a safety span, has to be determined on a case-by-case basis by examining the available residue data, but the CVMP guideline suggests that it should be between 10 and 30% of the time needed for all individual residues to deplete to below the MRL, or, alternatively, between one and three times the depletion half-life, added to this time. The former method of establishing a safety margin is more commonly used because sufficient data are not always available to estimate a half-life or the depletion kinetics may not be first order.

By whatever means are appropriate, separate withdrawal periods must be estimated for each edible tissue, including injection sites where appropriate, and the longest of these individual tissue withdrawal periods then becomes the overall withdrawal period for the product.

In addition to determining a tissue withdrawal period, it is also necessary to establish a milk withdrawal period for products administered to animals producing milk intended for human consumption. The animal used most commonly as a source of milk for human consumption is the cow and the paragraphs below were written with cows in mind. Nevertheless, much of what is written applies equally to other milk-producing species such as sheep, goats, reindeer and mares, whose milk is consumed in at least one EU member state. It should be noted that most regulatory agencies usually allow products to be contraindicated in animals producing milk for human consumption. In practical terms, this means that such products may be used in lactating animals, but the milk must not enter the human food chain. As such animals may produce milk for human consumption at some time in the future, it is sometimes necessary to specify an interval, often in terms of months, before milk may be collected for human consumption.

The linear regression statistical method used in the EU for establishing tissue withdrawal periods is not suitable for use with milk samples because one of the key requirements of the procedure is that individual residue concentrations are independent of one another, which does not hold true for milk where a continuous series of samples are collected from each animal. Therefore, in the EU, milk withdrawal periods are normally established using the Time to Safe Concentration (TTSC) method, which calculates a tolerance limit on the number of milkings per animal needed for residues to deplete to below the MRL (EMEA CVMP, 2000). This model requires milk residue concentration data from at least 19 animals and assumes a log-normal distribution of individual times to safe concentration. As concentrations of residues in milk sometimes deplete in a saw-toothed manner, it corrects by monotonic regression for any increases in concentration found in the depletion profile.

A second monotonic regression can be undertaken to smooth the relationship between the MRL and the resulting withdrawal period. This second monotonic processing is needed because a quirk of the TTSC method is that within the same data set reducing the MRL can bizarrely decrease the estimated withdrawal period due to statistical fluctuations: this is addressed using the second monotonic regression.

Thus in the EU the milk residue concentrations are usually processed by the TTSC method and the withdrawal period is calculated as the upper 95% confidence limit of the 95th percentile of the population of individual times to safe concentration. The calculated withdrawal period is rounded up to the next whole milking and may be expressed either in terms of hours from treatment or in the number of milkings from treatment. As with tissues, the EMEA has made freely available a program that regulators and pharmaceutical companies may use to calculate milk withdrawal periods by the TTSC method. The software contains the option of carrying out either or both of the monotonic regressions referred to above.

The TTSC method can be used in almost all circumstances except in a situation where all individual milk residues are below MRL at the first and all subsequent milkings after dosing. Because there is no variability between times

to safe concentration in this instance, then an alternative approach has to be used. One approach, which relies on a large proportion of milk samples containing quantifiable residues, calculates a tolerance limit for the first milking. If this is below the MRL then a 12-hour (1 milking) withdrawal period is applied, otherwise a longer withdrawal period will be needed. Where this approach cannot be used because most residues at the first milking are unquantifiable, and where also the LoQ is no greater than half-MRL, then it is usually acceptable to establish a withdrawal period of 12 hours (1 milking).

Provided the animals are milked at 12-hourly intervals, and they were dosed just after milking, then a 12-hour withdrawal period means that no milk needs to be discarded. However, in circumstances where the interval between treatment and first milking was less than 12 hours, then milk is only considered to be safe for consumption at the second milking after dosing. Similarly, in settings where cows are milked more frequently than 12-hourly, then milk must not be taken for human consumption during the first 12 hours.

In some cases where drugs used as veterinary medicines either deplete very rapidly from animal produce or leave very low residues that are below the MRL at all times after treatment, it may be possible for a zero withdrawal period to be established, meaning that no interval needs to be left between dosing of the animal and its slaughter or collection of its milk. When proposing zero withdrawal periods it is essential to take into account the pharmacokinetics of the drug in question. For example, after oral or intramuscular administration, concentrations of the marker residue may not reach peak levels for several hours or perhaps not until the next day. With topical treatments, often used to apply ectoparasiticides, slow transdermal penetration may mean that it is several days before the highest concentrations of marker residue are attained in tissues or milk. A delay to peak residues is also seen with eggs, irrespective of the route of administration, and this is addressed further below. Therefore, in these examples, samples taken very shortly after treatment may contain residues below the MRL whereas those taken sometimes afterwards may not. Thus residue studies conducted to establish zero withdrawal periods must examine a series of time points to detect the time of peak concentrations.

Eggs form a special case in that residues may partition into the yolk fraction of the eggs, or the albumen fraction, or both. Lipophilic residues favour the yolk while hydrophilic substances favour the albumen. When analysing eggs for residues, it is usual to combine the yolk with the albumen because the MRL relates to the whole egg and not just one part of it. Nevertheless, it is still useful to conduct separate determinations on representative yolks and albumen fractions to gain knowledge on how the residues are distributed between the two parts. It is known that the yolks develop in the follicles of hens over a period of several weeks, with the majority of growth occurring in the 2 weeks before ovulation when the individual yolks increase in weight from approximately 0.2 g to the final mature weight of around 17 g (Donoghue *et al*., 1996). At any one time, the ovaries will contain yolks in different stages of development, allowing the hen to lay one egg a day.

During this period of egg development, drugs administered to the birds can transfer to the yolks. Donoghue (2001) showed that the pattern of transfer of residues into the yolk was qualitatively similar for three substances with different physicochemical characteristics, namely, ampicillin, oxytetracycline and lindane. With each compound, the greatest quantities were transferred into yolks that were 4–5 days away from being laid in fully developed eggs. Smaller amounts were transferred to yolks that were further away from being laid. Consequently, for drugs that leave residues that favour the yolk, peak residues in eggs may not occur until 4–5 days after completion of treatment and may then persist for many days, or even weeks, afterwards. In contrast to the slow development of the yolk, the albumen fraction of an egg is only produced shortly before the egg is ovulated and therefore residues of hydrophilic drugs may be found in

eggs laid soon after treatment of the birds with a veterinary drug.

It is thus conceivable that storage of drug residues in the yolk can lead to long withdrawal periods for veterinary products used in laying birds. Because it is usual to treat an entire flock or shed with a drug, long withdrawal periods are very undesirable from the farmer's perspective and can cause marked economic losses. In some countries, for example Australia and the USA, it is policy that products may only be used with whole shed treatments of laying birds in situations where the residues are sufficiently below the MRL at all times after treatment to support a zero withdrawal period (Australian Pesticides and Veterinary Medicines Authority, 2004).

Milk residues are of particular concern in terms of establishing a zero withdrawal period. Commonly, cows are milked twice daily at approximately 12-hour intervals and drugs are dosed shortly after the morning milking. As already explained, with this regime, depending on the magnitude of the residues, it is sometimes possible to establish a 12-hour milk withdrawal period which has the advantage that no milk needs to be discarded from human consumption. However, being able to claim a zero milk withdrawal period can be of great commercial importance to pharmaceutical companies because it allows animals to be treated at any time prior to treatment, and for any milking regime to be used, without the need for the farmer to discard any milk.

However, in order to obtain a zero milk withdrawal period it is necessary to undertake residue studies where the effects of administering the drug at selected intervals of between 0 and 12 hours prior to milking are investigated. This is because drugs, and their metabolites, can transfer rapidly into milk from blood and the concentrations in milk will be in equilibrium with those in blood. Consequently, if cows are treated shortly before milking then the high concentrations in blood are mirrored in milk, and residues above the MRL could be found in the samples taken at the first milking after treatment.

Residues at injection sites can present particular problems when establishing withdrawal periods. Although estimates vary, it is generally accepted that residues in injection sites are only eaten on a very occasional basis. Using data from New Zealand, Brown (2000) calculated that injection site residues might be ingested between once every 1.8 years and once every 45 years, while Sanquer *et al*. (2006) estimated that the maximal likelihood of a consumer in Europe eating some or all of an injection site was about four times per year. Thus even from the European assessment, the ingestion of injection site residues is not a frequent occurrence and therefore the risk to the consumer is more akin to an acute risk than a chronic risk.

With this in mind, the regulatory authorities in Australia, Canada and the USA have developed procedures that allow, under defined circumstances, the residues in injection site muscle to be higher than those in muscle remote from the site of injection (i.e. 'ordinary muscle') (Reeves, 2007). In Australia, in cases where the total residues in injection site muscle exceed the MRL for ordinary muscle at the proposed withdrawal period, the estimated acute intake (EAI) is calculated for the injection site residue and this is compared with the acute reference dose (ARfD; sometimes referred to as an acceptable single daily intake (ASDI)) which assesses the safety to the consumer of ingesting a single dose of residue. If the EAI exceeds the ARfD then the Australian authorities impose an extended withdrawal period such that at the end of this time the EAI of total residues in the injection site is less than the ARfD.

The Canadian Veterinary Drugs Directorate also compares the total residues at the injection site at the proposed withdrawal period with the MRL for ordinary muscle. If the concentration of injection site residues exceeds the safe total residue level/MRL ratio by 10 times or more, the withdrawal period determined by the target tissue and the time required for injection site residues to deplete to the MRL for muscle are compared. The final withdrawal period is the longer of the two.

In the USA, the Center for Veterinary Medicine (CVM) accepts that residues may be present at

injection sites at up to 10 times the safe concentration established for ordinary muscle without this having any impact on the withdrawal period. However, where the injection site residues at the proposed withdrawal period are more than 10 times this safe concentration for ordinary muscle, then the withdrawal period is adjusted to the time required for injection site residues to have depleted to no more than 10 times the safe concentration for ordinary muscle. Alternatively, in cases where it is possible to derive an ASDI because the drug displays acute effects, then the CVM establishes a separate tolerance level for injection site muscle which can be used to calculate a withdrawal period necessary to ensure that injection site residues will be below this tolerance level.

Therefore, in some countries, it is sometimes possible that the injection site residues present at the withdrawal period could exceed the MRL established in those countries for 'ordinary' muscle. Because of the infrequency with which injection sites are eaten their residues should not pose any chronic risk to human safety, although consideration does have to be given to possible acute toxicological, pharmacological or allergenic effects arising from the consumption of an occasional injection site residue.

Nevertheless, while there will usually be no health concerns, residues in injection site muscle that exceed the MRL for ordinary muscle can create problems during residue surveillance because it can be difficult to distinguish between injection site and ordinary muscle and it is possible that a sample of an injection site could be inadvertently taken during routine residue surveillance and found to contain residues above the MRL for ordinary muscle. This could result in the entire carcase being condemned, possibly with the farmer being penalised. The finding of violative residues in muscle, on account of the unintentional excision of an injection site, could also have implications for international trade.

With this in mind, the EU has taken a position that is different to that in some other countries in that injection site muscle is treated no differently to ordinary muscle and at the withdrawal period the residues in injection sites should be below the MRL established for ordinary muscle. Besides simplifying the task of residue surveillance, this approach also eliminates any possible acute risks that the consumer may face from consumption of the occasional injection site and avoids the need to establish a separate acceptable daily intake (ADI) for acute intake.

A withdrawal period for injection site residues can be established using the statistical method in the same way that data from other tissues can be analysed by this approach. However, injection sites can show very marked inter-animal and inter-site variations in residue concentrations which can sometimes lead to the data being unsuitable for statistical analysis. In these cases, a non-statistical approach needs to be taken, as already discussed above.

It is important to note that the withdrawal period, whether established using a statistical method or whether estimated using the safety margin approach, cannot guarantee that every individual animal in a large treated population will have residues below MRL at the withdrawal period. At most, the statistical method only ensures that at the withdrawal period the required percentile of animals (e.g. 95%) will have residues below the MRL with the required degree of confidence (e.g. 95%). It is always possible that violative residues will be found in occasional samples despite the withdrawal period being observed by the farmer.

Special methods need to be used to establish withdrawal periods for antibacterial drugs that are infused directly into the udder after the last milking of the lactation period as the animals enter the dry period leading up to giving birth and restarting lactation. These products are intended to treat any subclinical infections that may be present and to provide prolonged protection of the udder against new infections arising during the dry period. Although most of the drug is likely to be systemically absorbed, or otherwise lost, from the udder during the dry period, when the animal gives birth then any residual deposits of drug remaining in the udder will be flushed out with the colostrum and the milk. For such

formulations, the withdrawal period must have two components. First, it must specify the minimum number of days between infusion and subsequent calving, and, second, it must define the number of hours after calving when the milk may be collected for human consumption.

The length of the dry period depends on the decision of the farmer on when to terminate the lactation, taking into account farm practices and animal welfare considerations. However, even within a herd where all cows are dried off at the same time in their reproductive cycle, there will inevitably be marked inter-animal variations in the length of the dry period because of the problems of accurately predicting calving dates. These differences in the length of the dry period can be reflected in the magnitude of residues secreted in colostrum and milk at the time the cows give birth.

Not all intramammary products are intended to provide the same duration of protection to the udder and some are designed for administration to cows with short dry periods, while others are aimed at those cows with long dry periods. To allow for variations in the length of the dry period, the EU guideline requires that if an applicant wants to specify that the interval between treatment and calving is x days, then residue data must be obtained from at least 19 cows with dry periods ranging between $^2/_3x$ and x days (EMEA CVMP, 2000). Given the problems in accurately forecasting calving dates, to obtain data from 19 cows with dry periods in this range requires starting the study with a larger number of cows and then excluding those animals with dry periods outside this range. After each cow has calved, then milk should be collected twice daily and analysed for residues to determine the post-calving milk withdrawal period. However, note that the milk collected in the first few milkings after lactation starts will be rich in colostrum and therefore will not be fit for human consumption. Also, in analysing such colostrum-rich milk, checks need to be made that the analytical method functions adequately because it will have been validated using regular milk and not the colostrum-rich type.

It is relevant to note that any animal producing milk for human consumption could conceivably be slaughtered for human consumption. This could happen as the result of an accident resulting in injury to the animal. Therefore, it is necessary that a tissue withdrawal period is established for all animals producing milk for human consumption. However, as already noted, it is not obligatory to define a milk withdrawal period for lactating animals provided milk from such animals does not enter the human food chain.

In Australia, and possibly elsewhere, it is also necessary to consider residues in calves arising from treatment of the cow during pregnancy or shortly after birth (Australian Pesticides and Veterinary Medicines Authority, 2001). Residues could enter the calf, either from in utero transfer or from the colostrum or milk fed to calves, and result in secondary residues in the tissues of the calf.

Withdrawal periods for fish can be assessed using the same techniques as used for tissues from other species. As explained earlier, the rate of depletion of residues from fish is temperature dependent and therefore withdrawal periods for fish are also temperature dependent. Consequently, quoting a fish withdrawal period in days is meaningless unless a temperature is also cited. However, an alternative is to refer to withdrawal periods in terms of degree days, which are the product of temperature and days. Thus a withdrawal period of 150 degree days equates to 15 days at a water temperature of 10°C or 10 days at a temperature of 15°C. This method assumes a linear relationship between the withdrawal period expressed in days and the reciprocal of water temperature which may not always be the case, particularly over a wide temperature range.

As was explained in Chapter 23, in the EU some veterinary products are assigned to Annex II of Council Regulation (EEC) No. 2377/90 which means that no numerical MRLs are necessary because residues of the substance are not considered to present a public health risk. Often, allocation to Annex II means that no withdrawal period is necessary before produce is used for human

consumption, but this must not be assumed to be the case in all situations. For example, with an Annex II drug given by intramuscular injection it is possible that a residue will be left at the injection site that could be harmful to human health. Therefore, residue depletion studies may be needed for some Annex II substances, although such studies can be complicated to carry out because there will be no established marker residue and no numeric MRL. Under these circumstances, it is necessary to obtain information on total residues and to then calculate the amount of residue consumed daily using accepted daily food consumption values, for example:

- 300 g of muscle;
- 100 g of liver;
- 50 g of kidney;
- 50 g of fat;
- 1.5 litres of milk;
- 100 g of eggs;
- 20 g of honey.

These are the values used in the EU (European Commission, 2005). The amount of residue ingested daily in the diet is then compared with the ADI and a withdrawal period established to ensure that the ADI is not exceeded. The finding of residues during surveillance that exceed the ADI would constitute a reportable offence under the requirements of pharmacovigilance.

Conclusions

In conclusion, this chapter has provided a short review of how withdrawal periods may be established for veterinary drugs that are administered to animals producing food for human consumption. The discovery of residues above the MRL in animal produce leads to pharmacovigilance issues which can result in prosecutions and financial penalties for farmers. Withdrawal periods are usually necessary to ensure that the residues have depleted to below the MRL at the time that the produce is sold for human consumption. It has been explained how studies are undertaken to investigate the depletion of residues and how the data can be analysed to define withdrawal periods. Although the emphasis has been on procedures used in the EU, the general concepts discussed are relevant to other parts of the world.

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25 Surveillance for veterinary residues

K.N. Woodward

Introduction

As noted in Chapters 2 and 23 of this book, violation of maximum residue limits (MRLs) in the European Union is an aspect of veterinary pharmacovigilance, as is the finding of residues of substances without an MRL or substances that are prohibited by virtue of inclusion in Annex IV of Regulation (EEC) No. 2377/90 (e.g. chloramphenicol and nitrofurans) or those banned or restricted under other EU legislation (e.g. clenbuterol and the anabolic agents used for performance enhancement).

Violative residues may occur because withdrawal periods have not been observed (or are inadequate), because higher doses or longer periods of administration than those authorised and specified in product literature have been administered or because illegal or unauthorised drugs have been given. The purposes of residues surveillance are to monitor the levels of compliance in a country or geopolitical area and, as with other areas of pharmacovigilance, to take corrective action when regular violations are discovered and signals and trends occur.

Residues in excess of MRL values or other tolerances and limits do not necessarily imply a public health (consumer) risk. Numerous safety factors are used in the elaboration of MRLs as described in Chapter 23. The acceptable daily intake (ADI) value is the gold standard for consumer safety, and not the MRL, which is in large part a regulatory implement. The ADI calculation utilises a large safety factor, usually of 100, and so even if the ADI is exceeded, the consumer may not necessarily be at risk and since ADI values are premised on the basis of life-time exposure, then occasional excursions above the ADI are unlikely to constitute a public health problem.

However, regulatory systems of any type depend on two main factors as a measure of success – compliance by those they are aimed at and public confidence by those they aim to protect. If lack of compliance comes to be regarded as the norm, then public confidence tends to collapse. Once that collapse has occurred, it is extremely difficult, and occasionally virtually impossible, to reconstitute the trust that has been lost. Establishing the safety and residue depletion profiles of veterinary drugs, and elaborating MRLs and subsequently determining withdrawal periods for food of animal origin, is an interesting but ultimately futile exercise, if those withdrawal periods are then ignored or if rendered useless by overdosing or by dosing for periods

longer than those recommended. Veterinary drug manufacturers spend huge sums on scientific and clinical studies to demonstrate the safety, quality and efficacy of their products when used as directed, and this is to no avail if recommendations subsequent to this expense and effort are ignored or, at best, treated as optional.

Similarly, the system will fall into disrepute, and again may be seen as failing, if drugs prohibited on the basis of potential health risks are employed to treat food-producing animals. Even if these abuses fail to materialise, food from third countries where different MRLs are employed, or where MRLs have not been established for some or all veterinary medicinal products used in food animals, means that consumers may be exposed at worst to potentially hazardous residues and at best to residues arising from drugs that have not been fully evaluated.

In many countries, consumer (or lay) representatives now sit on influential expert committees responsible for a number of regulatory areas, including those charged with the assessment of safety, quality and efficacy of medicines including veterinary medicines. This is certainly the case in the UK where the expert advisory body, the Veterinary Products Committee, has lay representatives among its membership. These individuals are often (but necessarily and not always) affiliated to consumer organisations and lobby groups. In Europe, the Bureau Européen des Unions de Consommateurs (BEUC, the European Consumers' Organisation) based in Brussels is heavily involved in lobbying European institutions on health and consumer matters. It is clearly in the interests of government institutions to ensure that legislation is enforced and seen to be enforced effectively, particularly on issues related to food safety. In the United States too, food safety lobbying is a reality and consumer-based advocacy groups lobby Congress in attempts to strengthen legislation and enforcement (Paige *et al*., 1997). None of this is surprising considering the spate of food safety issues and disease outbreaks in several countries in recent years, including salmonellosis, bovine spongiform encephalopathy and outbreaks of foodborne disease caused by *Escherichia coli* 0157 and *Campylobacter*.

Concerns over the safety of residues, particularly their potential toxic effects, have been expressed over the last 30 years (Brander, 1970; Van Dresser and Wilcke, 1989; Somogyi, 1992; Teske, 1992; Paige *et al*., 1997; Tomerlin *et al*., 1997; Mitchell *et al*., 1998; Paige, 1998; Miller and Flynn, 2000; Sundlof *et al*., 2000; Berg, 2001; McEvoy, 2001; Tennant, 2001; Reeves, 2005; Capleton *et al*., 2006). Some of these issues have been addressed elsewhere in this volume and particularly in Chapters 23 and 24 regarding the elaboration of MRLs and the establishment of withdrawal periods. However, it is concerns such as these that have initially led to and later refined legislation relating to the registration of veterinary drugs, the establishment of MRLs and surveillance of residues of veterinary drugs in food of animal origin.

This chapter focuses on residue surveillance in one member state of the European Union, the United Kingdom. The choice of the UK as an exemplar for this topic is manifold but not least is the availability of data and the transparency of that information.

Residues and residue studies

Residues are the metabolites of veterinary drugs, and their associated parent compounds, that remain in the animal or its produce (eggs, milk and honey) after treatment. Their behaviour depends on the nature of the drug and its metabolites and on the pharmacokinetics of the drug in the animal concerned. Those that are metabolised and excreted rapidly also rapidly deplete in the animal. Those that are slowly metabolised may also deplete rapidly if their excretion is not dependent on metabolism. Others may be subject to slow excretion, especially those that bind to macromolecules and are thus not available for metabolism and/or excretion. The majority of animals that are now farmed, including fish and shellfish, are susceptible to one or more types of bacterial, fungal or parasitic disease and there are

ranges of drugs available for the treatment of these conditions as indeed there are for a variety of non-infectious diseases. Some drugs may be metabolised largely to physiologically substances such as water, bicarbonate and carbon dioxide and be excreted relatively rapidly. Others may be converted to a variety of metabolites which together with any remaining parent drug may depurate over shorter or longer periods of time.

Residues may be found in all edible tissues (and in some considered non-edible, although these may eventually be consumed after processing into other products such as stocks and broths and processed food ingredients). Although the behaviour of drugs in animals may be examined through residues depletion studies, a more comprehensive understanding may be gleaned through well-conducted pharmacokinetic studies so that metabolism, distribution and excretion can be investigated, along with some of the determining factors (Clement, 1995). This also assists in demonstrating species differences, if any, between different animals and, together with results from similar studies in laboratory species, provides a better picture of the processes involved, the nature of the metabolites and the rates of clearance and excretion.

A good understanding of the pharmacokinetic behaviour of drugs, especially in the food animals to be treated, can underpin the design of formal residues studies and help to reduce costs and the need to repeat work. Furthermore, targeted analytical chemistry and other physicochemical methods of analysis for residues can only be attempted if the likely metabolites, or more appropriately analytes, are known and understood (Mercer *et al*., 1977; Perez, 1977; Morton, 1980; Ludwig, 1989; Jager and Vroomen, 1990; Shearer, 1990; Woodward, 1992a; Dixon, 2001; Cravedi, 2002; Hammel *et al*., 2008).

Milk and fish are frequently regarded as 'healthy' foods of high nutritional value. The presence of drug residues in these foods, and particularly the presence of antibiotic residues, is regarded by consumers as especially troublesome (if not hazardous) because of this perception. However, residues of veterinary drugs can and do find their way into these commodities (Feagan, 1966; Olson and Sanders, 1975; Braun *et al*., 1985; Booth and Harding, 1986; Horsberg *et al*., 1990; Sudershan and Bhat, 1995; Ibach *et al*., 1998; Talley, 1999; Intorre *et al*., 2002; Shaikh *et al*., 2003; Šinigoj-Gačnik *et al*., 2005; Unnikrishnan *et al*., 2005; Chinabut *et al*., 2006; Esposito *et al*., 2007).

Similarly, the presence of residues in eggs and poultry products gives rise to consumer concerns (Lashev & Mihailov, 1994; Anadón *et al*., 1995; De Wasch *et al*., 1998; Kan and Petz, 2000; Mortier *et al*., 2005), while residues of almost any substance regarded as a pesticide, despite its veterinary use, can lead to consumer concerns and such products are found in food of animal origin (Braun *et al*., 1985; Horsberg *et al*., 1990; Woodward, 1992a; Baynes *et al*., 1997; Szerletics-Túri *et al*., 2000).

In the EU, the hormonal growth promoters such as testosterone and trenbolone acetate were banned from use in food animals in 1988 (although they are still authorised in some non-EU countries). This occurred partly on consumer health grounds, although this was not considered at the time to be a critical issue, with socioeconomic and trade issues playing a suspect part in the story (Lamming *et al*., 1987; Farber, 1991; van Leeuwen, 1991; Waltner-Toews and McEwen, 1994; Miller and Leighton, 1996; Leighton, 1999), largely because residues of these drugs are low in concentration and because they are natural hormones, generally within normal physiological limits.

Directive 96/22/EC confirmed this prohibition and added other substances such as thyrostatic compounds, drugs with oestrogenic, androgenic or gestagenic activity, and some β-agonists. Some of these substances, for example testosterone, zeranol, trenbolone acetate and allyl trenbolone, had previously been used, some quite legally, as growth promoters or production enhancers, particularly in cattle (Patterson *et al*., 1985; Baker and Gonyou, 1986; Gray *et al*., 1986; Hunt *et al*., 1991; Jones *et al*., 1991; Zarkawi *et al*., 1991; Hayden *et al*., 1992; Peters, 1992; Herschler *et al*., 1995; Cranwell *et al*., 1996; Massart *et al*., 1996; Meyer, 2001).

A number of these possess potent endocrine activity (Le Guevel and Pakdel, 2001; Mantovani and Macri, 2002). However, a recent report by the UK's Veterinary Products Committee recognises some potentials hazards and associated risks arising from the use of these hormonal substances, but fails to give a firm endorsement of the EU-wide ban (Veterinary Products Committee, 2006). Others, such as the β-agonists salbutamol and clenbuterol, had been authorised for therapeutic purposes including tocolysis in cattle, but not for growth enhancement purposes. These drugs have repartitioning effects, reducing body fat while increasing lean tissue deposition (Buttery and Dawson, 1987).

Under Directive 96/22/EC the uses of many of these agents were restricted to therapeutic uses (e.g. testosterone and some β-agonist drugs) or prohibited altogether for use in food animals (e.g. trenbolone and its derivatives and zeranol). The milk-production enhancer bovine somatotropin (BST) was also prohibited in the EU, but this was largely for socioeconomic reasons, although animal welfare concerns were cited at the time (Waltner-Toews and McEwen, 1994). Regardless, BST has been used for several years in the US and in other countries without any major animal health problems.

To ensure regulatory compliance, residues surveillance is conducted in all EU countries (Macri and Marabelli, 1992; Woodward, 1993, 2000, 2005; Dixon, 2001). Under Directive 96/23/EC and Council Decision 97/47/EC, the competent authorities of EU member states are required to submit each year to the European Commission for approval an annual plan for sample collection and residues analyses to be conducted the following year. The numbers in each plan, and the analytes to be determined, are largely based on the results of previous years (Dixon, 2001). Applicants for MRLs are required to submit an analytical method suitable for determining reasons with their submission. This may be used, with or without adaptation, for residues surveillance for the drug in question. In addition, EU control and reference laboratories develop their own methods for products of interest, while there is a bewildering array available in the literature and in specialised texts (Heitzman, 1994; Kuiper and Andersen, 1994; Oka *et al*., 1995; Woodward and Shearer, 1995; Ellis, 1996; Dixon, 2001; MacNeil, 2003; Tuomola and Lövgren, 2004; van Hoof *et al*., 2004).

Residues surveillance for veterinary drugs in the UK

From what has already been mentioned, veterinary drug residue surveillance in the United Kingdom should be seen as part of a broader European Union exercise that is permanently in place. The competent authority for drug residue surveillance in the UK is the Veterinary Medicines Directorate (VMD) which has been responsible for the scheme for many years and which since 2001 has devolved parts of that task, including the provision of guidance and advice, to the Veterinary Residues Committee (VRC). The VRC is an independent committee that 'provides oversight into how the UK's surveillance for residues is carried out'. The reports of veterinary surveillance in the UK are published annually and provide a detailed source of data, one of the main reasons why the UK model was chosen to exemplify residues surveillance activities (VMD, 1996–2001; VRC, 2002–2007).

Under Directive 96/23/EC and Council Decision 97/47/EC, EU member states are required to submit each year an annual plan for residues analyses to be conducted the following year. The numbers in each plan, and the analytes to be determined, are largely based on the results of previous years (VMD, 2001). As an example, the targets for the UK for 2001 are shown in *Table 25.1*, with the actual numbers analysed and positive samples identified for the period 2003–2006 shown in *Table 25.2*.

In the UK, the exercise conducted under the EU legislation is known as the Statutory Surveillance Scheme. In addition to this there is a Non-Statutory Surveillance Scheme funded by the UK government which is based on UK rather than EU priorities. It is a more limited programme

Table 25.1 Target samples for residues surveillance in 2001.

Food product	*Number of samples*	*Number of analyses*
Milk	855	2,655
Game	200	350
Eggs	528	1,266
Fish	1,431	1,497
Poultry	8,318	9,872
Red meat	22,883	22,912
Total	**34,215**	**38,552**

Table 25.2 UK National Surveillance Scheme – Statutory Scheme, 2003–2006: number of samples and number of positive results*.

Year	*Analyses conducted*	*Samples at or above reference points*	*Positive samples***
2003	35,399	137	89
2004	29,475	137	75
2005	37,067	120	55
2006	38,257	101	50

*Based on VRC, 2007.
**Above the MRL or other action level, usually the limit of quantification of the assay for prohibited or non-authorised product where no MRL exists.

which examines residues in foods eaten by average consumers or of foods consumed by susceptible groups such as infants.

The results of the Statutory and Non-Statutory Schemes were originally published each year by the VMD in its Annual Report on Surveillance for Veterinary Medicines, up to and including the results for 2000 (published in 2001). In 2002 and subsequent years, the results were published under the auspices of the VRC.

In the Statutory Scheme the numbers of MRL violations were low in 2001. Most of these were related to tetracycline and sulphonamide residues in pig kidney, but these occurred in just 2–8 samples out of over 1,000 tested. Similarly, only a small number of samples of hen kidney and turkey kidney appeared to have residues of antimicrobial drugs above the MRL. One sample of cattle liver from 331 tested had residues of avermectin drugs above the MRL. Only one sheep sample, of 746 tested, had residues of organophosphorus compounds above the MRL. This might appear surprising in view of the numbers of sheep dipped in organophosphorus formulations each year. However, various surveys of organophosphorus residues in a number of food commodities have shown that concentrations of these compounds are generally very low (Woodward, 1992b). Several unauthorised or prohibited drugs were detected, but the numbers in all cases were low. These are summarised in *Table 25.3*.

In 2006, the overall numbers were again very low and only small numbers of samples from each category proved positive by exceeding the reference point (*Table 25.4*). The major finding of note was nicarbazin residues in 26 of 305 samples of broiled liver and 17 cattle with progesterone concentrations in excess of the reference point in 17 of 373 samples. In the latter case, the majority of the 17 samples only marginally exceeded the reference point of 0.5 μg/kg and it remains likely that the material was of endogenous rather than exogenous origin.

The Non-Statutory Scheme looked at a number of areas. Again, the number of residue violations was low and the main findings are shown in *Table 25.5*. In 2001, there were 1,320 samples included in the plan and 7,726 analyses, while in 2006 there were 1,483 samples and 5,030 analyses. In this latter scheme in 2006, some 34 residues were detected at concentrations above the action limits, and of particular interest and concern were residues of nitrofurans found in warm-water crustacean samples (*Table 25.6*), a finding that will undoubtedly promote further research and regulatory action.

Similar findings were made in the scheme for the 2002–2007 period (VRC, 2003–2008). These results provide significant reassurance on the safety of food of animal origin available in the UK. They are similar to the results obtained in previous years (VMD, 1996–2001). Although some of the MRL violations almost certainly arose from failure to observe withdrawal periods, there

Table 25.3 Occurrence of unauthorised or prohibited drugs in the Statutory Surveillance Scheme, 2001.

Commodity	*Analyte*	*Number of samples*	*Number with drug*
Salmon muscle	Ivermectin*	171	1
Salmon muscle	Leucomalachite green	30	6
Trout muscle	Leucomalachite green	69	11
Cattle urine	Nortestosterone**	209	5
Cattle retina	β-agonists†	107	1
Cattle bile	Zeranol††	301	17
Sheep bile	Zeranol††	85	1
Cattle serum	Progesterone†	178	9

* Not authorised for use in salmon.
** Prohibited drug.
† Prohibited drug except for some restricted therapeutic purposes.
†† A prohibited growth enhancer under EU legislation, but its residues can arise from contamination of feed with the fungal metabolite zearanolone, as happened in these cases.

Table 25.4 Occurrence of unauthorised or prohibited drugs in the Statutory Surveillance Scheme, 2006.

Commodity	*Analyte*	*Number of samples*	*Number with drug above reference point*
Egg	Lasalocid	249	4*
Egg	Nicarbazin	221	1**
Egg	Chlortetracycline	276	1*
Trout muscle	Malachite green/leucomalachite green	105	2**
Milk	Penicillin G	681	10*
Broiled liver	Nicarbazin	305	26†
Broiled muscle	Nicarbazin	62	1†
Broiled liver	Oxfendazole	130	2*
Duck muscle	Chlortetracycline	26	1*
Calf kidney	Oxytetracycline/chlortetracycline	199	1/2*
Cattle plasma	Phenylbutazone	275	1**
Cattle serum	Progesterone	373	17††
Cattle urine	Nortestosterone	615	2§
Cattle urine	Progesterone	51	3††
Cattle urine	Testosterone	62	1††
Cattle urine	Zeranol	342	2[5]§
Horse plasma	Phenylbutazone	49	1**
Pig kidney	Chlortetracycline	796	3*
Pig kidney	Sulphadiazine	799	2*
Sheep liver	Ivermectin	550	1§§
Sheep urine	Nortestosterone	161	16§

* In excess of EU MRL.
** No EU MRL.
† No EU MRL; in excess of JECFA MRL.
†† Prohibited drug except for some restricted therapeutic purposes.
§ Prohibited drug.
§§ Not authorised for use in salmon.

Table 25.5 Occurrence of unauthorised or prohibited drugs in the UK's Non-Statutory Surveillance Scheme, 2001.

Commodity	*Analyte*	*Number of samples*	*Number with drug*
Imported honey	Streptomycin*	50	5
Warm freshwater prawns	Tetracyclines	20	6**
Prawns	Chloramphenicol†	45	2

*Not authorised for use in bees in the UK; no honey MRL.
**Above MRL of 100 μg/kg.
†Annex IV of Council Regulation 2377/90.

Table 25.6 Main findings from the UK's Non-Statutory Surveillance Scheme, 2006 (VRC, 2006).

Produce	*Origin*	*Samples*	*Detected*	*Status*
Farmed trout	UK	137	Malachite green*, leucomalachite green, 1/137	No EU MRL
Cattle plasma	UK	275	Phenylbutazone, 1/275	No EU MRL
Horse	UK	49	Phenylbutazone, 1/49	No EU MRL
Farmed fish	Imported	300	Crystal violet**, 1/300	No EU MRL
Farmed fish	Imported	300	Leucomalachite green, 1/300	No EU MRL
Farmed fish	Imported	300	Nitrofurans, 2/300	Annex IV†
Farmed crustacean	Imported	246	Nitrofurans, 19/246	Annex IV
Warm water prawns	Imported	102	Nitrofurans, 3/102	Annex IV

*Malachite green is frequently illegally used as a fungicide in farmed fish; leucomalachite green is a metabolite of malachite green.
**Used illegally as a fungicide in farmed fish.
†Annex IV of Regulation (EEC) No. 2377/90 and prohibited from use in food animals.

is probably also a significant contribution from contamination of unmedicated feed with components of medicated feed at feed mills. MRL violations may occur as a result of the contamination (or carryover) of unmedicated feed with sulphonamide, chlortetracycline, penicillins and ionophore antimicrobials such as monensin (McCaughey *et al*., 1990a, b; Elliott *et al*., 1994; McEvoy *et al*., 1994, 1999, 2000; Kennedy *et al*., 1998a, b, 2000; McEvoy, 2002) which is of concern because of the implications for monitoring and control and as other contaminants, particularly microbiological varieties, might also be present (Moreno-López, 2002).

An ADI approach has been suggested to evaluate the impact of this problem (Nestmann and Lynch, 2007). The surveillance results are similar to those found for residues surveillance in the United States, although here, penicillin and streptomycin are major contributors. Failure to observe withdrawal periods was a major factor in the origin of violative residues in the US (Van Dresser and Wilcke, 1989; Paige *et al*., 1999a).

Residue violations in fish tissue might occur from environmental contamination with veterinary medicines. However, far more likely is contamination arising from environmental pollutants (Easa *et al*., 1995; Jensen and Greenlees, 1997). For example, a recent survey of residues in farmed salmon from around the world has revealed polychlorinated dibenzo-*p*-dioxins, dibenzofurans, DDT, chlordane and heptachlor epoxide (Hites *et al*., 2004). The health risks of these substances at the concentrations found in the fish are unknown, but the claim that they may pose a carcinogenic risk has been strongly denied.

However, environmental contamination with veterinary drugs has given rise to concern over the eventual occurrence of residues in food of animal origin (Kennedy *et al*., 2000), particularly from farmyard slurry (Berger *et al*., 1987). Concern has also been expressed over contamination of surface waters in the US by the anabolic growth promoter trenbolone, a constituent of feedlot effluent (Wilson *et al*., 2002), and whether oestrogenic growth promoters in the environment might evoke adverse events (Le Guevel and Pakdel, 2001).

Although reports of adverse effects in humans from residues of veterinary drugs in food are rare, they have occurred following ingestion of veal liver containing residues of the β-agonist drug clenbuterol (Pulce *et al.*, 1991; Brambilla, 1992), and in 2003, 39 people in Liaoyang, China, were affected by pork containing clenbuterol residues, with 29 requiring hospital treatment for symptoms including involuntary twitching and acute thirst (Anonymous, 2003). Concern has also been expressed over residues of lasolocid in eggs in the UK (Editorial, 2004). In general, however, it is difficult to associate human health problems with residues of veterinary drugs. Any adverse effects are likely to be acute rather than chronic, as illustrated by the example of clenbuterol (Paige *et al*., 1997, 1999b; Paige, 1998; Friedlander *et al*., 1999). The determination of NOELs involves laboratory animal studies and relatively high doses of test compounds, while the calculation of ADI values makes use of large safety factors, and so the elaboration of MRLs errs on the side of consumer safety. Hence, it is extremely unlikely that minor violations have any significant public health implications (McEvoy, 2001).

Residues surveillance indicates that residues concentrations, particularly those of antimicrobial drugs, are low in milk, but there are reports of so-called bulk tank failures (Biggs, 2000; Black and Cook, 2001). These occur not because of violation of any MRL by specific substances, but because the tests used by the dairy producers are inherently more sensitive and these are used as industry standards rather than as regulatory or consumer safety standards (Cullor, 1994, 1997; Cullor *et al*., 1994; Edmondson, 2001).

The Delvotest SP, a specific test used widely by the dairy industry, can detect several antibiotics used in cattle, including cloxacillin, framycetin, neomycin, penicillin G and sulphonamides, at concentrations below the MRL (Pott, 2000). Such tests can therefore cause major problems for farmers. Although they may have observed the requirements of the product literature, including the withdrawal period, and although the concentration of the antibiotic may be well below the MRL, the milk may fail the 'standard' imposed by the dairy industry and the farmer is then faced with a financial penalty (Pott, 1993, 2000; Hurst, 2000). This is complicated by the fact that some of the available tests are sensitive to natural inhibitory substances found in milk, such as those produced soon after calving (Pott, 2000). Although failure in these tests can often carry a financial penalty, they are not a pharmacovigilance issue unless confirmatory methods of analysis demonstrate that there has been a violation of the MRL.

Residues avoidance

Clearly, the most appropriate way of avoiding residues in food of animal origin is to use only those veterinary medicines authorised for the specific use in the species concerned at the recommended doses, for the recommended dosing periods and subsequently observing the recommended withdrawal periods. However, clinical necessity occasionally requires that animals, including food-producing animals, be treated with non-authorised drugs when there is no suitable alternative available, and this is foreseen and permitted under certain circumstances by EU legislation. Directive 2001/82/EC, as amended by Directive 2004/28/EC, requires EU member states to permit a veterinarian, under 'his direct personal responsibility', and specifically in the interests of animal welfare, to make some exceptions to the use of authorised products where

there is no suitable authorised product in the member state concerned, as follows:

- to use a product authorised in the member state for another species, or for the treatment of another condition in the same species;
- if no product exists, to use a product authorised for human use in the member state concerned; or
- to use a product authorised in another EU Member State for use in the same species or in another food-producing species for the condition or for another condition; or
- use a product prepared extemporaneously by a person authorised to do this under national legislation in the member state concerned.

If any of these alternatives, widely known as the cascade, are followed, then prolonged withdrawal periods, commonly referred to as standard withdrawal periods, must be applied in accordance with the Directive. These are:

- Eggs – 7 days
- Milk – 7 days
- Meat from poultry and mammals – 28 days
- Fish – 500 degree days.

Use of these extended withdrawal periods for extra-label use should ensure that residues have depleted to safe and non-violative concentrations in the commodity concerned and any risk must be seen as being restricted to produce from individual animals, as the cascade is not envisaged for use in large numbers – for the majority of diseases of livestock and other food animals, authorised medicinal products are available. Further reassurance can be obtained where necessary using a withdrawal estimator algorithm (Martínez-Jiménez *et al*., 2002).

As discussed in Chapter 24, withdrawal periods are established in the EU and in other countries using statistical methods which are the subject of EU Guidelines. Readers should be aware that other methods of determination are available (Concordet and Toutain, 1997a, b; Fisch, 2000; Martín-Jiménez *et al*., 2002; Buur *et al*., 2006). Suitable withdrawal periods, the awareness of the responsibilities placed on them by farmers and veterinarians, adequate record keeping and ensuring Good Agricultural (and Veterinary) Practice should serve to ensure that the chances of obtaining violative residues are minimised (Kavanagh, 1990; Van Miert, 1996; Anonymous, 2002; Early, 2004; Gaunt, 2006; Gehring *et al*., 2006; Payne *et al*., 2006; Price, 2006).

Some product formulations, especially those intentionally formulated for depot effects, can prolong residues depletion (Mawhinney *et al*., 1996; KuKanich *et al*., 2005). This is particularly true for products intended for intramuscular or subcutaneous injection, where prolonged absorption can be both a therapeutic benefit and a residues risk, especially at the site of the injection itself. Persistence of residues at the injection site is a major problem with injectable formulations (Banting and Baggot, 1996; Galer and Monro, 1996; Mawhinney *et al*., 1996; Nouws, 1990; Nouws *et al*., 1990; Brown, 2000; Beechinor *et al*., 2001; Sanquer *et al*., 2006a, b).

As a result of inter-animal variations, these products do not lend themselves easily, if at all, to the use of statistical methods for withdrawal period calculation. Under these circumstances, risk management techniques, including basing withdrawal periods on the temporal depuration of residues at the injection site to below the MRL for muscle, may be the only practical resort, even though this may result in exceptionally long withdrawal periods (Beechinor and Bloomfield, 2001; KuKanich *et al*., 2005; Sanquer *et al*., 2006a; Reeves, 2007).

This brings with it the problem of observance of withdrawal periods – they may well be ignored by farmers if there are what are considered to be overriding economic or animal husbandry considerations, even though the risk of eating injection site meat is low and the hazard presented is an acute one rather than the long-term option embodied in the MRL concept through the ADI. Consequently, it is in the interests of sound science to establish practicable withdrawal periods where injection sites are involved on the basis of residues depletion based on an acute factor rather than on the MRL (Sanquer *et al*., 2006b).

If residues do occur despite all of the efforts to prevent this, can they be removed or their concentrations be reduced by post-mortem processing? This issue has not been addressed to any great extent. What are the effects, for example, of processing, storage, fermentation or dilution with other materials and food commodities? However, the effects of cooking have been examined for a limited range of products. Some cooking procedures can lead to reductions in residue content, although the mechanisms involved are obscure as only small amounts of drug appear to be leached into the cooking liquids (which themselves may be used for culinary purposes).

For example, some cooking methods significantly reduced concentrations of residues of nicarbazin in some food commodities whereas other methods had little effect (Tarbin *et al*., 2005). Cooking had minimal or no effects on concentrations of chloramphenicol, oxytetracycline, streptomycin, sulphadimidine (sulphamethazine) or ampicillin in beef (O'Brien *et al*., 1981). Benzylpenicillin was stable at 65ºC but not at higher temperatures. Up to 50% of residues present in meat passed into cooking fluids (Rose *et al*., 1997a). Oxytetracycline and tetracycline residues were significantly reduced by cooking (Rose *et al*., 1996; Kühne *et al*., 2001), while sulphadimidine was found to be thermally stable (Rose *et al*., 1995a). Oxfendazole residues were seemingly reduced at high temperatures for prolonged periods, but this resulted in the formation of an amine derivative, formed from hydrolysis of the carbamate moiety (Rose *et al*., 1997b), which then raises questions over the safety of this material. The quinolone drugs oxolinic acid and flumequine were stable during cooking of fish (Steffenak *et al*., 1994). Levamisole and clenbuterol were stable in boiling water but unstable at 260ºC in cooking oil (Rose *et al*., 1995b, c). Ivermectin was also stable, although up to 50% of total residue was leached by the cooking liquids (Rose *et al*., 1998). Ronidazole was converted to a 2-hydroxy derivative in aqueous conditions, whereas dimetridazole was seemingly stable (Rose *et al*., 1999).

With most of these substances, the relevance to human food safety is unclear as the identities and nature of the degradation products are unknown (Moats, 1999). Some sulphonamide drugs appear to degrade on prolonged frozen storage but were seemingly stable for up to 3 months (Rose *et al*., 1995a; Alfredsson and Ohlsson, 1998). Sulphadimidine may be converted to the N^4-glucopyranosyl derivative on prolonged storage in pig liver (Parks, 1984), but once again, the implications of this for consumer safety are unknown.

All of this demonstrates that reliance on cooking and food processing to reduce residue concentrations in food is unwise. While processing may have some beneficial effects in reducing residue concentrations, too little is known about the fate of these residues and the safety of any degradation products to place any reliance on cooking, freezing or any other form of processing, in ensuring consumer safety. Prevention is certainly better than cure as far as drug residues are concerned.

Conclusions

Residues of veterinary drugs in food of animal origin carry a potential public health risk if the MRL is exceeded and potential regulatory and legal risks to farmers and others if their produce is found to contain violative residues. Residue surveillance schemes across the world help to minimise any risks and to provide welcome public reassurance that the food they consume is wholesome and safe.

Violative residues are unlike other areas of veterinary pharmacovigilance in that infringements are generally 'invisible'. A veterinarian or farmer cannot know if an animal has violative residues (although they may guess if the animal has been overdosed or a withdrawal period ignored), unlike the situation with adverse reactions where an obvious and reportable event usually occurs. In general, violative residues are only detected by government agencies in pursuit of surveillance

schemes of the types described here. However, other agencies do examine food for residues. These include milk suppliers and processors and food retailers and the onus is very much on them to report any residue violations that they detect to the responsible authorities. Under these restricted circumstances, reporting of residues violations is analogous to other areas of reporting in pharmacovigilance activities. Failure to observe withdrawal periods may lead to violative residues and subsequent recalls of affected food commodities, as happened recently in the UK with residues, including residues of doramectin in lamb where breeding animals were inadvertently sent to slaughter (Anonymous, 2007; Foster, 2007). However, with doramectin at least, residues may deplete at different rates in parasitised and non-parasitised sheep (Pérez *et al*., 2008) and this may be representative, or at least indicative, of residue depletion in other animals with other drugs and diseases.

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26 Adverse environmental effects and veterinary medicinal products

A. Tait

Introduction

Pharmacovigilance in the context of veterinary medicines is the gathering of information on adverse reactions that may occur after the administration of medicinal products to animals (Woodward, 2005) In the European Union (EU) Directive 2001/82/EC as amended by Directive 2004/28/EC (European Commission 2001, 2004) required member states (MS) to take all appropriate measures to encourage the reporting to the competent authorities of suspected adverse reactions to veterinary medicinal products. The Directive places particular emphasis on the collection of adverse reactions in animals and in human beings related to the use of veterinary medicines. However, Article 73 of the Directive describes several other areas concerning the use of veterinary medicines which are covered by the term pharmacovigilance. One of these areas is 'potential environmental problems related to the use of the product'. To assist in the recording and interpretation of the data collected under pharmacovigilance, the Directive (Article 77) indicates that the Commission with help from the European Medicines Agency (EMEA) and the MS will draw up guidance on the collection, verification and presentation of adverse reaction reports.

As a result of the commitment in the Directive a considerable number of guidelines and guidance documents have been prepared on pharmacovigilance by the EMEA on behalf of the Committee for Medicinal Products for Veterinary Use (CVMP). The guidelines, either published or draft, issued up until 2004 have been reviewed by Woodward (2005). Since then a number of new ones have been issued by the CVMP. In the guideline *Veterinary Pharmacovigilance in the EU – A Simple Guide to Reporting Adverse Reactions* (EMEA, 2006a), the importance of reporting adverse reactions, even if a relation to the product is only suspected, is emphasised. The guideline provides a list of what are considered especially important types of reaction including 'potential environmental problems'. Unfortunately there is no further guidance on what is meant by an 'environmental problem', and while 'lack of suspected efficacy' and 'off-label use' are somewhat self-explanatory, what is meant by an environmental problem is not. The guidance provided by the CVMP is more useful than that contained in a guideline published for consultation by the CVMP and developed by the

International Co-operation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH). In this guideline there was no mention of the environment whatsoever (EMEA, 2005a).

A veterinary medicine may reach the environment directly either during its administration to the target animal or during disposal of any unused or waste medicine. However, the most likely route of exposure of the environment is an indirect one where residues of the medicine reach the environment through urine or faeces after first passing through the animal to which it was administered. The main routes by which veterinary medicines are administered to animals are:

- by injection (intravenous, subcutaneous, intramuscular);
- orally, either directly or mixed with food or water; or
- by external administration to the skin.

Following administration, the active substance is absorbed into the bloodstream, distributed around the body, metabolised and then excreted. Absorption is 100% when the product is given intravenously and can be close to 100% via other routes of administration, depending on the formulation. Absorption can also be quite low particularly when the product is applied topically and the mode of action of the product does not require that the active ingredient is absorbed.

Products that are likely to enter the environment directly during treatment of the animals are those that are applied topically where there is potential for run-off from the treated animal soon after treatment. Plunge dips for sheep and products that are applied using a nozzle-type applicator to the body of the target animal, so-called pour-on products, are the most likely to enter the environment directly. The plunge dips also have the potential to enter the environment during disposal of the spent dip (the material remaining after dipping). In the UK, spent sheep dip can be disposed of to land providing that an authorisation for disposal has been obtained from the relevant authorities (Health and Safety Executive (HSE), 2007). In the case of dips and pour-on products the active ingredients are usually designed to kill external or internal parasites and have the potential to cause harmful effects to similar organisms living free in the environment.

Entry into the environment is inevitable after the administration of a veterinary medicine to animals and as a result it is possible that, in the words of the pharmacovigilance guidelines, potential environmental problems could arise. Unlike the situation in humans where the vast majority of the drugs excreted by human patients end up in the domestic sewage system, with veterinary medicines the treated animals will excrete residues of the medicine directly into their environment, which could be a stable, a field or a body of water (freshwater or marine). There is the potential for wide-ranging exposure and, depending on the active substance and the level of exposure, the potential to cause environmental problems. Such 'problems' could include the death of large numbers of animals, the death of a few but very noticeable animals, or the loss of or damage to plants. As these incidents are not related directly to the user of the medicine or the animal being treated it is questionable as to how they will be detected without them being visible and serious.

In this chapter the procedure for the environmental risk assessment of veterinary pharmaceuticals is discussed as these are more likely to cause potential problems than are veterinary vaccines. The presence of veterinary pharmaceuticals in the environment will be examined and whether there is any evidence that these have caused any environmental problems. The procedures in place for veterinary pharmacovigilance will be reviewed and compared with the procedures for pharmacovigilance of human medicines. Finally some conclusions will be drawn regarding the use of veterinary pharmacovigilance to address environmental concerns in the absence of continual 5-yearly renewals of marketing authorisations.

Environmental risk assessment

Legislation and guidelines

The requirement to evaluate the impact of the use of veterinary medicines on the environment is a relatively recent addition to the authorisation requirements for veterinary medicines. It was Directive 92/18/EC (European Commission, 1992) that introduced the requirement for an environmental risk assessment into EU legislation. The Directive specified that:

> 'The purpose of the study of the ecotoxicity of a veterinary medicinal product is to assess the potential harmful effects which the use of the product may cause to the environment and to identify any precautionary measures which may be necessary to reduce such risks.'

It was stated in the Directive that the study of ecotoxicity should be in two phases:

- Phase I would investigate the extent of environmental exposure.
- Phase II would provide further investigation of the fate and effects of the product on particular ecosystems.

The text of the Directive did not state how the risk to the environment was to be quantified or what constituted an acceptable or conversely an unacceptable risk to the environment.

As a consequence of the failure of the Directive to specify how the environmental risk assessment was to be carried out the CVMP developed guidelines on how to perform an environmental risk assessment for veterinary medicines. The first guidelines on environmental risk assessment were published by the CVMP in 1997 (EMEA, 1997). These were prepared in two sections, not surprisingly called Phase I and Phase II, following the nomenclature of the Directive. The guidelines were developed in the form of a decision tree where a positive response to a question in the decision tree was sufficient to end the assessment. As required by the Directive, Phase I assessment looked only at the potential exposure of the environment and each point in the decision tree allowed the assessment to terminate at Phase I as the questions concerned some aspect that influenced exposure. Questions early in the decision tree removed certain product types such as electrolytes and anaesthetics and products for species such as cats and dogs from the requirement for further assessment.

If a product proceeded to the end of the decision tree then calculations had to be carried out to determine the predicted environmental concentration or PEC. The PEC was calculated for the active substance or substances in the product. An assessment of the other constituents is not required unless there are good scientific reasons for requiring such an assessment. The key values in the Phase I assessment were the value of 10 μg/kg for the PEC of the active substance in soil and 0.1 μg/l for the PEC of the active substance in groundwater. If Phase I identified that a product required a Phase II assessment it was at this stage that information on the fate and effects of the active substance was required. When information on the exposure and the effect of the active substance on non-target indicator organisms was known, the risk could be assessed by comparison of the PEC with the concentration producing no effects in non-target species.

In 1996 the Steering Committee of the VICH (see Chapter 2) selected ecotoxicity as one of the five initial topics for harmonisation. In 1997 just as the CVMP was publishing its environmental risk assessment guidelines, the Ecotox Working Group was established under the VICH. This group included representatives from industry and regulatory authorities from the European Union, USA, Japan, Australia, New Zealand and Canada. The group was required to develop harmonised guidelines taking into account any existing guidance documents and any legislative/regulatory requirements. Just as in the CVMP guidelines, the VICH guidelines were split into two phases. The VICH process and its successes and failures in relation to veterinary environmental risk assessment are well described by Robinson (2007). The VICH uses a nine-step process to develop guidelines:

1. identification of the topic;
2. drafting recommendations, i.e. a guideline;
3. approval of the recommendations;
4. consultation;
5. revision of the guideline in the light of comments;
6. approval of a revised guideline;
7. setting an implementation date for adoption;
8. reporting back on implementation;
9. revision to take into account new scientific information.

For the Phase I and Phase II guidelines, the process of going from step 1 to step 7 took from 1997–2004 to complete. The Phase I guidelines, which were agreed by the VICH in 1998, were adopted in the EU and in the USA in 2000/2001, but not by Japan. The Phase I VICH guideline was published by the CVMP in 2000 (EMEA, 2000). The VICH Phase II guidelines took longer to complete and were not approved by the VICH Steering Committee until 2004. The VICH guidelines on Phase II (EMEA, 2005b) were published by the CVMP in October 2005. They have also been adopted for use in the USA, Australia and New Zealand. The translation process in Japan has delayed their adoption, but implementation of both the Phase I and Phase II guidelines remains on schedule. The VICH guidelines followed a similar layout to the earlier CVMP guidelines in using a decision tree approach. As in the CVMP guidelines, Phase I is concerned only with exposure. One of the main differences between the CVMP and VICH Phase I guidelines is that VICH guidelines contain only a single trigger value which is the PEC in soil. The value under VICH is 100 μg/kg. There are some differences between the original CVMP Phase II guidelines and those developed under VICH, but the basic principle of comparing exposure with effects has been retained.

The development of harmonised guidelines under VICH inevitably resulted in much of the detail provided in the original CVMP guidelines being removed in the cause for harmonisation. The process of harmonisation of guidelines has its critics within the VICH process, the Phase I guideline and the general regulatory framework of veterinary medicines being criticised in some quarters (Montforts and de Knecht, 2002). These authors were of the opinion that the regulatory framework for veterinary medicines was inferior to that of other product groups such as chemicals, pesticides and biocides. They were particularly critical of the VICH process, suggesting that the working group lacked experience, the participants from industry and from regulatory authorities would defend their specific interests perhaps to the detriment of the environment and the process itself was not transparent. It was considered that the VICH Phase I guideline was technically flawed as the PEC soil trigger had not been justified and there was no consideration of exposure of groundwater. The Phase II VICH guideline had not been published at the time Montforts and de Knecht published their article so this guideline escaped their scrutiny.

In an unusual move the case for VICH and the regulatory framework for veterinary medicines was presented by Long and Crane (2003). These authors presented detailed descriptions of the experience of the experts on the VICH working group, something Montforts and de Knecht did not criticise directly, and of the process of consultation within the EU constituency during the preparation of the Phase I guideline. It is interesting to note that Long and Crane had some sympathy with the view that the PEC soil trigger value was not rigorously justified, but they pointed out that parasiticides and fish medicines were identified as being of particular concern, and as a result the PEC trigger was not applied to these groups of medicines. Whatever concerns existed and perhaps still exist over the VICH process and the guidelines that were developed under VICH, both the VICH guidelines are now operational in the EU.

As indicated above, the VICH process resulted in a loss of much of the detail that had been present in the original CVMP guidelines. One particular area where further guidance was required was that of calculating PEC values for soil and the aquatic environment. There were

also a number of areas in the VICH Phase II guideline that required the reader to 'seek regulatory guidance'. For these reasons, the CVMP, through its Environmental Risk Assessment Working Party (ERAWP), recently published a guideline in support of VICH guidelines (EMEA, 2007) for use in Europe. This guideline does not add new requirements or deviate from VICH, but provides the technical details and specific European information which the VICH guidelines lacked. In particular the guideline identifies routes of potential exposure of the environment to residues of veterinary medicines and presents algorithms and suggested approaches for calculating the exposure of various environmental compartments. It also provides assistance in areas where VICH advice is to seek regulatory guidance.

The requirement of the EU legislation (European Commission, 2001, 2004) is to:

> '. . . assess the potential harmful effects which the use of the product may cause to the environment and to identify any precautionary measures which may be necessary to reduce such risks'.

This statement is expanded on in Annex I of the directive (European Commission, 2001) which states that:

> 'In a second phase, having regard to the extent of exposure of the product to the environment . . . the investigator shall then consider whether further specific investigation of the effects of the product on particular eco-systems is necessary'.

The legislation does not give any direction on what is considered to be 'particular eco-systems'. In other words there is no definition in the legislation of what are the protection goals of the risk assessment for veterinary medicines. These protection goals have almost been decided upon by default rather than being defined.

In the case of risk assessments for medicines used in the terrestrial environment the protection goal of the veterinary environmental risk assessment is to ensure that there are no unacceptable risks in the immediate area which is exposed to the residues of the medicine following excretion. For farm animal products this equates to the field onto which the residue is excreted or spread in manure, and the aquatic environment associated with this immediate area, i.e. groundwater and surface waters such as ditches, streams and ponds. The protection goal of medicines used for fish in aquaculture are in effect the same as those for medicines used in terrestrial animals except that the area that needs to be evaluated is not fixed, as all sites where fish are kept will be different. It is also true that a certain amount of environmental damage is accepted in fish farming. The deposition of organic waste on the seabed close to fish cages is unavoidable and some degree of impact has to be accepted (Scottish Environmental Protection Agency (SEPA), 2000).

Risk assessment procedure

An environmental risk assessment is required as part of the dossier for all applications for an EU marketing authorisation for a veterinary medicine. The assessment is carried out on the product, i.e. it is product specific. However, all the data required for the assessment are generated on the active substance or on rare occasions a major metabolite. It is possible that the environmental risk assessment for two products containing the same active substance can have different outcomes as a result of the different levels of environmental exposure to the active substance.

The Phase I assessment is intended to identify those products that will not lead to extensive exposure of the environment, this being the criterion for an assessment ending at Phase I which is set out in the Directive. The VICH Phase I assessment (EMEA, 2000) is based on a decision tree approach where the series of questions or decision points, built into the decision tree, enable the user to decide on whether or not exposure of the environment is extensive. The Phase I assessment can be carried out with information that is usually already available in the dossier and considers information such as:

- the basic physico-chemical properties of the active substance;
- the target species;
- the dose of the active substance;
- the duration of treatment;
- the clinical indications for the product;
- information on the routes of excretion of the active substance.

If the Phase I assessment identifies that exposure of the environment is likely to be extensive then the assessment moves into Phase II. The Phase I assessment is concerned only with exposure of the environment; it provides no information on the intrinsic hazards of the active substance and therefore does not provide any information on the risk to the environment. If the Phase I assessment identifies that a Phase II assessment is required then it is at this stage that environmental data on the active substance are required.

The Phase I assessment does not require any bespoke environmental information. The decision tree makes the basic assumption that for certain active ingredients, certain product types and for certain target species the exposure of the environment will not be extensive. As a result products containing natural ingredients such as inorganic salts, products indicated for use only in companion animals and products only administered to an individual animal or to a few animals in a flock or herd do not lead to extensive environmental exposure and do not require a Phase II assessment. Following the elimination of these product types the decision tree in Phase I splits into an aquatic branch covering products used in aquaculture and a terrestrial branch for food-producing animals.

Products that are intended for use in fish that are being raised for food in open water systems, either in the marine or freshwater environments, require assessment in Phase II as these products are administered by being placed directly into the environment. For food-producing animals on the terrestrial branch the first question is whether or not the product will be used in animals raised on pasture. Animals on pasture will excrete the veterinary medicine directly onto the field in which they are grazing. This fact is important for products that are intended to treat and control external or internal parasites as these may produce the same effect in similar organisms, e.g. insects, in the environment. For this reason products intended for control of external and internal parasites used in pasture animals require a Phase II assessment irrespective of other considerations. For all products that are used in farm animals that are raised intensively, particularly pigs and poultry, and any non-parasiticides used in pasture animals it is necessary to calculate the PEC in the soil in order to quantify the exposure of the environment. As already described, under the VICH guidelines there is a trigger value of 100 μg/kg for the PEC in soil. If the PEC in soil is at or above this value an assessment in Phase II is required. This trigger value was agreed during the VICH procedure, the rationale being that the value was below a level shown to have effects on earthworms, microbes and plants in ecotoxicity studies with veterinary medicines currently registered in the USA (EMEA, 2000).

The calculation of the PEC in soil has to be a conservative estimate and use worst case, but realistic values in the calculations of the Phase I assessment make no judgment on the hazard of the veterinary medicine. The calculation of the PEC in soil is the most important estimation of exposure which has to be made during the risk assessment procedure as it not only determines, for some veterinary medicines, whether or not a Phase II assessment is required, but also is the starting point for the Phase II assessment in terms of exposure estimates. As it is such an important value it is worth spending some time considering how the estimate is made and if it bears any relationship to actual concentrations in the field.

In the PEC calculation it is necessary to make some basic assumptions about the fate of the active substance following its administration to the target animal. It is assumed that whatever the route of administration, 100% of the administered dose is absorbed and then excreted in urine and faeces. It is assumed that there is no metabolism of the active substance by the animal. This

latter assumption is necessary as information on the fate and effects are only generated on the parent compound. This concept is referred to as the total residue concept (EMEA, 2007) and assumes that any metabolites are of lower toxicity than parent compound and that they behave similarly in the environment.

The routes of entry into the environment for the residues in excreta vary depending on the husbandry conditions of the animals during and after treatment with the veterinary medicine. For animals raised on pasture during and after treatment, for example sheep, the active substance is excreted directly onto the pasture. The proportion of the dose excreted in faeces and in urine is usually known, but in the Phase I calculation it is assumed that the residue is spread uniformly in the top 5 cm of the soil. A calculation method for determining the PEC in soil resulting from treatment of animals on pasture has been provided by the CVMP (EMEA, 2007). In this calculation the PEC is dependent on:

- the dose given to each animal;
- the number of times the animal is treated in a course of treatment;
- the fraction of animals in the herd or flock treated;
- the number of animals that are maintained on 1 ha of land (the stocking density).

The total dose applied per hectare of animals is divided by the weight of 1 ha of soil 5 cm deep (a default value of 750,000 kg). Values for parameters such as stocking density and animal body weight are available in the literature (Smith and Frost, 2000) and default values for standardising calculations have been provided by the CVMP (EMEA, 2007). The PEC value in soil is an annual value assuming one course of treatment per year and it is expressed in micrograms per kilogramme (μg/kg) of soil. This method of calculation results in a single deterministic value which represents an average value. It is clear that there is variability in some of the parameters used in this calculation, particularly the body weight of the animal and the stocking density. Kelly *et al*. (2003) suggested using a stochastic approach to the calculation of the PEC in soil for pasture animals. While their suggestions are very sensible, they have not been adopted as the VICH risk assessment procedure and the CVMP procedure prior to that required a single value for comparison with the PEC soil trigger.

Animals that are housed during and after treatment excrete the residue of the veterinary medicine into their pen or stable. These excreta may be mixed with straw or wood shavings in the case of cattle and poultry or they may be collected without other materials as in the case of pigs. For housed animals the residues of the veterinary medicine reach the soil when the manure or slurry is spread onto land as organic fertiliser.

A number of calculation methods have been proposed for estimation of the PEC in soil arising from spreading manure (see research papers by Spaepen *et al*., 1997; Montforts *et al*., 1999; Montforts and Tarazona Lafarga, 2003; EMEA, 2007). All have followed the same basic principle that the amount of manure that can be spread onto land is restricted by the amount of nitrogen that can be applied to the land. Although this nitrogen limit may vary between countries and between regions within a country there is a maximum limit set under the Nitrates Directive for many areas of the EU of 170 kg of nitrogen per hectare of land per year (European Commission, 1991).

Each of the papers provides default values for the body weight of different species of housed animal and for different categories within a species as well as for the annual quantity of manure produced by individual animal types and the quantity of nitrogen in that manure. There are differences between the calculations. For example, the method proposed by Montforts and Tarazona Lafarga (2003) assumed that the manure was stored for only 30 days and that the animals were treated during that period. The other methods assumed that the manure was stored for 1 year and treatment of the animals occurred sometime within the year. The CVMP (EMEA, 2007) in their guideline presented a calculation for the PEC_{soil} based on the calculation method proposed by Spaepen *et al*. (1997), except

that the values for nitrogen production by individual animal types takes into consideration the nitrogen lost as ammonia during the period of manure storage as described by Montforts (2006).

The use of the nitrogen spreading limit as a means of PEC calculation was evaluated for its validity as a model by Blackwell *et al*. (2005). In this study, PECs were calculated using the method of Spaepen *et al*. (1997) for three antibiotics – sulfachloropyridazine, oxytetracycline and tylosin – applied to arable land. These data were then compared with measured concentrations of the antibiotics in soil after spreading manure spiked with the compounds at levels intended to reproduce the results of the calculation method. Comparison of the results indicated that the PEC calculation tended to overestimate the actual measured concentration by about a factor of two. It was concluded by the authors that the PEC estimation was conservative and would provide a valuable safety margin when used in the environmental risk assessment.

At the conclusion of the Phase I assessment a decision is reached on whether or not the exposure of the environment is extensive as defined by the built-in triggers and decision points. For those products where exposure is not considered to be extensive the assessment stops and no environmental fate and effects data are required. As mentioned earlier, for products used in companion animals or for those used on an individual animal basis, such as anaesthetics, non-steroidal anti-inflammatories and general antibiotics, no environmental fate or effects data are required.

A Phase II assessment in accordance with the VICH Phase II guidelines (EMEA 2005b) is required for all fish medicines and for all parasiticides used in animals raised on pasture. For intensively reared food-producing animals and for those reared on pasture when the product is not a parasiticide, they will enter Phase II when the PEC in soil calculated in Phase I is 100 µg/kg or greater. The assessment in Phase II is structured in a step-wise fashion with the first step being known as Tier A. The Phase II assessment has three distinct branches to cover:

- products used in aquaculture;
- products used on intensively reared animals;
- products used in pasture animals.

The environmental exposure for each of the three branches is different, and obviously so for fish medicines. In Tier A of Phase II a basic data set is required which consists of information on the fate of the active substance in the environment and the effects of the compound on non-target indicator organisms tested in the laboratory.

The Phase II assessment requires certain information to be provided on the active substance present in the veterinary medicine, although, on rare occasions, it may be necessary to generate this information on a major metabolite excreted by the animal or on a major degradation product in soil. In this discussion it is simpler to consider only the active substance. For both fish medicines and products intended for use in farm animals a basic set of physicochemical data are required:

- water solubility;
- dissociation constant in water;
- UV/visible absorption spectrum;
- melting point/range;
- vapour pressure;
- n-octanol : water partition coefficient.

Studies are required to determine the fate of the active substance either in the soil or in the aquatic (usually marine) environment. The environmental mobility of the active substance is examined in a study to determine the soil adsorption and desorption of the compound. The rate of degradation, either in soil or in the marine environment, is also determined. Finally the effect of the active substance in tests with non-target organisms is determined. For active substances that enter the soil, the effect on soil nitrification by micro-organisms, the acute toxicity to higher plants and the subacute/reproductive toxicity to earthworms are determined.

In addition to these tests the effects on aquatic organisms, green or blue-green algae, daphnia and fish in freshwater and green algae, crustacea and fish in the marine environment are carried out. The Phase II VICH guideline recommends

that the study protocols used in all these experiments are those provided by the Organisation for Economic Cooperation and Development (OECD). These guidelines were chosen during the VICH procedure as being acceptable to all regions within the VICH process. It is a requirement of the legislation that all studies are carried out in compliance with Good Laboratory Practice regulations which ensures an audit trail for the study data.

The effects tests recommended in the VICH guidelines are all acute toxicity tests with the exception of the test in earthworms which is a subacute/acute (or reproduction) test and the test with soil micro-organisms which compares the function of soil microbes in control soil and soil amended with the test substance over a period of 28 days.

There is a specific test requirement for farm animals raised on pasture when treated with a product intended to treat and control ecto- or endo-parasiticdes. The effect of the active substance to dung insects has to be investigated unless it can be shown that the excretion of the compound is exclusively in the urine. Fresh dung from grazing animals provides a habitat for a diverse community of insects, flies and beetles which feed on the dung or on nutrients in the dung (Skidmore, 1991; Cox, 1999). Dung fly larvae feed either solely on micro-organisms associated with the dung or on micro-organisms first, followed by insects or solely on insects. Dung-feeding beetles may feed on dung or the micro-organisms within the dung or on other insects. Dung beetle larvae feed on undigested plant fibre (Floate *et al*., 2005). No test protocols are available at present to use in the evaluation of the effects of the test substance on dung fly larvae and beetle larvae as required by the VICH guideline. However, test protocols are under development, with a group known by the acronym DOTTS (Dung Organisms Toxicity Tests Standardisation) taking a strong lead (Römbke and Barrett, 2005; Römbke *et al*., 2007) with development to the ring test stage of an OECD draft guideline on an acute toxicity test for dung fly larvae which will provide an LD_{50} value for the compound under test. A screening index for predicting the effects of drugs on dung flies has also been developed (Boxall *et al*., 2007).

Once all the effects data are available a predicted no effect concentration (PNEC) is calculated for each test organism by applying an assessment factor (AF) to the endpoint value. The AF can vary between 10, for the subchronic study with earthworms, and 1,000, for acute studies with daphnia and fish. The assessment factor is designed to allow for different species sensitivity, the extrapolation of acute data to chronic exposure and the move from the laboratory into the field situation. An estimate of the risk is made by comparing the exposure of the environment, the PEC, with the hazard to a particular organism, the PNEC. The resulting value is known as the risk quotient (RQ). If the RQ is below 1 it is considered that the risk to that particular group is acceptable. This means in effect that the PEC has to be below the PNEC for the risk to be acceptable. This process of comparing the exposure with the effects to provide a measure of the risk is similar to risk assessment procedures used for pesticides, industrial chemicals and food additives (European Commission, 2002, 2003; European Food Safety Authority (EFSA), 2007).

Two examples will be used to illustrate the VICH risk assessment procedure: a medicine for use in farmed Atlantic salmon in the marine environment and administered in the feed, and a product containing a parasiticide for use in cattle when the animals are on pasture. Following the Phase I VICH guideline for both products there is no point on the decision tree that would allow the assessment to end at Phase I. The fish medicine is administered in feed to animals in open water and as a result a Phase II assessment is required. Similarly the parasiticide for cattle is used in animals on pasture and accordingly a Phase II assessment is compulsory.

In Phase II, the least complicated part of the assessment is the generation of the fate and effects data required in Phase II Tier A. Using OECD protocols, information would be produced on both the active ingredients for use in the assessment. What is more difficult and challenging,

particularly for the fish medicine, is identification of the environmental compartments that are exposed to the compounds and the quantification of the amount of the compound in that compartment, i.e. calculation of the PEC. In the case of the fish medicine, the compartment exposed is the aquatic compartment, specifically the area surrounding the cage in which the fish have been treated. The degree of exposure of the water and sediment from use of the medicine will depend on the properties of the active substance and whether it is more likely to be present in the sediment or the water column or both. The area exposed to the fish medicine will also depend on the movement of the water in the area around the cages.

The issues concerning the estimation of PECs for fish medicines are very specific and as there are so few fish medicines being developed and authorised there is no specific guidance on this aspect in the recently issued guideline from the CVMP (EMEA, 2007). In the UK, the Scottish Environment Protection Agency (SEPA) has experts in the area of regulation of fish farming and has developed models for estimating exposure of the water column and the sediment following use of fish medicines. Using these or similar models the PECs can be calculated and the RQ values determined for each of the non-target organisms. Should any of the RQ values exceed 1 then the first step is to refine the PEC using any information on metabolism together with more sophisticated models if required. If PEC refinement does not result in all RQ values below 1 then further studies on non-target organisms are required in Tier B of VICH Phase II.

For the product used in cattle the areas exposed to the active substance are the soil, the aquatic environment, both surface water and ground water and the dung of the treated animal which is a supply of food for coprophagous insects (Skidmore, 1991; Cox, 1999). The exposure of surface water can be either indirectly through leaching of the residue through the soil or directly when cattle enter water to drink and at the same time defecate into the water. Simple models for all these routes of exposure have been developed and published in the CVMP guidance document (EMEA, 2007).

Once the PECs for each of the compartments have been calculated then the assessment proceeds as for the fish medicine, with the RQ values being calculated for each of the non-target organisms tested. As for the fish medicine, if the RQ values are all less than 1 the assessment can end. If, however, there are any RQ values above 1 then further assessment is required, either refining the PEC values or providing further data to address the trophic level of concern. If any of the RQ values for aquatic organisms exceeds 1 then it is also necessary to investigate the exposure and effects on sediment-dwelling organisms initially using equilibrium partitioning to calculate a PNEC sediment from the lowest PNEC for the aquatic environment.

There are provisions in the directive for an application for a veterinary medicine to be refused only on the grounds that there is a serious risk to the environment from its use (European Commission, 2001, 2004). It is recognised that it is not possible to prevent the entry of veterinary medicines into the environment, but the assessment procedure should ensure that no unacceptable risk to the environment occurs as a result of their entry. The outcome of the environmental risk assessment is usually that the risk to the environment from use of the product is considered to be acceptable. In the overall analysis of the benefits of the product compared with the risks of authorising it, the environmental risk assessment will not present any obstacle to authorisation. This is clear from the number of veterinary medicines that are currently authorised in the EU.

In some situations, the environmental risk is only considered acceptable if some measures are put in place in the form of advice on the product literature which will mitigate any identified risk so the product can be used without producing unacceptable risks to the environment. Montforts *et al*. (2004) reviewed the legal obligations created by Directive 2001/82/EU (as amended) to ensure that authorities, MA holders and users of veterinary medicinal products followed labelling

advice on the environment which may have been placed on the product as an outcome of the environmental risk assessment. It was concluded that risk mitigation measures were not effective for a number of reasons:

- precautions were not legally binding on veterinarians and farmers;
- the keeper of the animals and person who is responsible for manure handling may not be responsible for treating the animals;
- the risk mitigation measures cannot be shown to be effective using the risk assessment methodology;
- precautions may not be practicable under good agricultural practice.

Despite these criticisms, risk mitigation/management measures are still included in the literature for some products.

Occurrence of veterinary residues in the environment

Environmental entry

A large number and wide variety of veterinary medicines are available throughout Europe to treat animal disease and to protect animal health. These medicines in turn contain a significant number of active substances of different chemical classes. During an exercise to prioritise veterinary medicines as a first stage towards including them in a national monitoring scheme for the UK, Boxall *et al*. (2003a) identified 56 active substances used in veterinary medicines for the priority list. These were mostly antimicrobial compounds and those used to treat either internal or external parasites. These active substances were prioritised on the basis of usage or predicted hazard and there were many more active substances used in veterinary medicines in the UK which were not on the priority list (Boxall *et al*., 2002).

If products for food-producing species are considered as products that result in extensive environmental exposure then the residue of the veterinary medicine will reach the environment either directly, for example in aquaculture when fish are treated either via feed or topically as in a bath treatment, or indirectly as happens with both terrestrial food-producing animals and in fish when the product administered to the animal is excreted in the hours or days after treatment. The active substance that enters the animal will be metabolised to a greater or lesser degree before it is excreted. Studies have shown that ivermectin is excreted mainly as unchanged drug in the faeces of the treated animal (Chiu and Lu, 1989; Halley *et al*., 1989a, b) whereas many antibiotics such as sulphonamides, trimethoprim and tetracyclines are excreted mainly unchanged in the urine of the treated animals (Baggot, 1983). There are other compounds that are metabolised to a large degree in the target animal. Salinomycin, for example, is reported to be extensively metabolised in chickens (EFSA, 2004).

If the products are used in pasture animals or in aquaculture, residues will enter the environment directly in urine and faeces and in fish feed in aquaculture. Animals that are intensively reared such as poultry and pigs are housed during and after treatment with veterinary medicines. In this situation the residues of the veterinary medicine, parent compound and metabolites which are excreted in the urine and faeces are stored for a period of time, which can be anywhere from 1–12 months (Menzi, 2002; Boxall *et al*., 2003b) before the excreta, as slurry or manure, is spread onto the land as a source of organic nitrogen. It is possible that during this period of storage the excreted parent drug and any metabolites undergo further degradation. On the other hand, some drugs are fairly stable in the slurry and there is little degradation before the slurry is spread onto land.

Environmental distribution

Once the parent compound plus any metabolites and degradation products enter the environment they distribute initially in the soil and subsequently move to groundwater, surface waters

and sediment. Such movement is governed by the properties of the substances concerned: physico-chemical properties, the rate of degradation and the mobility of the compounds in soil and water. For example, the sorption coefficient (Kd) of veterinary medicines varies from 0.2 l/kg for chloramphenicol in marine sediment to 5,610 l/kg for enrofloxacin in soil (Boxall *et al*., 2003b).

For veterinary medicines the sorption is not always related to the organic carbon content of the soil, and Koc (sorption normalised to organic carbon content) values do not always correctly describe the sorption potential as other processes such as ion exchange and hydrogen bonding play a role. Degradation of veterinary medicines in the environment is variable. For example, emamectin, olaquindox and tylosin rapidly degrade (SEPA, 1999; Ingerslev and Halling-Sørensen, 2001); ivermectin, and ceftiofur are moderately persistent (Bull *et al*., 1984; Ingerslev and Halling-Sørensen, 2001); and sarafloxacin is highly persistent (Boxall *et al*., 2003b). Degradation may be affected by environmental conditions, such as temperature, soil type and pH. For example, the degradation half-life for ivermectin under winter conditions is more than six times greater than during summer conditions, and the compound degraded faster in a sandy soil than in a sandy loam soil (Bull *et al*., 1984; Halley *et al*., 1993).

Environmental monitoring

Monitoring of veterinary medicines in the environment is not carried out routinely. In the UK the Environment Agency for England and Wales (EA) has a programme of monitoring pesticides and other chemicals in the environment. Some of the pesticides that are monitored by the Agency are also used as veterinary medicines. These include cypermethrin, permethrin, deltamethrin and diazinon. The Agency reports the results of its monitoring on an annual basis. The published reports do not provide information on the concentration of the compounds in the environment. Instead, they report the number of times the Environmental Quality Standard (EQS) for the compound has been exceeded.

In the EA report for 2006 which reported the results of monitoring from 2005 there were a large number of EQS failures for the sheep dip active substances diazinon and cypermethrin (Environment Agency, 2006). These failures were mainly in Wales and in an area of North East England centred on the River Aire. The failures in Wales appeared to be due to the use of the product, while the failures in the River Aire area were probably the result of the discharge of effluent from wool processing plants located in that area.

As well as the formal monitoring of chemicals in the environment, a significant research effort has been committed to the investigation of veterinary medicines in the environment. Veterinary medicines have been measured in surface waters, groundwaters, sediments, slurry/manure and biota. Monitoring studies have focused on veterinary products used in sheep dips and aquaculture and as antibiotic treatments for livestock.

A number of studies have investigated the presence in the environment of veterinary medicines used in aquaculture. A discussion of monitoring studies that have been conducted, including measured environmental concentrations, is presented below.

Emamectin benzoate is the active substance in Slice® which is used in aquaculture for the control of sea lice. It is effective against several life stages of sea lice. As part of an environmental risk assessment of emamectin benzoate carried out by SEPA (1999) field monitoring studies were conducted at a fish farm sited on a Scottish loch to determine chemical residues in sediment, flocculent material retrieved from the loch bed, water, particulate matter and indigenous fauna. Most samples collected and analysed contained no measurable concentrations of either the parent compound or its major desmethylamino metabolite (limit of detection (LOD) of water 0.2 µg/l; LOD sediment, flocculent material, particulate matter, deployed and indigenous fauna 0.25 µg/kg). However, a maximum concentration of 5.0 µg/kg of emamectin benzoate was recorded

one week post treatment in hermit crabs, and of 1.23 and 1.99 µg/kg in dogfish and the crab species *Munida rugosa*, respectively, at the same time interval. Emamectin benzoate was also detected in sediment samples collected up to 12 months following treatment at levels of up to 2.73 µg/kg and just above the limit of detection (0.25 µg/kg) in 5 out of 61 samples of flocculent material collected and analysed. Water and particulate components collected from silt traps suspended 2 m above the loch bed were analysed separately. The parent compound was detected at 1.06 µg/l in water and at 75.1, 154 and 366 µg/kg in the particulate component.

In the same study, the desmethylamino metabolite was detected infrequently in sediment and mussel samples above the limit of quantification (0.5 and 1.0 µg/kg respectively). A peak concentration of 30 µg/kg of the metabolite was detected in the particulate component of samples collected in silt traps and at a concentration of 2.4 µg/l in the water component of a pre-treatment silt trap sample.

Studies have shown residues of oxolinic acid to be present in the surrounding wild fish population and other marine animals during and after the medication of cultivated fish (Samuelsen *et al*., 1992b; Ervik *et al*., 1994). In both studies, wild fauna were captured and monitored within the vicinity of aquaculture facilities off the west coast of Norway, following treatment with oxolinic acid. Maximum reported concentrations of oxolinic acid in the two studies were 15.74 µg/g for fish muscle, 3.77 µg/g for crab muscle and 1.48 µg/g for mussel tissue. Samuelsen *et al*. (1992b) demonstrated that tissue concentrations had declined to low levels 12 days after treatment. In a study conducted off the south-west coast of Finland (Björkland *et al*., 1991), residues of oxolinic acid were detected in anoxic sediments collected below three out of five fish farms where fish had been treated. Maximum concentrations of 0.05–0.2 µg/g were measured in sediments for 5 days after treatment of the fish.

The environmental fate of oxytetracycline following its use in aquaculture has been extensively researched (Jacobsen and Berglind, 1988; Björklund *et al*., 1990, 1991; Samuelsen *et al*., 1992a; Coyne *et al*., 1994; Capone *et al*., 1996; Kerry *et al*., 1996). A limited number of studies have investigated residues of oxytetracycline in wild fauna (Björklund *et al*., 1990; Capone *et al*., 1996). Concentrations of oxytetracycline in samples of bleak and roach, obtained from around a Finnish fish farm on the last day of medication, ranged from 0.06–3 µg/g (bleak) and 0.05–0.1 µg/g (roach) in muscle tissue (Björklund *et al*., 1990). In bleak, concentrations declined to levels at or near the limit of detection soon after treatment had finished, whereas in roach, measurable concentrations were observed in some fish samples up to 13 days after treatment.

Similar, low concentrations of oxytetracycline in wild fauna were also observed in a study conducted in Puget Sound, Washington (USA) (Capone *et al*., 1996). Only trace oxytetracycline residues (about 0.1 µg/g) were found in oysters or Dungeness crab. However, the authors reported drug residues of between 0.8 and 3.8 µg/g in the edible crabmeat red rock crabs up to 12 days after treatment. Trace concentrations were detected in two red rock crabs collected at 41 and 75 days.

There is considerable evidence to show that the enriched sediments, often present under fish farm cages, contain residues of oxytetracycline (Jacobsen and Berglind, 1988; Samuelsen *et al*., 1992a; Coyne *et al*., 1994; Capone *et al*., 1996; Kerry *et al*., 1996). Rapid sedimentation is a process characteristic of many aquaculture facilities, due to debris (mainly faeces and uneaten food) leaving the cages and accumulating underneath. Consequently, sediments containing oxytetracycline may be quickly buried and the drug may persist indefinitely.

In Norway, oxytetracycline has been found at concentrations ranging from 0.1–4.9 mg/kg dry matter (Jacobsen and Berglind, 1988). The authors indicated that antimicrobial effects might be expected at these concentrations. In a study located in the Baltic Sea, sediment samples collected from two farms on the last day of medication were shown to contain oxytetracycline at concentrations ranging from 0.05–3.8 µg/g (Björklund *et al*., 1990). Eight days after

medication had ceased, drug levels at one farm had decreased to below the detection limit (0.05 μg/g). However, up to 16 μg/g was measured in sediments taken at the other farm on day 8, and at 308 days the bottom deposits still contained between 1.0 and 4.4 μg/g sediment. The authors indicated that lower temperature and stagnant, anoxic conditions were probably responsible for the high concentrations observed.

In a separate study conducted off the southwest coast of Finland, five separate fish farms were monitored during and up to 12 days after treatment (Björklund *et al*., 1991). The maximum concentrations of oxytetracycline detected in the sediments were between 2.0 and 6.3 μg/g. Twelve days after the end of medication, levels of the drug had decreased to between 0.8 and 2.5 μg/g. Similarly, low concentrations were reported in an investigation conducted at a marine salmon farm situated in Galway Bay, Ireland (Coyne *et al*., 1994). Oxytetracycline was detected in the top 2 cm of sediment samples collected from under two adjacent cage blocks following the therapeutic use of the drug. Peak concentrations of 10.9 ± 6.5 and 9.9 ± 2.9 μg/g were detected on the tenth day of treatment and 3 days after its last use, from under cage blocks 6 and 7, respectively. Approximately 1 month after treatment, mean concentrations had decreased to between 1.6 ± 0.4 and 2.3 ± 0.5 μg/g. At 66 and 71 days after the end of therapy, concentrations were below the limit of detection.

In a later cage block study at the same site in Galway Bay, oxytetracycline was detected at concentrations ranging from 1.3–4.5 μg/g in the top 2 cm of four of the eleven sediment cores collected 5 days after the last administration of medicated feed (Kerry *et al*., 1996). The authors noted that the lower concentrations were probably as a result of the reduced treatment rate: 20 kg of oxytetracycline was used in this study as opposed to the 175 kg used previously.

Capone *et al*. (1996) presented an extensive study consisting of field investigations at three salmon facilities in Puget Sound, Washington (USA). The farms studied were chosen to represent a gradient in the magnitude of antibacterial usage. The frequency of detection of oxytetracycline was shown to parallel drug use. Residues were rarely detected beneath a farm that used very little oxytetracycline (3 kg). However, concentrations of between 0.5 and 4 μg/g were commonly detected at a farm that used 186 kg in a single prophylactic treatment period. Significantly, oxytetracycline residues (0.2–2 μg/g) were measured in surface and subsurface sediments prior to treatment. The authors believe that these persistent residues were probably due to drug usage during the previous summer or earlier.

In contrast to the above investigations, much larger concentrations of oxytetracycline were detected by Norwegian researchers under a salmon farm situated off the west coast of Norway (Samuelsen *et al*., 1992a). Following a single 10-day therapeutic use of the drug, peak concentrations of 189 and 285 μg/g were detected in undercage sediment cores collected over a period of 18 months, following medication. The disparity in results obtained in this study compared with previous studies was considered an artefact of gross overfeeding at the farm (Coyne *et al*., 1994; Kerry *et al*., 1996; Smith, 1996).

Following oral administration, ivermectin is mainly excreted in fish in the unchanged form (Høy *et al*., 1990). Given this, a variety of modelling approaches have attempted to estimate the extent to which orally administered ivermectin will accumulate in sediments under fish farms (Davies *et al*., 1998). The presence of ivermectin in sediments has also been investigated at a small number of commercial fish farms. Unpublished work from two studies (for which a limit of quantitation of 10 and 50 ng/g was achieved) failed to detect any ivermectin residues in sediments (Kwok, unpublished; E. Nixon, unpublished, cited in Canavan *et al*., 2000).

In a third monitoring study, quantifiable residues of ivermectin (measured as H2B1a, the secondary butyl compound of ivermectin) were detected in sediments under and adjacent to salmon cages situated approximately 1 km off the west coast of Ireland (Canavan *et al*., 2000).

Sediment cores were collected on the final day of a 4-month period in which the drug was administered twice weekly. Ivermectin was detected at concentrations of between 1.4 and 6.8 ng/g to a depth of up to 12 cm in cores collected from under cages and up to 31 m away from the edge of the cage block. In addition, analysis of the top 2 cm of three sediment samples that had previously been collected from the same farm but stored for 4–5 years revealed H2B1a concentrations of between 1.4 and 5.6 ng/g.

Extensive monitoring studies have been performed in the UK for sheep dip chemicals (e.g. Environment Agency, 1998, 2001). Monitoring data for England and Wales in the year 2000 demonstrated that the organophosphorus substances diazinon and propetamphos were detected in surface waters more frequently than chlorfenvinphos and the synthetic pyrethroids. Diazinon was detected in 498 out of 4,186 samples at concentrations ranging from 1–550 ng/l whereas propetamphos was detected in 168 out of 3,773 samples at concentrations ranging from 1 ng/l to 11 mg/l. Chlorfenvinphos, cypermethrin and flumethrin were detected in much fewer samples at concentrations ranging from 1–242, 1–85,100 and 1–2,190 ng/l respectively. Chlorfenvinphos, diazinon and propetamphos were detected infrequently in groundwaters and marine waters, with maximum reported concentrations being 20, 240 and 58 ng/l respectively.

Several veterinary drugs have been detected in soil that has been amended with animal manure. In three separate investigations in Germany, soil samples collected from regions with intensive livestock production were analysed for frequently used drugs (Hamscher *et al*., 2000a–c). In the first study, soil samples were collected at various depths from eight fields in the Lower Saxony region that had been manured with slurry 2 days prior to sampling (Hamscher *et al*., 2000a). In the upper 10 cm of the soil samples, 9–12 µg/kg of chlortetracycline, oxytetracycline and tetracycline were detected, with trace concentrations of tylosin also being found. Concentrations of the three tetracycline compounds decreased with depth to around 1 µg/kg below 60 cm.

In a subsequent study conducted in northern Germany, soil samples were collected and analysed from 12 different agricultural fields, 4–5 months after being treated with animal slurry (Hamscher *et al*., 2000b). Tetracycline and chlortetracycline were detected in the top 30 cm of nearly all samples at concentrations of between 1 and 32.2 and 1.2 and 26.4 µg/kg, respectively. In a follow-on study, conducted by the same researchers, the average distribution of tetracycline in the top 30 cm of soil amended with animal slurry was between 20 and 40 µg/kg (Hamscher *et al*., 2000c). Levels of chlortetracycline were generally below 5 µg/kg, although a peak concentration of 41.8 µg/kg was detected at a depth of 0–10 cm in one soil sample.

Elsewhere, American researchers detected trace amounts (approximately 0.1–2 µg/kg) of ivermectin in the top (0–3 inches) of soil in a cattle feedlot housing animals treated 28 days previously (200 µg/kg body weight) (Nessel *et al*., 1989). The authors suggest the concentrations detected in the soil are probably as a result of the faeces being trampled into the mud and subsequently being protected from light, thus retarding degradation.

Contamination of water

Whilst screening sewage treatment work effluents and associated receiving surface waters for 18 different antibiotic substances, residues of chloramphenicol were detected by German researchers at concentrations of 0.06 and 0.56 µg/l (Hirsch *et al*., 1999). The authors pointed out that as its use in human medicine is extremely limited, the two positive samples were more likely to result from its sporadic veterinary use in fattening farms.

In studies for the Centers for Disease Control and Prevention (CDC), the US Environmental Protection Agency (USEPA) and US Geological Survey (USGS) sampled and analysed liquid waste from hog lagoons (13 in three states) and surface and groundwater from areas associated with intensive swine and poultry production (52

from seven states) (Meyer *et al.*, 2001). All samples were analysed for chlortetracycline. Whilst the compound was detected at up to several hundred micrograms per litre in lagoon samples, it was only found in one surface water sample at a concentration of 0.5 µg/l (limit of detection).

In a recent national monitoring study in the US (Kolpin *et al.*, 2002) a wide range of medicines were monitored in watercourses. A number of substances that are used as veterinary medicines, including sulphonamides, fluoroquinolones, tetracyclines and macrolides, were detected in the nanograms per litre range. Many of these substances are also used as human medicines so the concentrations may result from a combination of inputs from both human and veterinary sources. The majority of surface monitoring studies involve grab sampling on a number of occasions across a variety of sites.

There are only a few reports of veterinary medicines being detected in groundwater (Hirsch *et al.*, 1999; Hamscher *et al.*, 2000b, c). In an extensive monitoring study conducted in Germany, a large number of groundwater samples were collected from agricultural areas in order to determine the extent of contamination by antibiotics (Hirsch *et al.*, 1999). The data show that in most areas with intensive livestock breeding, no antibiotics were present above the limit of detection (0.02–0.05 µg/l). Sulphonamide residues were, however, detected in four samples. Whilst the source of contamination of two of these is considered to be attributable to irrigation with sewage, the authors conclude that sulphadimidine, detected at concentrations of 0.08 and 0.16 µg/l, could possibly have derived from veterinary applications, since it is not used in human medicine.

In the investigations of Hamscher *et al.* (2000b, c) soil water was collected and analysed from four separate areas of agricultural land: two belonging to livestock farms and treated with animal slurry; and two where no animal manure had been applied for approximately 5 years. Chlortetracycline, oxytetracycline, tetracycline and tylosin were all found at the limit of detection (0.1–0.3 µg/l) in water samples collected at 80 and 120 cm depth, independent of soil treatment. In addition, no biologically active residues could be detected with microbiological assays that had approximately fivefold higher detection limits.

Veterinary medicines have been shown to leach from landfill sites. In Denmark, high concentrations (parts per million) of numerous sulphonamides were found in leachates close to a landfill site where a pharmaceutical manufacturer had previously disposed of large amounts of these drugs over a 45-year period (Holm *et al.*, 1995). Concentrations dropped off significantly tens of metres away.

So far, only one study has investigated the occurrence of veterinary drugs in surface/subsurface run-off. In a post-approval study carried out for Merck and Co., the run-off from a cattle feedlot following injection of five steers with ivermectin at 200 µg/kg body weight was collected and analysed for six separate time periods (Nessel *et al.*, 1989). Samples were collected during the 7 days prior to treatment (to establish baseline data) and during four consecutive 7-day periods following injection. The authors reported trace amounts of ivermectin (1.1–1.2 ng/l) detected in two surface water samples collected 0–6 and 14–20 days post treatment and 2 ng/l of ivermectin in the surface water of a pen flood irrigated on day 28 after the treated animals had been removed. In the 7-day period prior to treatment ivermectin was detected at concentrations of 3.2–4.4 and 0.8–1.5 ng/l in surface and subsurface water, respectively.

Veterinary drugs in topically applied formulations have the potential to be washed off the dorsal surfaces of treated animals exposed to rain shortly after dosing. In a wash-off study conducted by Merck and Co., animals were treated with a topical dose of ivermectin (500 µg/kg body weight) and then 6 hours later subjected to 12.5 mm artificial rainfall over a 10-minute period (Bloom and Matheson, 1993). Approximately 0.6% (714 µg) of the applied dose was recovered in the wash-off water (5.4 l). The average concentration of ivermectin was determined to be 1.32 µg.

Comprehensive monitoring

Recently, the results of a monitoring study of veterinary medicines in the environment have been reported by Boxall *et al*. (2006). As a result of previous work (Boxall *et al*., 2002) a priority list of compounds used in veterinary medicines in the UK was developed for further consideration of the need to monitor veterinary medicines in the environment. In the recent report this list was refined and a total of 18 compounds were selected for further investigation. Products containing these active substances were chosen, farms where these compounds were used were identified and a monitoring programme which lasted for an 11-month period was established. The final monitoring sites were chosen using the following suitability criteria:

- soil and hydrology representing potential high exposure;
- area where manure or slurry was applied in high proportion to the site;
- potential inputs of veterinary medicines from other sources;
- animal type and treatment;
- number of medicines used.

In the end four sites were chosen:

1. an indoor intensive pig facility using lincomycin, oxytetracycline and trimethoprim/sulfadiazine;
2. an outdoor pig unit using chlortetracycline and ivermectin;
3. a cattle unit with animals on pasture using doramectin and ivermectin;
4. a poultry farm using amoxicillin and enrofloxacin.

The concentration of the active substances in faeces, soil, sediment and water was measured, although not all compounds were measured in all matrices.

At the intensive pig facility after the manure was applied to the soil lincomycin, oxytetracycline, sulfadiazine and trimethoprim were detected in soil, water and sediment (from a field drain). In soil and sediment, oxytetracycline was present at the highest concentration – 305 and 813 μg/kg respectively. The peaks occurred about 2 weeks after slurry application. In the ditch water lincomycin was present at the highest concentration of 21.1 μg/l. The peak occurred 2 days after application of the slurry.

At the outdoor pig facility using ivermectin the compound was found in soil at concentrations of between 5.9 and 46 μg/kg, with the highest concentrations found 60 days after treatment had stopped. Ivermectin was not detected in samples of water taken from a stream running through the field (LOD 0.0002 μg/l).

At the site where cattle were being raised on pasture faeces collected weekly contained highest concentrations of doramectin 7 days after treatment (112 μg/kg). The concentration declined to 11 μg/kg by 35 days after treatment. Doramectin was not detected in water samples collected from a stream (LOD 0.001 μg/l), but was found in sediment at a maximum concentration of about 3 μg/kg. The cattle were observed entering the stream to drink and to stand in and around the margins of the stream. Cattle were treated twice with doramectin and the compound was found in sediment on every occasion after the second treatment (7 weeks after the first). The pattern for ivermectin was similar to that of doramectin, with the compound being found in faeces (maximum 1,800 mg/kg) and sediment (maximum 4.9 μg/kg), but not in water samples.

At the poultry farm enrofloxacin and its major metabolite ciprofloxacin were found in turkey litter at concentrations of 2.92 and 0.28 μg/kg respectively, but neither were found in soils.

This study is interesting because it related the use of veterinary medicines in a field situation to measurements made in the environment. The authors of the report also attempted to provide some context to the results by comparing the maximum concentrations measured with PNEC values produced using the most sensitive test organisms. In general, the maximum measured concentrations of the compounds in soil and water were below the PNEC values, suggesting

there was unlikely to be a cause for concern. The one exception to this was lincomycin where the concentration in soil was higher than the PNEC for micro-organism. In sediment the absence of effects data made comparison of the concentration with the PNEC impossible. The impact of the compounds on sediment-dwelling organisms cannot be ruled out.

There is a large body of data which shows that active substances that are used as veterinary medicines can be found in the environment. This fact is not surprising given that some of the products are introduced directly into the environment and all others will enter the environment sometimes during treatment and always following excretion by the target animals. The environmental risk assessment for veterinary medicines recognises this fact and attempts to estimate this exposure as part of the risk assessment. Detection of a veterinary medicine does not automatically mean that there is a concern or adverse effect associated with the presence of the compound. Veterinary pharmacovigilance is not concerned with the presence or absence of a veterinary medicine in the environment. The concern of pharmacovigilance is to record and report any adverse events that occur in the environment due to the presence of veterinary medicines.

Effects of veterinary medicines in the environment

Effects in the marine environment

In order to investigate the effects of authorised sea lice treatment on the ecology of the environment in which these products were used, a large-scale monitoring trial was conducted between 1999 and 2004 (Black, 2005). Sampling programmes were set up at four fish farm sites in western Scotland – Lochs Sunart, Diabaig, Craignish and Kishorn – where treatments for sea lice were being used. Later in the study the monitoring efforts were concentrated on Lochs Sunart and Kishorn. The active ingredients in use were mainly cypermethrin and emamectin benzoate. The sites were selected as they represented a spectrum of sea loch types varying in scale, current speed, salinity, exposure, coastal exchange and latitude.

At each of the sites the hydrographic parameters were examined to allow modelling of dispersion and to determine the substrate type and bathymetry. A number of ecological sampling programmes were initiated, each focusing on a different ecosystem:

- examination of effects on settlement of flora and fauna;
- sampling sediment for meiofauna and macrofauna;
- zooplankton sampling before and after sea lice treatment;
- time series measurements of phytoplankton.

Each sampling programme was carried out at different distances from the fish farms in order to detect any differences that may have been attributable to sea lice treatment.

There were no detectable adverse effects of sea lice treatment on zooplankton and in particular copepods. Changes that were observed were attributed to seasonal trends and not to treatment. Phytoplankton, while not directly affected by the products used in the study, may be indicators of effects on other zooplankton species. The study found that phytoplankton communities were very similar between the four sea lochs and presence or absence of a particular species could not be attributed to treatment. Changes were related to season, temperature, salinity and nutrients. The effects on meiofauna and macrofauna in sediment depended on the site under investigation. There were no clearly discernible effects of sea lice treatment on the meiofauna in any of the lochs, although there were changes in populations which could be the result of organic enrichment. There was no evidence that the settlement of barnacles and mussels had been adversely affected by the use of the sea lice treatments. The low settlement of barnacles at one site in Loch Kishorn may have been the result of discharge from the fish farm site, but also may have been due to a number of other processes.

The study was not able to detect any long-term changes in ecology due to use of veterinary medicines employed to treat sea lice. The processes of species succession and population dynamics were well within the range for normal variation. It was considered that any changes were likely to be so subtle as to require long-term monitoring together with a close relationship with the individual fish farms. The study did show that wide-scale changes due to the use of veterinary medicines, if they occurred at all, were likely to be in the same order of magnitude as natural changes and hence very difficult to detect.

As discussed above, the environmental risk assessment for medicines used in fish relies on the estimated PEC in water or sediment being compared with the PNEC values for non-target species generated using laboratory studies. Many of the studies are acute studies and it relies on the assessment factor to take into account the extrapolation to chronic exposure. The study carried out by Black (2005) under field conditions failed to find any adverse effects of sea lice treatments on flora and fauna in the sea lochs studies. This suggests that the risk assessment procedure has not grossly underestimated the potential for environmental risks. Hence, the veterinary medicinal products thus authorised do not pose major environmental risks.

The conclusion of the study was also interesting when considering the role of pharmacovigilance in noticing adverse effects on the environment when the product is used as intended. In the world of fish farming it is almost impossible to expect that the use of a fish medicine which has been approved on the basis of an acceptable risk assessment would cause such adverse effects in the environment that it would be detected by a user of the product or member of the public.

Effects on birds

Populations of the Oriental white-backed vulture (*Gyps bengalensis*), long-billed vulture (*Gyps indicus*) and slender-billed vulture (*Gyps tenuirostris*) have declined by over 95% in the Keoladeo National Park in India during 1997 and subsequent years (Prakash, 1999; Prakash *et al*., 2003). It was not until 2004 that Oaks *et al.* (2004) identified the cause of the decline. These workers carried out post-mortem examinations on vultures and found a high proportion of the birds (85%) had characteristic signs of visceral gout on the surface of internal organs probably as a result of renal failure. These observations correlated with findings of high concentrations of the non-steroidal anti-inflammatory drug diclofenac in the kidneys and other tissues of birds that had died of renal failure (Taggart *et al*., 2007a, b). Diclofenac was confirmed as the cause of death when birds fed the drug developed renal failure and visceral gout. It was hypothesised that the morbidity and mortality in the vultures was due to the animals scavenging on dead livestock which had been treated with diclofenac prior to death. Oakes *et al*. (2004) reported that in Pakistan diclofenac was widely available for veterinary use as an over-the-counter drug for use in all types of livestock. Diclofenac is toxic to both the Eurasian and African Gyps vultures (*Gyps fulvus* and *Gyps africanus*) (Swan *et al*., 2008).

In 2006 in recognition of the decline of the vulture population caused by the use of diclofenac the Indian Ministry of Health ordered the country's drug manufacturers to stop producing and selling veterinary diclofenac formulations. The Ministry is withdrawing manufacturing licences for the production of diclofenac for veterinary use (Anonymous, 2006). However, the effects appear to be widespread across India (Schultz *et al*., 2004; Green, 2006; Cuthbert *et al*., 2007; Green *et al*., 2007). There are concerns that other drugs, including antibiotics, may exert adverse effects on vultures and other scavenging birds (Lemus *et al*., 2008).

Is the environmental risk assessment used during the approval procedure for a new product capable of detecting an unsuspected environmental impact such as that seen in vultures? An application for a marketing authorisation for a new product for food-producing animals raised either on pasture or intensively would contain an

environmental risk assessment carried out in accordance with the VICH guidelines. If the exposure of the environment was low (PEC_{soil} < 100 µg/kg) and the active substance was not a parasiticide then the assessment would end at Phase I with no information, except for laboratory animal studies in the basic toxicity package, on the hazard presented by the active ingredient. If a Phase II assessment was necessary because the PEC_{soil} was >100 µg/kg or the active substance was a parasiticide then the studies required in Phase II, Tier A (described above) would be required. The acute/sub-acute studies are carried out on invertebrates and fish.

If the risk assessment indicates that all the RQ values are <1 then the environmental safety of the product is acceptable and assuming all other aspects of the dossier are acceptable a marketing authorisation will be granted. Even if the Tier A assessment indicates that there is a risk to the terrestrial compartment, any additional studies will not include effects on birds or mammals. The environmental risk assessment would not detect the adverse effect seen in vultures which has arisen from a unique set of circumstances which are only found during use of the product in the field.

The exact type of adverse event reported in India is not likely to be repeated in Europe as there are no vultures in Europe and it is not usual for carcasses of dead animals to be left in the field to decompose. However, what would happen if an adverse event of this type was to occur in Europe? For example, a drug used in pasture animals some of which died in the fields, the carcasses left for a short period and some scavenging birds exhibited the same sensitivity to the drug as vultures. It could be possible for birds to be adversely affected. The question then is would this adverse event be detected and would it be reported under the EU pharmacovigilance scheme?

There is evidence from the UK that the presence of dead birds will be reported if a scheme is well publicised. In the UK the Wildlife Incident and Investigation Service (WIIS) has reported adverse effects of pesticides for many years. Since 1997 it has been accepting reports of dead birds caused by the suspected misuse of veterinary medicines. In the reports of the UK Veterinary Medicines Directorate (VMD) Suspected Adverse Reactions Surveillance Scheme (SARSS) for 2004–2006 there were a number of reports of dead birds (Dyer *et al*., 2004–2006). The conclusion is that if the death of birds or large mammals is the adverse event then there is a good chance of it being detected by someone and possibly it being reported to a pharmacovigilance system. However, for small birds and mammals the chances of detection and reporting would be much lower.

Further evidence to suggest that this type of adverse event would be reported and could lead to changes in the use or labelling of a product comes from the USA. The Center for Veterinary Medicine (CVM) in the United States which is responsible for the authorisation of veterinary medicines has included an environmental warning on pentobarbital-containing euthanasia products. This warning followed the reported deaths of 34 eagles as a result of them feeding on carcasses of animals euthanised with pentobarbitone which had not been disposed of properly (Otten, 2001; Anonymous, 2003).

Effects on invertebrates

Parasiticides are used extensively in veterinary medicine to treat and control external and internal parasites of domestic livestock, particularly cattle and sheep which spend a good part of the year on pasture. Veterinary parasiticides used in ruminants include anthelmintics such as the benzimidazoles, levamisole and morantel and the avermectins and milbemycins (McKellar, 1997). This latter group provides control of both external and internal parasites.

The first endectocide, ivermectin, was introduced into the market in the early 1980s. At this time there was no requirement in the veterinary legislation in Europe to carry out an environmental risk assessment as part of the approval procedure. Since the introduction of ivermectin there

have been other endectocides introduced for use in farm animals, mainly cattle and sheep. These include abamectin, eprinomectin and doramectin, which are all avermectins, and moxidectin, which is classified as a milbemycin. This group of compounds is known collectively as the macrocyclic lactones. The introduction into the market of some of these other macrocyclic lactones was at a time when an environmental risk assessment did in fact form part of the authorisation procedure.

Despite the fact that there was no requirement in Europe at the time of authorisation of ivermectin to provide an environmental risk assessment, information on the environmental fate and effects of ivermectin has since been generated and subsequently published by the discoverers of the compound Merck Sharpe and Dohme, now Merial (Halley *et al*., 1989a, 1993; Nessel *et al*., 1989). Most of the information required under the recent VICH Phase II guideline (EMEA, 2005b) is available, although the studies may not have been conducted in accordance with current OECD guidelines. Environmental information is also available in the public domain for abamectin (Halley *et al*., 1993). However, very little information has been published on the environmental fate and effects of other macrocyclic lactones.

All of the compounds listed above are used in farm animals, cattle, sheep and pigs and in horses. Endectocide products used in the terrestrial species listed above are administered by a number of different routes depending to some extent on the target species and the active ingredient. The routes of administration include:

- subcutaneous injection;
- oral administration either in liquid form as an oral drench or as a paste or as a slow release bolus formulation;
- topical administration by pour-on application where the active ingredient is absorbed through the skin.

Whatever the route of administration the active substance will be metabolised by the target animal to a greater or lesser degree and then excreted in urine and faeces. The majority of animals treated with these products are grazing on pasture during and after treatment and as a result residues are deposited directly onto the pasture.

Ivermectin undergoes some metabolism in the target species, but the majority of the dose, irrespective of the route of administration and the target species, is excreted in faeces as unchanged ivermectin (Halley *et al*., 1989b; Chiu *et al*., 1990; Alvinerie *et al*., 1999; Perez *et al*., 2001). The same is true for the other endectocides for which published information is available (Perez *et al*., 2001; Kolar *et al*., 2006).

The concentration of ivermectin in the faeces of animals after treatment has been reported by a number of authors. The concentration of ivermectin in cow pats after either subcutaneous injection at 0.2 mg/kg bodyweight or pour-on administration at 0.5 mg/kg bodyweight was determined by Sommer and Steffansen (1993). The ivermectin concentration in dung collected 1 day after treatment was 9.0 mg/kg dry weight after subcutaneous injection and 2.8 mg/kg dry weight after pour-on application. Five days after treatment the concentration of ivermectin was 2.8 and 2.7 mg/kg dry weight for subcutaneous and pour-on treatments respectively. Assuming that fresh cattle dung contains about 80% water the concentration in dung 1 day after treatment was 1.8 mg/kg (subcutaneous) and 0.56 mg/kg (pour-on) on a fresh weight basis. The concentration of ivermectin in faeces is variable depending on parameters such as the breed of the cattle and the diet.

In a study in cattle where ivermectin was administered to two groups of cattle fed on different diets, the peak concentrations of ivermectin in dung were about 0.35 and 0.2 mg/kg for pasture and grain-fed animals respectively (Cook *et al*., 1996). In a study in horses using ivermectin, moxidectin and doramectin, the concentration in faeces was determined after oral treatment of all three compounds at a dose of 0.2 mg/kg bodyweight (Gokbulut *et al*., 2001). The concentration of all three compounds in faeces (on a dry weight basis) peaked 24 hours after administration at 19.5, 20.5 and 16.6 mg/kg for ivermectin,

doramectin and moxidectin respectively. By 48 hours the concentration of all three compounds was below 5 mg/kg. In another study in horses with moxidectin and ivermectin given orally at 0.2 mg/kg the concentration in faeces peaked 2.5 days after administration at a concentration of 2.5 mg/kg wet weight (Perez *et al*., 2001).

The excretion of abamectin and doramectin has been studied in the faeces of sheep following a single subcutaneous dose of 0.2 mg/kg bodyweight (Kolar *et al*., 2006). The maximum concentration of abamectin of 1.3 mg/kg dry weight was detected on day three after treatment, while the maximum concentration of doramectin of 2.2 mg/kg dry weight was detected on day two after treatment.

The pattern of excretion of the macrocyclic lactones is such that when veterinary medicines containing these actives are used to treat cattle and sheep the dung of these animals contains high concentrations of unchanged parent drug for some time after the animals have been treated. Shortly after the introduction of ivermectin the results of studies carried out using the dung of treated animals showed that there were adverse effects on insects that utilised cow dung (Wall and Strong, 1987; Ridsdill-Smith, 1988; Strong, 1992). Similar effects on dung insects have been shown to occur with other macrocyclic lactones (Miller *et al*., 1994; Wardhaugh *et al*., 2001; Steel and Wardhaugh, 2002; Floate *et al*., 2005). The information available indicates that macrocyclic lactones can be toxic to dung insects for anything from a few days to a number of months.

Some of the variability is probably due to the different methods used in the numerous experimental studies, but the evidence does indicate that the individual compounds have differing toxicity to dung insects. In the paper by Floate *et al*. (2005) the compounds were ranked in order of toxicity as abamectin > doramectin ≥ ivermectin > eprinomectin > moxidectin. The potential adverse effects of avermectins and milbemycins on dung insects have been clearly demonstrated. However, what is less clear-cut is whether or not there is an adverse effect in the wider environment. There are clear divergences of opinion on this, with a number of authors indicating their concern that the compounds are affecting dung flies and beetles (Herd, 1995; Wardhaugh and Ridsdill-Smith, 1998; Suárez *et al*., 2008), whereas others take a different view (Barth *et al*., 1993; Forbes, 1996). It is interesting to note that the two groups can be divided almost exactly along the lines of those not involved or employed by the manufacturers of endectocides and those that are so employed.

In their reviews on the effects of avermectins and milbemycins, Edwards *et al.* (2001) and Floate *et al*. (2005) listed a number of areas where further research was needed in order to elucidate the effects of these compounds on the environment. These include:

- studies on nematodes, mites, fungi and rare invertebrates;
- studies on other active substances used in veterinary medicines to control invertebrates such as synthetic pyrethroids, and insect growth regulators;
- long-term field studies to monitor the effects on populations of insects and dung degradation;
- development of ecological models to assess long-term risks.

Abamectin and doramectin have been shown to be toxic to earthworms, isopods and other soil invertebrates (Kolar *et al*., 2008).

The VICH guidelines (VICH, 2005) require that for any parasiticide product used in pasture animals and excreted in dung, the effects of the compound on the development of the larvae of a dung fly and dung beetle species has to be investigated. So it is very likely that the basic tests at Phase II Tier A will detect compounds that are toxic to dung insects. However, it is likely that many compounds developed for their activity against parasites will prove to be toxic to larvae and adult insects that utilise animal dung. This fact alone is not likely to result in the product not being given an authorisation.

When a risk is identified at Tier A the first step in refining the risk assessment is to refine the PEC. However, this is not possible in the dung

pat unless there is significant metabolism in the treated animal. For all the active substances on the market to date, significant metabolism does not occur so it will be necessary to carry out studies in Tier B, such as field trials. Should these trials provide a way of refining the risk assessment then the product would be approved for use. It is likely based on the precedent of existing endectocides that some adverse effects would be considered as acceptable.

Once approved and on the market it could be expected that any adverse effects on the environment would be identified and further monitored to perhaps confirm that these were no more serious than expected. This after all is the role of pharmacovigilance. It is possible that in areas of special scientific interest there would be monitoring programmes in place which may detect adverse effects on dung insects. However, in most farming areas the decline in dung insects would have to be highly significant for this to be noticed and reported.

Effects on aquatic organisms

In several countries including the UK sheep are treated for various infestations of external parasites by plunge dipping. The practice of treating sheep in this way has been carried out for over 100 years. In the UK the number of active substances used in sheep dips has been reduced over the years until, in 2005, there were only two left on the market – diazinon, an organophosphorus compound, and cypermethrin, a synthetic pyrethroid. Both of these are acutely toxic to non-target organisms in the environment particularly invertebrates and fish (WHO, 1989, 1998) and diazinon has the additional problem of being neurotoxic, which can affect humans.

As well as being acutely toxic it has also been found that both diazinon and cypermethrin can have subtle non-lethal effects on fish at very low concentrations. Cypermethrin at a concentration of <0.004 μg/l reduced or inhibited the priming response of male salmon parr to prostaglandin F2α (Moore and Waring, 2001). It can also adversely affect other aquatic organisms (Saha and Kaviraj, 2008). Similar effects on male salmon parr to those produced by cypermethrin were produced by diazinon, but at much higher concentrations of between 2.0 and 10.0 μg/l (Moore and Waring, 1996). In vitro diazinon has also been found to have cytotoxic and endocrine disrupting effects on adrenocortical steroidogenic cells of rainbow trout (Bisson and Hontela, 2002).

After dipping sheep in a plunge dip the animals have to be retained for 10 minutes in a holding pen which drains the dip back into the dip bath (DEFRA, 2001). The sheep can then be released back into the fields which may contain watercourses such as streams, ponds and ditches. Sheep dip can enter the environment during the normal authorised use of the dip when the treated animals re-enter the wider farm environment. In a study carried out in 2006 (Sinclair *et al*., 2007) it was shown that cypermethrin was found in the run-off from an area of hard-standing in which dipped sheep had been held before being returned to the field. These sheep had already been held in a draining pen next to the dip bath as required. The other findings of the study were that such run-off could occur some time after the sheep had left the pen. It is also possible for dip to enter the environment directly as a result of accidental, or deliberate, loss of the dip from the dip bath. As a result, both diazinon and cypermethrin have been found in the aquatic environment at concentrations above their respective EQS values, indicating the potential to produce adverse effects (Environment Agency, 2006).

The EA in England and Wales and SEPA in Scotland are responsible for reporting and investigating any suspected pollution incidents caused by sheep dip chemicals. In turn these incidents are reported to the competent authority in the UK (the VMD) as required under Article 73 of Directive 2001/82 (as amended). The VMD publishes the results of its SARSS annually in the *Veterinary Record*. These publications include the reports of environmental pollution incidents caused or suspected of being caused by sheep dips.

The reports for 2004–2006 show a large number of pollution incidents resulting in adverse effects to aquatic invertebrates in England and Wales caused by sheep dips (Dyer *et al*., 2005–2007). Both cypermethrin and diazinon were identified in these incidents. However, there were more incidents categorised as major or serious (the most serious in terms of environmental damage) where cypermethrin was identified than those where diazinon was present. The causes of incidents, when a cause could be identified, were given as poor dip bath construction, inappropriate use and disposal, run-off from hardstanding resulting in dip solution entering surface water and dipped sheep crossing surface water after dipping. The large number of serious environmental incidents caused by cypermethrin sheep dip resulted in the VMD suspending the marketing authorisations of these products in February 2006.

The environmental risk assessment of a sheep dip, should an application for a marketing authorisation for a new product be made, would enter Phase II as the product is a parasiticide used in pasture animals. The exposure assessment of such a product appears to be relatively straightforward, as after dipping, the animals are held near to the dip bath until they are no longer dripping. The dip wash lost at this time drains back into the dip bath which is disposed of either by contract waste disposal or by application to land. In the UK, this latter route is under the control of the EA or SEPA. The disposal would not form part of the assessment under VICH guidelines. The routes of exposure of the active substance from the treated sheep would be as outlined in the VICH guidelines. A PEC_{soil} would be calculated and from this value PEC values for groundwater and surface water would be derived. The fate and effects studies required in Tier A of Phase II would be presented and the risk evaluated.

When a sheep dip is used in the field the information from reports of environmental pollution incidents suggests that the exposure scenarios used in the risk assessment and which are based on how the product should be used do not address all the potential routes of exposure when the product is used in the field. The study on run-off discussed above (Sinclair *et al*., 2007) demonstrates that there is the potential for exposure of surface water and the possibility of adverse effects long after sheep have been dipped in accordance with label instructions. The reports from Dyer *et al*. (2005–2007) indicate that sheep crossing streams after treatment can cause problems yet there was no evidence that the dipping had not been carried out as required by the label. There is also the potential for pollution to occur due to poor dipping techniques.

It could not be expected that the environmental risk assessment carried out before authorisation would address the exposure scenarios reported from the field. The adverse effects in the environment were detected and reported as required by pharmacovigilance legislation which resulted in the suspension of the products in question. However, as noted in the report from Dyer *et al*. (2006) all the incidents were reported by those responsible for the environment – either the EA or the SEPA. It has to be questioned as to whether or not such incidents involving invertebrates would be spotted and reported by the general public.

Adverse event reporting in the UK and Europe

Veterinary medicines

In the UK there has been a procedure for reporting suspected adverse reactions caused or suspected of being caused by veterinary medicines for many years (Gray and Knivett, 2001–2003; Dyer *et al*., 2004–2007). The pharmacovigilance responsibilities of the competent authority are discharged by the VMD through the SARSS, which monitors suspected adverse reactions to veterinary medicinal products in all animal species, in humans and in the environment. Under the SARSS anyone, for example members of the public, veterinary surgeons, farmers and

doctors, is encouraged to report to the scheme. Pharmaceutical companies are legally required to keep a record of any information they receive about suspected adverse reactions to their products.

In the guidance note published by the VMD concerning pharmacovigilance (VMD, 2005) environmental incidents are mentioned as a type of incident that may be caused by a veterinary medicine and which should be reported to the scheme. It advises reporters to collect as much information as possible, including information on the product, the date of the incident and a description of what the problem in the environment was and how the possible entry into the environment had occurred. A special reporting form is available for reporting adverse environmental effects.

In France, pharmacovigilance of veterinary drugs is administered by the Agence Nationale du Médicament Vétérinaire and has been fully operational since 2002. As part of the system operated by the French Agency, risks presented by the use of veterinary medicines to the environment should also be reported.

In the USA the pharmacovigilance of veterinary medicines is overseen by the Center for Veterinary Medicine (CVM) which is part of the Food and Drug Administration (FDA). The primary purpose of the 'adverse drug event' monitoring system is to detect side effects or problems associated with the use of approved veterinary medicines (FDA, 2004) including lack of effectiveness. The scheme is a voluntary one; however, Federal regulations require manufacturers of approved animal drugs to send the FDA all information concerning adverse drug events which come to their attention. The FDA system does not mention the reporting of adverse effects on the environment.

In Australia, the Australian Pesticides and Veterinary Medicines Authority (APVMA) operates an Adverse Experience Reporting Program for veterinary medicines (AERP Vet) established by the APVMA to facilitate responsible management of veterinary medicines throughout their lifecycle. The scope of the AERP Vet covers adverse experience reports involving animal health issues, human health issues, lack of efficacy, residue issues and environmental damage (APVMA, 2007a). The adverse reaction reports can be provided by the manufacturer of the veterinary medicine, by veterinary surgeons, by users of the product and animal owners (APVMA, 2007b).

The main responsibility of the European Medicines Agency (EMEA) and its veterinary scientific committee, the CVMP, in post-marketing surveillance of veterinary medicinal products in the EU is for products that reach the market by authorisation through the centralised procedure. In addition, the CVMP Pharmacovigilance Working Party (PhVWP-V) regularly meets at the EMEA. The mandate of the PhVWP-V now enables the group to form the scientific platform on pharmacovigilance of all veterinary medicinal products. Experts on veterinary pharmacovigilance from the competent authorities of each member state contribute to this forum. This expert group assesses pharmacovigilance issues for centrally authorised products on behalf of the CVMP as well as for products that have been authorised by the member states via the national, mutual recognition or decentralised procedures, or the procedure of mutual recognition (EMEA, 2006b).

The UK VMD publishes reports of adverse reactions due to veterinary medicines on an annual basis. Inspection of the report indicates that environmental incidents only began to be reported in 2003 (Dyer *et al*., 2004). Of the reports published by the VMD since 2004 only a single report has been submitted by a member of the public, out of a total of 163. There have been no reports of environmental problems from manufacturers of veterinary medicines. The reports from the UK in the main involve sheep dips and poisoning of birds either deliberately or accidentally. However, one report from 2004 (Dyer *et al*., 2005) was not on this theme. The report concerned an unexplained lack of trout fry in the upper reaches of a stream. Cattle were allowed to drink from the stream and these animals had been treated with a product containing ivermectin. The report from the VMD concluded that it was not possible to establish a positive link

between the two events. However, in the environmental risk assessment for parasiticides used in cattle one of the exposure scenarios considered is direct excretion into a surface water body when the animals enter the water to drink (EMEA 2007). This report suggests that this route of exposure may perhaps occur in practice.

Published information on the results of pharmacovigilance from other competent authorities is scarce. The EMEA has recently established a computer system known as EudraVigilance Veterinary. The system is described as a European data-processing network and database management system for the exchange, processing and evaluation of Suspected Adverse Reaction Reports related to veterinary medicinal products authorised in the European Economic Area. From July 2005 competent authorities of the member state have been required to send relevant data to the network, with the veterinary pharmaceutical industry following. Procedures are currently being developed to evaluate and make available the information contained within the network. Relevant safety information with regard to the use of veterinary medicines will become publicly available.

The EMEA has also published some information on veterinary pharmacovigilance (EMEA, 2007) on products that were authorised by the centralised route. The EMEA received 738 reports of adverse reactions in animals and in humans in 2006. Of these, 300 reports were from EU countries and the remainder were from the USA, Canada and some other countries. The majority of the reports were of adverse reactions in the target species (638), with the remaining reports (100) being for adverse reactions in humans. There were no reports of centrally authorised veterinary medicines causing any adverse effects in the environment.

Conclusions

Veterinary medicines are administered to animals to treat and prevent disease and after administration the active substance and any metabolites are excreted by the animal in urine and faeces. The residues of the medicine will, as a consequence of excretion, enter the soil environment either directly because the animal is outdoors when excretion occurs or indirectly in manure or slurry in the case of food-producing species that are housed during and after treatment. For medicines used in fish the route of entry can be in excreta of the fish, but all fish medicines are placed directly into the environment.

As a consequence of the exposure of the environment it has been a requirement since 1992 that as part of the authorisation process for veterinary medicines the safety of the environment should be assessed. The assessment of environmental risk is part of the evaluation of the quality, safety and efficacy of the product and has to be considered in the overall benefit : risk assessment which is undertaken before approval of the marketing authorisation. It is possible under the legislation to refuse the approval of a marketing authorisation on the grounds of a serious risk to the environment.

The environmental risk assessment is carried out using a tiered approach of first evaluating exposure of the environment (Phase I) and, if this exposure is considered to be extensive, then evaluating the toxicity and fate of the active substance and the risk to the environment (Phase II). The assessment is carried out following internationally recognised guidelines and using the principle of comparing exposure with toxicity to derive a risk quotient. The approach is similar to that of assessment for other groups of chemicals. The Phase I assessment eliminates from further consideration those veterinary medicines whose use does not result in extensive exposure of the environment. The Phase I procedure has to be conservative as it does not provide any information on the potential risk to the environment from the use of the product.

There are many veterinary products where the assessment does not progress beyond Phase I and the largest group of products that do not receive any further consideration are those indicated for use in companion animals, i.e. cats and dogs.

This means that there are a number of active substances entering the environment following use for which there is no information on their environmental properties and effects. It is assumed that irrespective of the toxicity of the compounds the *risk* is acceptable because exposure is low. For products that end assessment at Phase I the environmental risk is considered acceptable and it will not be considered as a risk in the benefit : risk analysis. It is accepted that the active substances used in products that end assessment at Phase I will enter the environment without anything being known of their environmental properties.

If it is necessary to carry out a Phase II assessment then laboratory data on fate and effects are produced. The assessment follows a tiered approach and will move to the next tier if a risk is identified. The assessment procedure uses data from acute laboratory studies together with assessment (safety) factors to evaluate the risk to the terrestrial and aquatic environments. The exposure of the environment to active substances in products that enter Phase II is extensive (otherwise Phase II would not be necessary), but the aim of the risk assessment is to determine that the concentration of the compound in the environment – terrestrial and aquatic – will be below the concentration that may produce adverse effects. If this condition can be demonstrated by the risk assessment then the risk to the environment is considered acceptable and in the analysis of benefit : risk the outcome of the assessment is not considered as a risk.

As discussed, the active substances of veterinary medicines enter the environment, soil, freshwater and seawater as a result of treating animals. There has been much evidence gathered to show that a number of active substances used in veterinary medicines are present in the environment. Residues of active substances used in fish medicines are found in the marine environment. These include antibiotics and parasiticides which, depending on their properties, are found in the water and in sediments. However, the presence of these substances in the environment does not mean that they will produce adverse effects. It can be argued that if the environmental risk assessment has been carried out correctly then there should be no unacceptable risk to the environment. The study carried out in Scotland to try to identify any adverse effects of sea lice treatments was not able to detect any adverse effects that were due to the treatment with the veterinary medicines. The results suggest that as far as can be certain the process of environmental risk assessment during authorisation correctly concluded that there would be no detectable risk.

Residues of active substances have also been measured in soil from fields where manure from treated animals has been spread or where treated animals are grazing and in surface water in the pastures. The only documented effects of veterinary medicines adversely affecting the terrestrial environment, groundwater or surface water are those produced by the macrocyclic lactones on dung flies and dung beetles. The adverse effects on the larvae of dung flies and dung beetles was not detected during the authorisation of the first of the avermectins, ivermectin, but was reported by research workers in the years immediately following its introduction. All recently authorised macrocyclic lactones will have been assessed for their effects on dung insects before authorisation. The adverse effects produced would have been considered as part of the benefit : risk assessment of the product and it has to be concluded that the benefit : risk was considered positive for approval of the products. If the effects of the macrocyclic lactones had not been detected as a result of research work, and following the requirements of environmental risk assessment, would the effects have been reported under the pharmacovigilance system? It is possible that if all dung insects were wiped out by residues of these compounds in dung then this would have been noticed, but it would be unlikely that the immediate thought would be that a veterinary medicine was to blame. As the effects of the macrocyclic lactones appear not to adversely affect populations of insects, the subtle effects would probably not be detected.

Pharmacovigilance does appear to have played a role, however, in the identification of the adverse effects caused by cypermethrin and diazinon

sheep dips. The observations and reports from the field appear to suggest that it is not possible to use these dips, in particular cypermethrin, without the potential to produce adverse effects in the environment. It is very unlikely that such adverse events could be predicted from the environmental risk assessment because although the hazard of the active substances is easily identified, the routes of possible exposure of the environment and in particular water courses cannot be predicted. This is more evident when some of the exposure routes result from the product being used exactly as described on the label.

In the case of the vultures in South East Asia the situation is unique and it is difficult to envisage it happening in Europe. However, the observations on the death of the vultures and the subsequent elucidation of the cause is the sort of event that should be detected by a robust pharmacovigilance system. The death of large birds or mammals would be noticed and reported, but for smaller creatures the same may not be true.

Veterinary medicines enter the environment as a result of their use. The environmental risk assessment should demonstrate that the use of these medicines will not result in unacceptable risks to the environment, although this does not mean that there will not be any local adverse effects which overall is considered to be an acceptable level of risk. The role of veterinary pharmacovigilance is the gathering of information on adverse reactions which may occur after the administration of medicinal products to animals. This is relatively easy to achieve for adverse reactions in the target animal and the user as the link to the product causing the problem is usually clear. For the detection of adverse environment events there is the difficulty of linking a potential effect on the environment with use of a particular veterinary medicine. Unless the potential incident is spotted soon after the product is used or there is a prolonged effect in the environment it is very unlikely that pharmacovigilance will detect adverse environmental effects.

The new EU legislation introduced in 2005 places more emphasis on ensuring the safety of products through pharmacovigilance. All authorised products will now have only one renewal of the marketing authorisation and then will hold an authorisation indefinitely. It will be the role of pharmacovigilance to detect any adverse effects produced by the product. For the environment it is not at all certain that pharmacovigilance is up to this job.

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27 Causality in pharmacovigilance and expectedness of adverse reactions

K.N. Woodward

Introduction

Most regulatory schemes for spontaneous adverse reporting for human or veterinary medicines include a requirement to assign causality and to report unexpected adverse drug reactions, frequently with some degree of urgency. To put it another way, there is a need to know, if at all practicable, if a particular product was responsible for a particular adverse reaction and, if so, whether that reaction was expected and therefore listed on the label and product literature, or otherwise.

Causality

Marketing authorisation holders for veterinary medicinal products in the EU 'may comment' on causality using the ABON system. The ABON system categorises adverse reactions as probable (A), possible (B), unlikely (O) or unclassifiable (N). The original Volume 9 of the *Rules Governing Veterinary Medicinal Products in the European Union* provides some general guidance, or more accurately criteria that 'should be complied with', but this can be confusing, and it lacks specificity, particularly for the B, O and N categories.

The revised criteria, A, B, O, O1 and N are proposed in the draft of Volume 9B (EMEA/CVMP/PhVWP/430286/2007 – draft 13) and here further guidance is given. These criteria are summarised in *Table 27.1*, but whatever criteria of causality are used, and regardless of whether the country involved is in the EU or elsewhere, guidance in some form or other is frequently required.

At first the issue of causality may appear straightforward, but this is certainly not always the case. We can take an example. An elderly dog with a bacterial infection dies soon after the injection of an antibiotic. This raises the question of whether the animal died from the disease, from an adverse effect of the drug, from old age or from a combination of two or more of these – or from something else entirely. A cat suffers a seizure and brain damage after a routine inhalation anaesthetic – was it an adverse drug reaction, a reaction to surgery or lack of oxygen? How can the gender, disease-state, age, species and breed related factors be dissected out or tied in with any adverse drug reaction signs?

The issue of causality extends far beyond pharmacovigilance. It applies across medicine in its

Table 27.1 Criteria for ABON classification of suspected adverse reactions in animals.

Classification	*Criteria*
A	Probable All of the following: • Reasonable association in time between drug administration and onset and duration of the event • Positive challenge/dechallenge • Clinical or pathological phenomena should be consistent with the adverse reaction, or at least plausible, given the known pharmacology and toxicology • No equally plausible explanation. Concurrent use of other drugs or intercurrent disease, exclusion of other causes • Where any of the above cannot be satisfied, consider B, N or O or O1
B	Possible • Drug causality is one of other possible or plausible causes but • Data do not meet inclusion criteria for A
O	Unclassifiable/unassessable • Insufficient data to draw any conclusions
O1	Inconclusive • Other factors prevented a conclusion, but an association with product treatment could not be eliminated
N	Unlikely to be product related • All cases where there is no reliable or adequate evidence with which to make an assessment of causality

widest sense, and to epidemiology specifically. Can exposure to various chemicals, environmental agents, radiation, foods, lifestyles, recreational factors, infectious agents and so on cause (or contribute to the cause of) a disease? More specifically, what is the association between exposure to an agent and the aetiology of the disease, or is it a coincidental, chance finding? What criteria can be applied to convert a suspicion into an association and an association into a cause? Perhaps put a little more elegantly, 'one must still ask how to know a cause upon seeing one, and how not to confuse the real thing with an impostor' (Hume, 1739, quoted in Susser, 1991).

Often, there are many factors that might lead up to a disease state (Rizzi and Pedersen, 1992). Drug reactions can be categorised into type A, that is those that are an extension of the pharmacological or toxicological activity of the drug. Examples would include a drug that induces prolonged and excessive tachycardia eventually leading to myocardial damage, possibly through anoxia, or hepatic necrosis caused by a high dose of paracetamol (acetaminophen). These reactions are predictable from a knowledge of the drug's pharmacology and toxicology. Even so, species differences must be borne in mind! On the other hand, type B reactions are those that cannot be predicted from the drug's pharmacology or toxicology, and may be bizarre; these include allergic reactions and those determined by pharmacogenetic factors (Routledge, 2000). Type A reactions might be regarded as singular causality (e.g. overdose) while type B could be seen as general causation (e.g. the properties of the drug, the genetic make-up of the patient) or multifactorial and certainly idiosyncratic.

Unfortunately, analysis of spontaneous adverse reactions seldom has anything like this simplicity (if any of this can be regarded as simple), and although type B reactions might have a complex background determined by predisposition to hypersensitivity reactions or other states with a genetic component, type A reactions are often

more complex than might otherwise seem logical as they may be determined (for example) by pharmacokinetic factors related to age or pre-existing disease (e.g. reduced drug metabolising ability due to liver disease, reduced capacity for drug excretion due to renal disease, reduced organ function due to old age or inadequate organ function in neonates including pre-ruminant function or immature hepatic metabolising capacity, increased susceptibility due to cardiac failure) or to drug–drug interactions, or to breed-specific factors (Pirmohamed and Park, 2007). They may also be determined by pharmacodynamic determinants brought about by disease or age-related deterioration (Dayan, 2000; Routledge, 2000). Both pharmacokinetics and pharmacodynamics may be influenced by, and may influence, drug–drug interactions. All of these factors conspire to make the attribution of causality more complex than it might otherwise appear both in humans and in animals, and they emphasise the need to consider all possible contributory causes in addition to the primary cause, except in the simplest of cases (Riegelman, 1979).

The association between an event and a causal factor may therefore be difficult to determine, for many of the reasons already described. The identification of associations may eventually be dependent on statistical analysis (or analyses) as well as biological considerations. An event or a series of events must have a relationship in time with a potential causal factor, if there is to be any association made, and though it may seem obvious, the relative direction of time with respect to an event (Susser, 1991). If an animal develops hepatitis the day *before* taking a drug it is not a good indication that the two are related. Developing hepatitis a few days after a course of treatment might give rise to some suspicion. On the other hand, there is unlikely to be a causal relationship if hepatitis develops months after treatment, although clearly it cannot be totally excluded. It very much depends on the mode of action or mode of adverse action of the drug in question. Some reference to Koch's postulates, formulated in 1891 with regard to the origin of infectious diseases, is both appropriate and useful (Reid, 1997). In fact, to assist in the analysis of causality, a number of relevant criteria can be applied.

Temporal relationships

As suggested above, there should be a plausible relationship in time between a drug exposure and an adverse event. The elapsed time should be realistic for the onset of the adverse event, although, as already indicated, deciding on what might be realistic is not without its own problems. For example, it might be realistic to assume that severe tachycardia followed almost immediately by myocardial infarction was associated with a drug administered to a dog 30 minutes previously. However, development of primary carcinoma of the urinary bladder 1 week after administration of a drug is extremely unlikely to have any causal association (although it might have a diagnostic, non-causal association). However, many adverse reactions, particularly in humans, do have long latency periods and this must not be overlooked for adverse reactions in animals. Examples include the induction of cancers and renal papillary necrosis resulting from the use of certain non-steroidal anti-inflammatory drugs (Stephens, 2000), while teratogenic effects, depending on the species, the timing of organogenesis and the length of gestation, will always be evident after a latency period, and iatrogenic cancers usually after a long latency period.

Dechallenge/rechallenge

If an ongoing adverse reaction ceases on dechallenge, that is on drug withdrawal, in an animal or in a group of animals, it constitutes reasonable evidence that the drug *may* have been responsible for the effects seen. The strength of that evidence increases if the adverse effect reappears on rechallenge (re-administration) and resolves again on subsequent dechallenge. Delayed challenge and reduced dosing may also help to provide further

supporting evidence. However, it must be recognised that challenge–dechallenge–rechallenge are not without ethical considerations. These may be minor in the event of relatively minor suspect adverse reactions, for example a skin rash, but it would be unethical to rechallenge an animal that had suffered hepatic necrosis or severe pulmonary oedema or similar on initial administration. Such considerations must be taken into account when investigating causal relationships between a possible adverse reaction and drug administration.

Anatomical site

If the anatomical site of injury or of an adverse pharmacological effect is in agreement with the known target organs for toxicity or pharmacology, then this is strong supporting evidence for causality. This is particularly relevant for drugs that produce adverse effects at the site of application, for example at injection sites or in the mouth, or close to administration sites such as the oesophagus in the case of oral administration (Venulet *et al.*, 1986). The association of an adverse drug reaction with a particular drug may be strengthened with the knowledge that a drug is distributed to or accumulates in a particular organ or tissue, and, conversely, the association may be weakened by the knowledge that the drug is not distributed to a tissue or organ. However, remote effects must also be considered: for example, cardiovascular collapse due to central nervous system depression, effects mediated by as yet unidentified metabolites or those mediated through the immune system such as toxic epidermal necrolysis.

Time course of the reaction

This may be indicative or characteristic of a reaction associated with a particular drug or class of drugs in a given species or across a range of species. Data from pharmacokinetic and pharmacodynamic studies in laboratory animals, as well as information generated in the target species may be helpful in examining time-course relationships. If the drug is also used in humans, there may be useful contributory data available from this source.

Previous adverse drug reaction

If the drug has resulted in an effect that has previously been reported, and especially if this is in the same species and breed, or it has resulted in similar effects to those caused by another drug in the same class, then this is significant and it increases the possibility and the degree of suspicion that an adverse reaction was caused by the drug. The 'previous' adverse events may have been reported to a regulatory authority, published as a peer-reviewed paper in a scientific journal, or be based on a letter submitted to a periodical.

Type of adverse reaction

Some adverse drug reactions are relatively common conditions *as* adverse drug reactions but not necessarily in terms of incidence or frequency. Examples include toxic epidermal necrolysis, aplastic anaemia and angioedema in humans, and hypersensitivity reactions or vomiting in animals.

Drug–drug interactions

Drug interactions may occur and the chances of their occurrence increase with the numbers of drugs or types of drugs administered. Some drugs may have drug interactions that may characterise the combination and aid in determining causality.

Toxic concentrations

If the concentrations found in blood or plasma are known to be typical of concentrations that are

associated with toxicity, then the plausibility of the argument increases. As toxicity often varies from species to species, this kind of relationship is likely to be more useful when the comparison is made with data obtained from the same species and, where possible, from the same breed. However, species differences must also be considered. For example, concentrations of a drug that may be simply therapeutic in one species may be positively toxic to another. This must be seriously considered with any adverse drug reactions in cats and other felines.

Algorithms for causality

Taken together, the criteria for assessing or establishing causality should amount to a test of biological plausibility that the drug may or may not have been involved in an adverse drug reaction or reactions. In assessing the causality of veterinary drug reactions, the approach described above is an integral and intuitive aspect of the interpretation made by an informed reporter. For human medicines, however, there are a number of rather more formal approaches involving differential diagnosis, probability ratings, decision trees, and methods involving computer algorithms, Bayesian calculations and fuzzy logic reasoning (Naranjo *et al.*, 1990, 1992; Naranjo and Lanctôt, 1991; Lanctôt and Naranjo, 1994, 1995; Stephens, 2000; Sproule *et al.*, 2002).

For veterinary medicines, and arguably for human medicines, some of these methods are probably overly complex and certainly inadequately validated. It is easy to lose sight of biological and medical considerations and to focus on the outcome of the algorithm. Those trying to make the analyses must still consider biological plausibility regardless of whether the outcome is 10, 15 or 22 (whatever the output of the algorithm might be), even if a degree of biological plausibility is built into the algorithm. Algorithms should be considered as useful tools that might point the investigator in a possible direction, rather than definitive solutions governed by a series of numbers and pass or fail points. Nevertheless, some of the methodologies developed for use in human pharmacovigilance may be useful in determining the strength of association rather than the presence or absence of association, and thus may contribute to or complement a more intuitive and scientific component.

A number of relatively simple methods are available for causality assessment of human medicines and, with some adaptation, these can be extended to veterinary medicines. Of these, one of the most readily adaptable is known as RUCAM – the Roussel Uclaf Causality Assessment Method (Bénichou *et al.*, 1993; Danan and Bénichou, 1993; Bénichou and Danan, 1994). This depends on a weighting system based on a series of factors, many of them already mentioned, and it builds on the criteria developed for other approaches (Venulet *et al.*, 1986). It is shown in modified form in *Table 27.2*.

In this system, as extended to potential adverse events in animals, some thoughts have to be given to species and species-specific effects and, perhaps more rarely, to breeds and breed-specific effects, as indicated in *Table 27.2*. The course of the reaction may not be the same from species to species. Collie dogs are more susceptible to the effects of some avermectins so this would probably not lend supporting (or otherwise) evidence for an effect seen in a different breed of dog, although the possibility should not be completely dismissed. Cats and other felines, including lions and lynxes, are unable to conjugate many toxic materials through glucuronidation and are thus more susceptible to the toxicity of specific substances that are detoxified through this pathway in the liver (Burchell and Coughtrie, 1992; Mackenzie *et al.*, 1992; Miners and Mackenzie, 1992). Hence, a lack of toxicity in one species, for example the dog, might not be 'against the role of the drug' as defined in point 2 of *Table 27.2* in another species or even across a range of species.

The main criticism of this scheme concerns the scoring system and, more specifically, how well the score supports causality. The maximum score is 15 so we can now take the example of a

Table 27.2 The RUCAM approach to causality assessment, modified for veterinary purposes (after Danan and Benichou, 1993).

Criteria		*Score*
1. Time to onset		
	Highly suggestive	+3
	Suggestive	+2
	Compatible	+1
	Inconsistent	0
If incompatible, case 'unrelated'		
If data not available, 'insufficiently documented'		
2. Course of the reaction*		
	Highly suggestive	+3
	Suggestive	+2
	Compatible	+1
	Against the role of the drug	–2
	Inconclusive or not available	0
3. Risk factors for drug reaction*		
	Presence	+1 to +2[††]
	Absence	0
4. Concomitant drug(s)**		
	Time to onset incompatible	0
	Time to onset compatible but unknown reaction	–1
	Time to onset compatible and known reaction	–2
	Role proven	–3
	None or no information	0
5. Non-drug related cause(s)**		
	Ruled out	+2
	Possible or not investigated	+1 to –2[†]
	Probable	–3
6. Previous information on drug*		
	Reaction unknown	0
	Reaction published but unlabelled[§]	+1
	Reaction labelled[§]	+2
7. Response to rechallenge		
	Positive	+3
	Compatible	+1
	Negative	–2
	Not available or not interpretable	0
Or plasma concentration of drug known to be toxic*		+3
Or validated laboratory test with high specificity, sensitivity and predictive value*		
	Positive	+3
	Negative	–3
	Not interpretable or not available	0

* In that species and/or breed.
** Sum of negative values cannot be lower than –4.
† Depending on the nature of the reaction.
†† One additional point for each risk factor to maximum +2.
§ Discussed further in section on expectedness.

non-steroidal anti-inflammatory drug given to a dog for musculoskeletal disorders, which 12 hours after the second dose vomits blood. The intuitive response is gastrointestinal bleeding due to the adverse effects of a NSAID, a response well known in the dog and, indeed, in many other species. There is even a label warning regarding the potential for this to happen. Treatment was stopped and the reaction stopped. However, it reoccurred when treatment was again attempted. The scores based on the RUCAM model are as follows for each point:

1.	+3
2.	+3
3.	+1
4.	0
5.	+1
6.	+2
7.	+3
Total	13/15

This is undoubtedly strong support, and so one may be strongly inclined to place this in the 'probable' (A) category – but where does probable end and possible (B) begin, and so on, until we reach 'unlikely' (O)? Is 11 still 'probable' or merely 'possible'? Do other approaches assist or confuse the issues? Consider, for example, if the animal had vomited on rechallenge but without evidence of blood, or even had not vomited at all. Intuitively, one might still have a serious degree of suspicion, but the RUCAM score would be lower.

The method developed by Naranjo and colleagues (Naranjo *et al.*, 1981) is of interest because it too is based on a probability approach, with a consensual, content and concurrent validity. It uses a relatively simple scoring system depending on 'Yes', 'No' or 'Do not know' answers to responses, as shown in *Table 27.3*.

The numbers in italics refer to the example of the NSAID drug in a dog used earlier. Although there is a strong intuitive feeling that the drug caused the gastrointestinal bleeding, the total score is only 5 of a possible total of 13, although this could be increased to 6 if

Table 27.3 The Naranjo approach to causality assessment, modified for veterinary purposes (after Naranjo *et al.*, 1981).

Criteria	*Yes*	*No*	*Do not know*	*Score*
1. Are there previous *conclusive* reports on this reaction in this species?	+1	0	0	*+1*
2. Did the adverse effect appear after the drug was administered?	+2	−1	0	*+2*
3. Did the adverse reaction improve on dechallenge or after the administration of a specific antagonist?	+1	0	0	*+1*
4. Did the adverse reaction reappear on rechallenge?	+2	−1	0	*+2*
5. Are there alternative causes other than the medicine that may have caused the reaction?	−1	+2	0	*−1*
6. Did the reaction reappear when placebo was given?	−1	+1	0	*0*
7. Was the drug detected in blood or other body fluids at concentrations known to be toxic in this species?	+1	0	0	*0*
8. Was the reaction more severe when the dose was increased, or less severe when decreased?	+1	0	0	*0*
9. Did the animal have a similar reaction to the same or similar drugs in any previous exposure?	+1	0	0	*0*
10. Was the adverse reaction confirmed by any objective evidence?	+1	0	0	*0*
			Total score:	*5*

the 'objective evidence' was taken to be the confirmation, rather than (say) biopsy or findings at necropsy.

A further method developed for assessment of human adverse drug reactions, TAIWAN (Triage Application for Imputologists Without an Interesting Name), makes use of assessments based on the answers to a series of questions each of which relate to the usual criteria of causality (Stephens *et al.*, 2000). These questions are associated with scores linked to a likelihood of association:

- A: probable = 2
- B: possible = 1–1.99
- O: unclassified = 0.99

as set out below:

- Was there a known biological association for the adverse event? (yes = 2.5; no, but hypothesis exists = 1.5; no = 1).
- Was there a temporal association between the drug and adverse event? (yes, strong, = 2.5; plausible = 1; weak = 0; no = –2).
- Did the adverse reaction resolve or reduce on drug withdrawal or dose reduction? (yes = 3; partially resolved but not specified = 2; yes, but with treatment = 1; no, natural lesion found = –2; no = –1).
- Did the adverse event reoccur on rechallenge? (yes = 3; no, but adverse event treated (or prevented) = 2; yes, same therapeutic area = 1.5; no = –1).
- Is the adverse event known or expected? (yes, expected and labelled = 2.5; yes, a few publications = 1.5; some spontaneous cases = 1; no = –1).
- Is the adverse event known to occur with intercurrent disease? (yes = 0; rarely = 1.5; no = 2).
- Is the adverse event known to occur with the concomitant drug with a temporal relationship? (yes = 0; rarely (<1 drug) = 1; rarely (1 drug) = 1; no = 2; interaction published or expected = 2.5; interaction hypothetical (i.e. might be reasonably expected on basis of available knowledge) = 1.5).
- Does the patient have any relevant medical history? (yes, significant = 0; yes (adverse event rare) = 1; no = 2).

This too could be adapted for use with animal adverse reactions. It would lead to a strong 'probable' with the example of the non-steroidal anti-inflammatory drug mentioned above and has the attraction of broad categorisation rather than a numerical result which might be difficult to understand.

Another approach to examining adverse reaction causality is to refer to the Bradford-Hill criteria. In the mid-1960s, Sir Austin Bradford-Hill put forward a number of criteria for a causal association in relation to the environment and disease (Bradford-Hill, 1965). There is absolutely no reason why the concept of the environment cannot be extended to cover a drug environment. Indeed, the nine criteria proposed lend themselves well to the concept of causality in epidemiology, pharmacovigilance and pharmacoepidemiology (Shakir and Layton, 2002), and equate to four criteria of enumerative and eliminative induction, deduction and analogy (Vineis, 1991). They are set out in *Table 27.4*.

The application of these criteria, in a reasoned and considered manner and bearing in mind the inherent difficulties in interpretation of causality associations, can strengthen an opinion as to whether or not there is a causal relationship. However, in applying these criteria (or indeed any others, or indeed any algorithm), the general shortcomings of pharmacovigilance data must be considered, especially significant under-reporting, poor quality of data and misclassification (Shakir and Layton, 2002). To these must be added misdiagnosis, lack of reliability and paucity of data particularly with regard to clinical chemistry, biopsy, gross pathology and histopathology, and post-mortem findings.

Seeking absolute causality is a difficult task and frequently the aim of the majority of examples is to make a qualitative determination of probability (Hutchinson and Lane, 1989). For example, a drug may not cause adverse reactions in normal healthy dogs, but it might cause them

Table 27.4 The Bradford-Hill criteria (based on Shakir and Layton, 2002).

Criteria	*Attributes*
Strength	Strong associations are more likely to reflect cause while weak ones are likely to suggest bias
Consistency	Repeated observations of a finding in different populations with different circumstances provide more support for causality
Consistency	A cause leads to a specific effect, not multiple effects, e.g. a drug is responsible for a specific type of tumour, not several types of tumour (although a drug might lead to a number of iatrogenic conditions – hepatic necrosis and nephrotoxicity)
Temporality	The cause must precede the effect – a consistent pattern with exposure followed by effect is highly suggestive of a causal relationship
Biological gradient	Essentially a dose response – the higher the exposure dose, the more likely there will be a response, and the severity will increase with increasing dose
Plausibility	Biological plausibility must be assessed before a causal relationship is claimed. This can be difficult in circumstances where the mechanism of toxicity is unknown, and with type B reactions.
Coherence	The cause and effect explanation where the data should not conflict with what is known about the reaction and its associated biology. However, it is important to recognise that any such assessment depends on current knowledge, and so an apparent lack of coherence may be related to a gap in the knowledge base rather than a conflict with it and this may need further investigation
Experimental evidence	Studies in biological systems will provide evidence (or otherwise) to support a conclusion of causality (or lack of it). It must be emphasised that in any such pursuit, the choice of biological model must be made carefully. For example, a study in geriatric rats to investigate an adverse effect reported in older dogs might be more informative, and ultimately more supportive, than a study in young beagles, if the effect in the older dogs is a true age-related (rather than species-related) effect
Analogy	Bradford-Hill considered that analogies could be helpful, and in pharmacovigilance some support can be obtained by comparing a drug in a class with other drugs in the same class. However, some drugs do provide exceptions and prove to be the only toxic member of the class and so the analogy option needs to be used with care. It might work well with NSAIDs and β-lactam antimicrobials, for example, but not with other drug classes. Furthermore, interspecies analogies might be flawed in some cases, but comparisons might be made between the results of preclinical toxicology studies in laboratory species and the patient species

in elderly dogs or in animals with renal insufficiency, but not in all dogs with renal insufficiency and even with the same degree of renal insufficiency. In other words, there is often a tendency to look for a single cause for what is in effect a complex reality (Gori, 1989). The renal insufficiency is a risk factor rather than a certainty factor, and the causal factor of the drug is better for being viewed as a determinant. In fact, some authorities favour the determinant rather than the causal approach (Susser, 1991; Weed, 1997; Attena, 1999).

All of this is particularly true of spontaneous reaction reporting where, essentially, data acquisition is followed by data assessment and interpretation. These latter processes are often undertaken as part of a two-step process:

1. assessment of each case; and
2. aggregate assessment of a group of cases.

Aggregate assessment carries with it the possibility of making a causal relationship based on a trend examination of a number of reports, rather than trying to assign causality on the basis

of a single report, which in the early days following the launch of a new product might be difficult, if not impossible, particularly if the adverse reaction(s) is not expected. Consequently, experimental or analytical or epidemiological studies may be necessary to confirm the association between administration of a drug and a suspected adverse drug reaction (Meyboom *et al.*, 1997; Meyboom, 1998).

One might question the need for any algorithms or mechanistic criteria for causality assessment, but there are three reasons why they might offer positive benefits.

First, while the NSAID example mentioned above is a 'good' example of an adverse reaction in veterinary medicine, it is poor from the point of view that the effects are well known, well documented and expected, and follow-up and further examination are rare in practice, with the possible exception of fatal cases or where evidence to support litigation is being sought. So, the extra data from blood concentrations and other investigations that might push the total scores towards causality are not available, and hence the scores are relatively low. This could cause an inexperienced investigator to err against an intuitive conclusion, and possibly lead to an opinion that the adverse reaction is unrelated to the drug. On the other hand, for new drugs and vaccines, where signals develop slowly over time and where there may be very little intuitive feel for their biological properties, or at least for their adverse effects, then an algorithmic approach may well provide useful supporting evidence that could tip the conclusion in one direction or another, particularly in those cases where additional clinical and pathological information is available.

The second reason is that an algorithmic approach is already used in the US for the evaluation of adverse reactions to veterinary medicines. The Center for Veterinary Medicine (CVM) employs a method published by Kramer and colleagues in 1979 for the assessment of adverse reactions in human patients, adapted for use with animal patients (Kramer *et al.*, 1979; Bukowski and Wartenberg, 1996). The method uses a number of decision routes or 'axes' for the evaluation process, and each axis bears some resemblance to the RUCAM (Benichou) and Naranjo models discussed above. Thus, these come under the headings of:

- previous experience with the drug;
- alternative aetiological explanations;
- timing of events;
- drug levels;
- evidence for overdose;
- effects of dechallenge and rechallenge.

Movement through each axis, depending on yes, no or do not know answers, leads to the addition or subtraction of points and, where appropriate, movement on to the next axis. The total scores can vary from –7 to +7, and the NSAID example used previously scored +6 even without any adaptations for veterinary use, suggesting a strong association between the drug and the adverse reaction.

The third reason is that in the European Union, the EMEA has introduced a guide to causality assessment to assist in the allocation of A, B, O or N classifications. This was originally issued as a consultation document and adopted by the CVMP in April 2004. This guide is definitely not an algorithm, nor is it intended to be an algorithm. It is a questionnaire, each question within a series of boxes, which leads the investigator towards one of the ABON categories. It shares some of the approaches used by the algorithms in that different answers to the questions lead to other options which increase or decrease the tendency towards one or other categories, but it is not based on a points system and so the ultimate challenge posed by the algorithmic approaches – *What does this number mean?* – does not arise. Helpfully, the EMEA's document comes with some worked examples to provide practical guidance on how the system should be used (EMEA, 2004), and the EMEA is undoubtedly hoping that it will assist in the causality classification. However, it has also indicated that should this approach fail, it will consider introducing an algorithmic approach in the future (Freischem, 2004).

In general, it seems that an intuitive approach, based on expert knowledge of the drug and its properties, may often be the most efficient approach to the ABON classification. Nevertheless, the following factors should always be considered:

- the chronology of administration of the drug, and the intervals between beginning and ending treatment and the onset of the reaction;
- the course of the reaction once the drug has been withdrawn;
- the role of both the drug and diseases in the possible aetiology of the reaction;
- the response to rechallenge with the drug;
- the results of laboratory tests, where available;
- any pre-existing conditions in the animal that was treated, including its age;
- any known adverse reactions in animals with specific conditions or with age;
- previous knowledge of the toxicity or effects of the drug (International Consensus Meeting, 1990).

In human medicine, the opinions of physicians have often been proven to be a better causality assessment tool than some algorithmic methods and in some cases there was little agreement, and in some cases only 6% concurrence (Miremont *et al.*, 1994; Benahmed *et al.*, 2005), although other studies have shown wide disagreement among experts (Arimone *et al.*, 2005). Others have concluded that the routine uses of algorithms may be of little benefit, particularly when there is lack of agreement between approaches used (Louik *et al.*, 1985), although studies of the Naranjo probability approach compared with the Kramer scoring system approach suggest that they are reliable under most conditions and circumstances (Busto *et al.*, 1982), and it may well be that the differences noted in assessment by some observers may be related to investigator effects such as lack of familiarity with the algorithm or even with the drug. The Naranjo approach may lack validity for some types of adverse reaction (García-Cortés *et al.*, 2008).

Furthermore, if the documentation is poor, then over-analysis of individual reports may not be practicable or useful (Fescharek *et al.*, 1996). Their use may also be limited when the available data are restrictive in nature or of poor quality (Liu *et al.*, 2001), and in human medicine, more detailed information is often available from published reports rather than from spontaneous reporting data (Haramburu *et al.*, 1990), and this is likely to be reflected in the veterinary sector.

All of the approaches discussed here incorporate the elements of the Bradford-Hill criteria, and so they should serve as useful tools in the assessment of causality, when used along with judgement, expert knowledge and intuitive thinking. The Bradford-Hill criteria have proved to be useful in assessing the causality of cisapride-induced arrhythmia in humans (Perrio *et al.*, 2007).

Even after all of these considerations, proof of causality is frequently lacking and what remains is a high degree of suspicion, which in itself may be all that is needed for regulatory action such as changes to the terms of a marketing authorisation or even drug withdrawal (Auriche and Loupi, 1993). Moreover, there is often a lack of agreement between the outcomes from several algorithmic approaches (Macedo *et al.*, 2003).

Some of these factors may be explained by the failure of many algorithmic approaches to take into account the relationships with probabilities. This results in approaches that are as limited as those that fail to consider biological plausibility. To address this, a method has been developed that makes use of factors involved with making expert judgements *and* a scientific weighting using multilinear regression so that both biological and statistical components are considered, and this has shown promise as an approach to causality assessment (Arimone *et al.*, 2006).

The Veterinary Medicines Directorate (VMD) in the UK has used the ABON system for a number of years. For reports received in 2002, around 18% of spontaneous reports for adverse reactions to veterinary medicinal products fell into the A category, with approximately 16% in the B category (Knivett, 2003). Only 3% were in

the N category and the vast majority, 63%, were classified as O, insufficient information. Interestingly, the VMD further classifies the O causality reports into B (possibly associated), and then further into B-Factor (other factors played a role such as disease or stress) and B-Multi where an adverse reaction occurs but several products have been used at or around the preceding period.

Finally, the VMD has an Opru category where a spontaneous report is filed in association with a particular drug but where the VMD believes that a co-administered drug is more likely to be the causative agent. In the 2002 period, around 47% of O category reactions were categorised simply as O, with 40% as B-Factor, 13% as B-Multi and 0.1% as Opru. It is unlikely that algorithms could be practicable in defining this degree of categorisation, and judgmental and intuitive decisions are thus a necessary input. Moreover, from an epidemiologic viewpoint, it is important to appreciate that confounding factors may lead to confusion, and that, ignoring the most simple of cases, establishing cause and effect may be very difficult (Maldonado and Greenland, 2002). For example, knowing that an exposure (treatment) has occurred may not be sufficient to associate cause and effect. One may need to know the degree of exposure, the duration of exposure and the nature of the exposed population.

Expectedness

One of the key requirements for pharmacovigilance reporting for most regulatory authorities is the determination of whether or not the adverse reaction was 'expected'. For human medicines, the response to this depends largely on what appears in the company core safety information (CCSI), in current labelling or in the summary of product characteristics (SPC), depending on where the product is marketed (Castle and Phillips, 1996; Brown *et al.*, 2001).

Veterinary medicines lack a formal CCSI, although individual drug sponsors may have an equivalent referred to using another name, and so expectedness is based on the information that appears on the label, in the product literature or in the SPC. In general, an adverse reaction to a veterinary medicine can be regarded as 'expected' if it is noted on the product label or in the SPC. Hence, such reactions are often referred to as 'labelled'. Conversely, 'unexpected' adverse reactions are those that are not described on the label, in the product literature or in the SPC, and are sometimes referred to as unlabelled.

From the above, it is clear that expected adverse reactions are those that have been observed previously. This may have been during clinical trials, in which case they will have been added to the product literature and label at authorisation, or they may have been noted after marketing commenced and added to the literature and label subsequently. So, and by extension, an unexpected adverse reaction is one that has not been seen previously. Thus, the classification as one or the other appears to be straightforward and easy. Nevertheless, confusion can occur, and even with human pharmacovigilance, where arguably there has been significantly more experience than with its veterinary counterpart, confusion can occur (Castle and Phillips, 1996).

Consider the labelled warning 'May cause gastric discomfort' while the spontaneous report has 'Signs of epigastric pain', then this would almost certainly be regarded as 'expected'. However, if the reported event was gastric ulceration, then this would almost certainly not be regarded as being equivalent to gastric discomfort, and it would be classified as 'unexpected'. There are a number of examples in human pharmacovigilance where equivalence, or lack of it, may determine the outcome of 'expectedness' classifications (Castle and Phillips, 1996) and these can serve as illustrations of some of the principles involved which can be applied to veterinary pharmacovigilance:

- Pulmonary fibrosis of the upper left lobe is equivalent to lung fibrosis.
- The labelled warning refers to hepatic necrosis, but biopsy reveals necrosis with eosinophils. This would be regarded as

'expected', as eosinophils would themselves be expected to occur in the presence of necrosis.

- On the other hand, if an additional sign is not associated with a type of adverse reaction, then this would be unexpected; for example, gastric irritation and melaena would not be considered equivalent to irritation.
- However, if an additional sign is usually associated with an adverse reaction then this would be classified as expected even if it is not mentioned in the product literature.
- A fatal outcome is not 'unexpected' even if the fatality is unlabelled, if the adverse event in question is often associated with a fatal outcome, e.g. the labelled adverse reaction is myocardial infarction, and the reported event is death due to myocardial infarction.
- If an adverse reaction is more severe than the product literature suggests, then an 'unexpected' classification is appropriate. For example, the product literature refers to 'elevated hepatic enzymes' and the reported event is hepatic necrosis, or even if the labelled term is hepatic necrosis and the reported effect is 'death due to hepatic necrosis'.
- By contrast, if the effects were less important and severe than the product literature suggests, it would be unlikely to be classified as unexpected. For example, the product literature referred to hepatitis, but raised hepatic enzymes were noted.
- If death is a normal outcome of a condition that was diagnosed prior to treatment (e.g. cancer), then the death itself, while it may certainly be considered as an adverse event, is not an adverse drug reaction, and so requires no classification as expected or unexpected. However, if the condition is exacerbated by the treatment it is an adverse reaction, and if it was not in the product literature then it is unexpected.
- A group of symptoms is observed, which could be related to a specific condition, but the symptoms alone are not definitive of that condition. For example, the product literature refers to wheezing, hypotension and urticaria, and the report refers to anaphylaxis. In this case, wheezing, hypotension and urticaria, although all symptoms of anaphylaxis, cannot be equated to 'anaphylaxis', unless the term appears definitively in the product literature and, under these circumstances, anaphylaxis would be unexpected.
- On the other hand, if anaphylaxis appears in the product literature, then an observation of wheezing, hypotension and urticaria would be regarded as synonymous and therefore expected.
- If the product literature refers to a reaction that is transient, and the report refers to the same condition but it persists, then this too is unexpected.

There are numerous other examples as to what might constitute expected/labelled and unexpected/unlabelled, but very little regulatory guidance. Indeed, there is very little guidance of any description for either human or veterinary medicines. The exception is a publication by Brown and colleagues in 2001 in which she examines some examples, criteria and contingencies drawn from human medicine (Brown *et al.*, 2001), and again these can be adapted for use in veterinary medicine pharmacovigilance. Some suggestions, based closely on Brown's publication, are offered for consideration below and these build upon those already described above.

- **For an adverse reaction to be considered expected, it must be stated unequivocally in the SPC or product literature.** For example, the SPC states that potential adverse effects in the dog are wheezing, skin rash and hypotension and the report states anaphylaxis; the latter would not be regarded as expected in these circumstances and it should not be assumed that the group of signs referred to in the product literature constitute anaphylaxis, or even that they were seen in the same dogs; in clinical trials, some dogs may have experienced wheezing, some may have had hypotension and others may have suffered skin rashes. Hence, anaphylaxis would be regarded as 'unexpected'.

- **If an adverse reaction is listed in the product literature, then it is evident that the signs and symptoms that constitute it are 'expected'.** For example, the product literature refers to anaphylaxis in the dog, and the signs reported are anaphylaxis, with breathing difficulties, hypotension and skin rashes. In this case, the anaphylaxis is expected and the breathing difficulties, hypotension and skin rash are assumed to be part of the associated signs accompanying the condition, and not additional signs. However, if an additional sign not attributable to anaphylaxis is seen, then this is 'unexpected'.

- **The effect is listed in a third country's product literature, but not in the local material.** This should form the basis for discussion with the regulatory authorities. It may, for example, be a disputed effect in the third country, and its appearance in the product literature there may reflect a particular analysis by its regulatory authority that is not shared by other regulatory authorities in other countries. In such cases, the adverse reaction would be regarded as 'unexpected' in the local area, but expected in the country that constitutes the exception.

- **If an adverse reaction occurs with greater specificity than is described in the product literature, then it should be considered unexpected.** For example, if the product literature indicates that the drug may cause birth defects but phocomelia is reported, then this should be considered to be 'unexpected'.

- **Where an adverse reaction occurs with a specific drug but the product literature only refers to class effects, then the reaction is unexpected.** For example, if the SPC warns that 'Non-steroidal anti-inflammatory drugs may cause gastric ulceration in treated dogs' and the reaction occurs following treatment with the specific product, then the reaction should be regarded as unexpected. As a further example, a corticosteroid product available in the UK for use in horses carries several warnings including:
  - Use of the product in horses may induce laminitis . . .
  - Systemic corticosteroids have caused deposition of calcium in the skin (calcinosis cutis)

  Should the first of these occur, it is clearly an expected adverse reaction as it has been seen and reported with the product, whereas should the second occur, it is unexpected as the labelling is class based, and the reaction was reported previously with the corticosteroid class, but not with the actual product.

- **If an adverse reaction is associated in the product literature with a specific species (or breed), then it is expected only when it occurs in that species (or breed).** For example, if the product literature for a product authorised for use in dogs, cats and ferrets warns 'May cause vomiting in dogs', then should this occur in cats it is an unexpected reaction.

- **If an adverse reaction occurs with greater severity than is recorded in the product literature, then this should be considered unexpected.** For example, if a product for use in dogs suggests that hepatitis may occasionally occur, then hepatic necrosis is 'unexpected'.

- **The duration of an adverse reaction, as described in the product literature, is markedly different or, more specifically, is markedly longer.** For example, the product literature describes 'pain lasting a few hours', while the adverse reaction report mentions pain lasting up to a week. This would constitute an unexpected reaction. This type of statement may be difficult to interpret when numbers are replaced with adjectives or adverbs, e.g. transient pain or prolonged inflammation, as these are very subjective, and it is desirable that numbers and units are used where possible (lasting up to an hour, for 2–3 days, etc.) and that product literature

be written in these more specific terms (Nakao and Axelrod, 1983).

- **Lack of efficacy is only 'unexpected' when the product's lack of pharmacological activity is related to an authorised indication.** For example, if the product is authorised for the treatment of respiratory disease caused by *Pasteurella multocida* and *Actinobacillus pleuropneumoniae* in pigs, it is not 'unexpected' when it fails to treat a respiratory infection in pigs caused by *Mycoplasma hyopneumoniae*. If a drug is being used in a clinical trial to develop a new indication, failure to produce efficacy to support that indication is *not* an unexpected adverse reaction.
- **Death is not an adverse reaction term, expected or otherwise; it is the outcome of an adverse reaction.** For example, a dog is given a non-steroidal anti-inflammatory drug and after 2 days of treatment, the dog undergoes a massive gastric haemorrhage and dies. In this case, the adverse reaction is the gastric haemorrhage, the outcome of which is death. Similarly, a cat treated with penicillin experiences a myocardial infarction which proves fatal. Regardless of whether penicillin is the causal agent, the adverse reaction term is myocardial infarction, and the outcome is death. Thus death in itself should never be regarded as an adverse reaction and it should not be reported as such except where 'death' is the only information available.
- **Adverse effects noted in preclinical studies, that is in studies with laboratory animals, are not expected if seen in treated animals in a clinical veterinary setting.** For example, adrenal atrophy occurring in a treated dog is not 'expected' if the only situation where it was previously seen was in a 90-day feeding study in rats. However, it could be considered 'expected' had it been observed in a laboratory study in dogs.

The situations set out above are only meant to serve as general examples and many others can be envisaged. On the other hand, synonyms for adverse reactions are expected. There are no good reasons why environmental effects should not be treated in the same manner as any other adverse effects, and the same principles will apply. Similarly, for violations of MRL values, these would only be regarded as unexpected if the product had been used fully in accordance with the terms of the marketing authorisation. Nor should a causal association be a prerequisite. If the product literature states that an adverse reaction has been noted, 'but no causal relationship established', then if it is noted in a treated animal it is 'expected'.

Discussion

Faced with sick animals which are then treated with veterinary medicinal products, it is not always apparent if any ensuing adverse event is as a result of that animal's pre-existing disease, if it is a new disease unrelated to any drug administration, or if it constitutes an adverse reaction to a medicine. Consequently, the assignment of causality, as required by regulatory authorities, is rarely easy. The difficulties are compounded when faced with a report of what might be an adverse drug reaction rather than the animal itself, and, as a result, being without a full medical history or, indeed, a full knowledge of the course of the events following administration of the drug. Nevertheless, with information on a drug's pharmacological and toxicological profiles, with information on the course of a possible adverse event and information from the product literature and from published sources, it is possible on many occasions to decide if an adverse event is in fact an adverse drug reaction, and, if so, if it was related to a particular product administered to the animal.

A number of factors should be taken into account when such situations are being reviewed and analysed, and these are integral to ascribing causality as required in the EU. In problematic cases, the weight of evidence may be quantified by applying algorithmic approaches originally

developed for human pharmacovigilance purposes, but these may be of limited utility when attempting to decide if a particular numerical score is likely to encourage an assignment of A, B, O or N. Although more complex on first examination, the multiple decision matrix approach used in the USA may be more helpful as it integrates the accepted criteria of causality, which in turn incorporate the classical Bradford-Hill criteria, into the functioning logic of the approach, hence removing problematic barriers of deciding whether or not a particular numerical score is more likely to weigh in favour of A or B, or O or N.

European Union legislation requires (as does other national legislation) that unexpected adverse drug reactions are given a degree of priority in being reported. However, this requires some interpretation of what is and what is not expected. On first examination this too may appear to be relatively straightforward. If a drug reaction is described in the product literature it is expected, and if it is not mentioned, then it is unexpected. However, it is often difficult to reach decisions on this issue in human pharmacovigilance where only one species is being treated, and the adverse reactions mentioned in the product literature apply only to that single species. It is much more difficult for veterinary medicinal products where several species might be indicated in the product literature – is an adverse reaction expected if it is described for one species and occurs in another? When does a set of signs and symptoms constitute a specific adverse reaction?

By applying some simple rules, again initially developed for human pharmacovigilance purposes but modified to take into account multi-species treatments, and even multi-breed treatments, some better idea of whether a specific adverse drug reaction is expected or otherwise can be established. Again this is not entirely an academic pursuit because, and as mentioned above, regulatory authorities, and certainly EU authorities, do expect promptness in the reporting of unexpected adverse drug reactions, in accordance with the requirements of the legislation, so-called expedited reporting (Sachot, 2002).

It is also important to consider medical terminologies used in both product literature and in the reporting of adverse reactions. Lack of standardisation of terminology, imprecise use of terminology and misuse or abuse of synonyms can lead to confusion both in terms of ascribing causality and in the interpretation of 'expectedness' (Anonymous, 1990). All approaches to causality assessment must be treated with a degree of caution. In the field of human pharmacovigilance, it has recently been concluded that there is still no fully accepted algorithm for causality assessment (Agbabiaka *et al.*, 2008), and any method employed should endeavour to ensure that clinical and biological plausibility are fully taken into account.

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28 Quantitative aspects of veterinary pharmacovigilance

K.N. Woodward

Introduction

The quantitative handling and treatment of pharmacovigilance data, including veterinary pharmacovigilance data, is a crucial part of pharmacovigilance analysis and reporting, and particularly in reporting to regulatory authorities. However, in the veterinary sector, it is poorly developed and may be considered to be in the development phase of growth and use.

The reasons for this are probably manifold, but they include the relative youth of veterinary sector pharmacovigilance compared with its human medicine counterpart and the availability of resources to lead developmental methodologies. Fundamental research, including statistical and other quantitative aspects of human pharmacovigilance, is vibrant as evidenced by the nature and frequent appearance of related articles in specialist human medicine and pharmacovigilance journals such as *Pharmacoepidemiology and Drug Safety*, the *European Journal of Clinical Pharmacology* and *Drug Safety*, not to mention the numerous articles that appear in either less specialised journals or in journals whose area of specialisation is one other than pharmacovigilance, for examples journals dealing with oncology, dermatology or cardiology. There are currently no dedicated veterinary pharmacovigilance journals, and veterinary pharmacovigilance papers, including case reports and related articles, tend to appear in veterinary journals with a more general topic coverage. However, even here, the number of articles dealing with quantitative aspects of veterinary pharmacovigilance is surprisingly small. Those interested in this aspect have to rely on knowledge and techniques derived from the human sector.

This situation is not altogether surprising. The available information suggests that the number of adverse reactions associated with human medicines reduces those observed with veterinary medicines almost to the point of insignificance. A periodic safety update report (PSUR) for a veterinary drug might have tens or possibly low hundreds of adverse drug reaction reports; a corresponding human drug PSUR might contain thousands of reports. For example, in the period 1986–2001, the French Pharmacovigilance System for human medicines received 197,580 adverse reaction reports (Thiessard *et al*., 2005), which one can average (incorrectly as it happens as the trend over that period was a linear increase) to approximately 12,400 reports a year for a single species, humans. Compare this with the total of 7,096 adverse reaction reports reported to the UK's

Veterinary Medicines Directorate (VMD) over the period 1991–2002 (Woodward, 2005), or 650 per year across seven species (dogs, cats, horses, cattle, sheep, pigs and fish), with the majority occurring in two species (dogs and cats), and the discrepancy in terms of sheer numbers can be easily seen and the pattern is repeated across most other countries.

Consequently and evidently there might appear to be more of a pressing need to marshal and analyse pharmacovigilance data from human adverse drug reporting systems than from the veterinary drug reporting. Nonetheless, this should not prevent logical attempts to analyse veterinary data where this is required and where it makes sense, which is often within a regulatory reporting context. To this extent there are four areas where analysis may be useful:

1. signal detection;
2. incidence calculation;
3. data mining;
4. benefit : risk analysis.

All of these will be discussed in turn during the course of this chapter, while some aspects have already been reviewed in Chapter 13.

Signal detection

Following the launch of a new drug, there will be an initial surge in adverse reactions noted and reported, and even with a new indication for an existing drug, or even a new presentation of an existing drug, there will more than likely be an increase in adverse drug reactions reported.

The purpose of signal recognition is two-fold – to recognise when this is occurring (it may be regarded as too late if it has occurred to any great extent) and, if necessary, to do something remedial about it (Stephens, 2000; Shakir, 2007). In veterinary medicine it is also likely that a new signal will begin to occur when an additional species is added to the label of a product already authorised in another or in other species. Monitoring the trend or trends in these signals is important if the adverse reaction profile of the drug is to be known, if not understood, and if precipitant or unexpected regulatory action from a government agency is to be avoided. So, how does a company (or an agency) monitor signal emergence and development?

The essence of signal detection and monitoring is regular observation (Waller and Lee, 1999; Hauben and Zhou, 2003; Evans, 2007; Singh and Trivedi, 2008). There is little point in examining the adverse reaction record for a new drug or addition of a new species 1 year after launch only to discover an alarming number of adverse drug reactions (although a regulatory authority may have already raised the issue by the time that this happens). It is essential that the numbers and nature of adverse drug reactions received after the launch of a new product or the addition of a new species or, indeed, the implementation of any major change are monitored on a regular basis, even if this is weekly at first. As the real incidence becomes apparent, and depending on the severity of the adverse reactions, and whether or not these were expected, then the frequency may be reduced or, rarely, if the data give rise to specific concerns, maintained or even increased. The periodic safety update reports serve a number of purposes, but an important function is the identification of trends, including the detection and development of emerging signals (Klepper, 2004; Verpillat and Toumi, 2007).

Signal detection may need to be, and indeed should be, tailored according to the nature of any adverse reactions seen. For example, unlabelled (unexpected) severe reactions will deserve more attention than mild, expected reactions. However, even when reactions are expected, and regardless of being mild, severe or otherwise, they should be monitored for their frequency, particularly when a specific rate appears in the product literature. This is not a major issue if the frequency is less than stated (although this may have commercial value if the evidence suggests that the product literature should be amended to reflect a lower incidence than the label and product literature suggests), but it can have significant regulatory and commercial impact if the incidence is

greater than claimed, and if the state of affairs persists, regulatory action can be expected.

These regular reviews of trends should be structured. The incidence, severity, nature, degree of expectedness, species specificity and seriousness should all be taken into account, preferably by reviewing line listings and causality and through examining case reports. Depending on the nature of the events reported, they should be reviewed by veterinarians, pharmacovigilance experts, pharmacists, experienced pathologists and toxicologists so that the biological and medical relevance and causality associations of the adverse reactions can be assessed.

However, this does raise the issue of what constitutes a signal, or at least a genuine valid signal. For human medicines, the World Health Organisation (WHO) suggests three index cases, where an index case is one that contains information on 11 key areas (Edwards *et al*., 1990). For veterinary medicinal products, these criteria should be extended to 14 to include species, breed and numbers treated and reacting (for flock or herd treatments) to give a number of criteria, as shown below:

1. Species
2. Breed
3. Source of case data
4. Case identification
5. Description of the reaction
6. Name of veterinary medicinal product
7. Treatment dates
8. Reaction dates
9. Numbers treated/numbers reacting
10. Age
11. Gender
12. All concomitant drugs and dates
13. Indication
14. Eventual outcome.

By extension from the human criteria, the first nine items would constitute a 'feasible' case if they occur together, while if the remaining five items also occur, the case would be regarded as 'substantial'. An index case would then be determined by a combination of feasible and substantial criteria. For the purposes of veterinary pharmacovigilance, an index case would consist of two cases with substantial ranking or four feasible with feasible criteria.

Unfortunately, the manual and periodic review of data as described above can be, and frequently is, marred by under-reporting, incomplete data and variations in human judgment (Hauben and Zhou, 2003). To avoid these pitfalls, and to facilitate the signal detection process, automated signal recognition systems have been used in human pharmacovigilance. These are based on statistical models and fall into two broad categories: numerator and denominator-based methods. Denominator methods generally use drug exposure estimates and changes with time in reporting rates or frequency of reports as a fundamental approach. Numerator-based methodologies are frequently more complex and include proportional reporting ratios and Bayesian data mining techniques. One of the common aspects of these is how the observed frequencies of adverse reactions differ from the expected rates (Tubert and Bégaud, 1991; Meyboom *et al*., 1997; Collet *et al*., 2000; Bate *et al*., 2002a, b; Hauben, 2003; Hauben and Zhou, 2003; Chan and Hauben, 2005; Hauben and Reich, 2005; Roux *et al*., 2005).

All of these methods have applicability to veterinary medicine pharmacovigilance and, indeed, some commercially available database products now have this capability. However, it must be recognised that automatic signal recognition methods are not infallible, and they may be affected by excessive signal detection, lack of recognition due to failures to use standardised reporting and reporting terms (although this is less critical with the adoption of more and more standardised terminologies and dictionaries in both the human and veterinary fields), and providing sufficient data to demonstrate the validity for the predictive value of information derived from data mining (Waller *et al*., 2005; Henegar *et al*., 2006; Stephenson and Hauben, 2007). Moreover, problems may arise with small databases, and signal detection is likely to improve as the database size increases (Hammond *et al*., 2007).

There is another problem. This is what is known as the Weber effect or the Weber curve (see also

Chapter 13). Weber noted that soon after the introduction of a new human drug, there is a rise in the number of adverse drug reactions which then peaks, usually in the second year of sales, and falls away, despite continued increases in sales and prescribing (Weber, 1984, 1986; Hartnell *et al*., 2003). This is not restricted to new products. It occurred when an omeprazole formulation used in human medicine was removed from the Dutch market and replaced, with much publicity, with an alternative formulation (de Graaf *et al*., 2003). It has also been noted with non-steroidal anti-inflammatory drugs for use in human medicine in the UK (Hartnell and Wilson, 2004) and among a range of human drugs in France, the UK and the USA (Sachs and Bortnichak, 1986; Haramburu *et al*., 1997; Ajayi *et al*., 2000), although it is by no means universal. An illustration of a Weber curve is shown in *Figure 28.1*. A word of caution is necessary here. Not all adverse drug reactions may give rise to a classical Weber effect even though a rise in the number of reports may occur shortly after marketing begins or a new indication is added (McAdams *et al*., 2008).

Verbal communications and experience suggest that it is also frequently seen in veterinary medicine, and in both human and veterinary pharmacovigilance it is frequently related to a number of factors including heightened initial awareness due to a new drug or class of drug becoming available, heightened interest in a new drug's clinical performance and expectations, and any degree of controversy associated with the product (as with the Dutch omeprazole experience) followed later by a loss of interest as familiarity with the drug's adverse reaction profile grows. Those involved in pharmacovigilance activities should be aware of the Weber effect, and should even anticipate it when any degree of novelty is introduced. However, and as always, such a peak should not be dismissed as 'just the Weber effect'. Other more sinister explanations (the drug really does have a problem) should be considered!

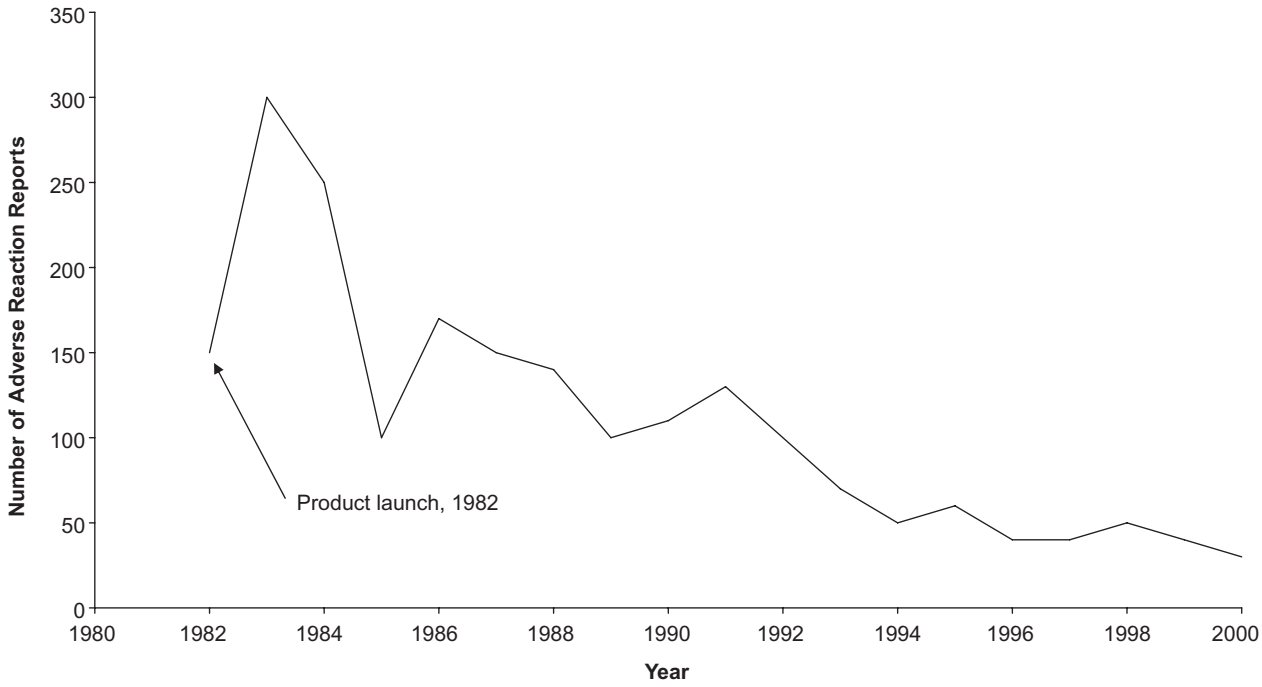

Fig. 28.1 Idealised Weber curve for adverse reactions to a hypothetical canine non-steroidal anti-inflammatory drug.

Calculation of incidence

The title of this section is accurate to a degree. Calculations are involved and the results of those calculations produce values that approximate to incidence criteria. However, when one considers the vagaries normally encountered in pharmacovigilance activities, as discussed elsewhere in this book (see Chapters 6, 10, 11 and 12), including (but not limited to) under-reporting, incomplete reporting, difficulties in diagnosis, doubts over causality, cause of death and concomitant drug use and drug–drug interactions, it is clear that 'incidence' signifies a degree of sophistication that frequently does not exist and might be better replaced with 'occurrence', while 'calculation' would be better replaced by 'estimation'. Nevertheless, calculation of incidence is a frequently employed term and one that will be used here. It is generally required by regulatory authorities for inclusion in PSURs and the value most frequently used is the crude rate.

Crude rate

This is the simplest index used to summarise the occurrence of adverse drug reactions and is essentially the number of patients reacting divided by the number of treated patients, expressed as a percentage (Chuang-Stein, 2000). It is employed in or required by most veterinary pharmaceutical reporting systems, but it has several shortcomings, which have already been alluded to.

The two main defects associated with this calculation are concerns arising from the degree of under-reporting of adverse drug reactions and the other weaknesses of pharmacovigilance reporting mentioned earlier, and doubts over the total number of patients treated (Bégaud, 2007). The latter is usually derived from sales data. Sales data themselves may not be quite temporal with the adverse reaction data. For example, for a hypothetical drug, adverse reaction data may be available for a period January to the present which we will assume is December, but the sales data may cover a slightly different period because, although the sales may have been made between those dates, the actual product sold and accounted for in those sales figures may have been stored at a wholesaler's or on the veterinarians' own shelves. Hence, and inevitably, there will always be a slight mismatch, although this will 'average out' to some extent over time.

The standard calculation, shown below, is currently the most practicable approach available and has the most utility when comparing data, but it should be recognised that obtaining reliable data on sales for a particular product, or even estimating total veterinary drug usage, is fraught with difficulties (Kools *et al*., 2008):

$$\text{Incidence (\%)} = \frac{\text{Number of animals reacting}}{\text{Number of animals treated}} \times 100$$

It is possible to calculate 95% confidence intervals for the true but unknown crude incidence rate by normal approximation or by what is known as the rule of 3, which indicates that if there are no events of a given type in $3n$ patients treated with the drug, then the one-sided 95% confidence limit derived for the crude incidence rate is $1/n$ (Chuang-Stein, 2000). Whether or not this has any real utility remains unclear in view of the limitations already discussed.

Refinement of the crude rate

There are a number of ways that the crude rate can be refined. In most countries it is expressed as a national value. However, there is no reason (other than practical ones such as obtaining specific sales data) why it cannot be broken down further into a regional or state crude rate value to allow for comparisons. For example, in PSURs prepared for the EU, it is common for the crude rate to be expressed for each individual EU member country where the product is sold and as a pan-EU total. These values might then be compared with rest-of-world figures, or even with individual third-country crude rates.

Where the product is marketed individually for different species, then the crude rate for each species can be estimated to determine if the product has species-specific problems. However, this approach is sometimes complicated by the fact that products are frequently marketed for more than one species in the same presentation (e.g. cattle and pigs; dogs and cats) and so individual sales figures are not available, or the product may be supplied for multi-species use in one region or country and for individual species use in another. However, the latter situation does allow the calculation of species ratios from those countries where individual species are indicated, thus allowing for extrapolation for those countries where they are not.

The simple calculation shown above makes it clear that it is the number of animals reacting that forms the basis of the estimate rather than the number of adverse reactions. There are two reasons for this:

1. A single veterinary adverse reaction report may include several animals and for intensive poultry farming or aquaculture, for instance, hundreds or occasionally thousands.
2. The categories of adverse reaction need to be singled out. For example, adverse reactions in treated animals should be handled independently from those occurring in exposed humans, and in countries or regions where other issues constitute pharmacovigilance activities, then these too need to be separated. Thus, in the EU, environmental incidents and maximum residue limit violations should be estimated separately, where they occur.

Care too should be taken when dealing with products with global brand names as they may have similar formulations but not necessarily identical ones. This may not be a problem if a series of adverse reactions is due to a common active ingredient or excipient, but it can (and will) be problematic when it is formulation-related or due to an excipient used in one geographical region but not in another. Problems may also arise with vaccines that at first appear to be identical in different regions and may even share a common name, but on closer consideration contain region-specific antigens such as organism strains or serotypes. Such products should not automatically be considered to be identical and treated together in a crude rate calculation.

With many toxic insults, the critical factor is duration of dosing rather than dosing per se. This may be because the drug accumulates or because the damage done by the drug is cumulative, or for a variety of other reasons. Hence, there may be no or very few adverse reactions noted in patients given a single dose or short course of treatment with a specific product, but they may be seen or increase in frequency with longer periods of administration.

Consider the case of a hypothetical drug used in dogs. Myocardial infarction has been shown to be an adverse drug reaction in treated animals, but only in dogs given the drug for longer than 30 days. In a group of 700,000 treated animals there were 300 cases of myocardial infarction. Hence, the crude rate is $300/700{,}000 \times 100 = 0.042\%$. However, only 100,000 dogs were considered to be at risk because the remainder were treated for substantially less than 30 days. Now, the adjusted rate = $300/100{,}000 \times 100 = 0.3\%$. Alternatively, if the at-risk 100,000 dogs were treated for 60 days, then this equates to 600,000 patient days or 1,644 patient years. The incidence rate for myocardial infarction is therefore $300/1{,}644 \times 1{,}000 = 183$ cases per 1,000 patient years. This gives a better assessment of, and clearer feeling for, major irreversible effects including deaths, and is therefore an alternative to concentrating on the crude rate alone, *if there are* adequate data to allow for this analysis.

These are not the only methods available for comparing rates, occurrence and frequencies, but they have the most utility. Other methods include the life table estimates which are useful in generating a hazard versus risk function, and which provide some predictive power for future events (Abt *et al*., 1989; Salsburg, 1993) and calculations of potential recurrence rates within certain time frames (Tremmel, 1996). Although these are used

to an extent in human pharmacovigilance, they probably have less value in the veterinary sector.

Data mining

Data mining is a tool devised to extract useful information, whatever that might be, from massive data sets (Smyth, 2000; Hand, 2007). As noted earlier, in veterinary pharmacovigilance, and unlike human pharmacovigilance, it is rare to be faced with enormous arrays of data, but those rare occasions may result in a requirement for a data searching or knowledge discovery technique. Moreover, the value of data mining in pharmacovigilance is evident because previously unidentified or unexpected signals are unlikely to be uncovered by relatively simple database searches (Bate and Edwards, 2006). It is extensively used in human pharmacovigilance, and especially in international databases such as those operated by the WHO (Bate *et al*., 1998), or where investigators wish to determine the role of different but interrelated factors in the occurrence of adverse drug reactions (Cerrito, 2001).

The mathematics behind data-mining techniques are relatively simple, but, nonetheless, such discussion is beyond the scope of this chapter. Suffice to say that the major approaches are:

- the proportional reporting ratio (PRR);
- the reporting odds ratio (ROR);
- Bayesian and empirical Bayesian methods (Almenoff *et al*., 2005).

All of these methodologies attempt to identify statistical associations between medicinal products and the occurrence of adverse reactions (or other events) contained in databases. In general, the methods make use of automated and computer-based approaches using commercially available software (Zupan *et al*., 1999; Bate *et al*., 2002a, b; Almenoff *et al*., 2005; Bate and Edwards, 2006). These approaches are not restricted to pharmacovigilance and they have found applicability in other areas of medicine and biomedical sciences such as toxicology (Zupan *et al*., 1999; Helma *et al*., 2000; Helma, 2004). The majority of these data-mining approaches employ variants of disproportionality analysis, that is, methods that estimate or identify the disproportionality in reporting between drug-adverse event pairs that occur in databases at higher frequencies than are actually expected (Almenoff *et al*., 2003). They are also useful in signal detection and in identifying drug–drug interactions (Lindquist *et al*., 2000; Bate *et al*., 2002b; Gould, 2003; Hauben, 2003; Hauben and Zhou, 2003).

Of the techniques currently available in human pharmacovigilance, Bayesian methods have found the most utility. These are based on Bayes' Theorem, or more correctly on variants or special forms of Bayes' Theorem, a statistical concept developed in the mid-eighteenth century by Thomas Bayes, a Presbyterian clergyman. Simplistically, approaches based on Bayes' Theorem attempt to establish a relationship between the prior probabilities of a symptom and of a disease, and the probability of the symptom in the presence of the disease or in pharmacovigilance terms and, for example, the same relationships for exposure and adverse reaction or drug–drug interactions (Morgan, 1988; Szarfman *et al*., 2004; Almenoff *et al*., 2005; Berry, 2005; Connor and Berry, 2005; Goodman, 2005; Hauben, 2003, 2004; Louis, 2005). Frequently, the models are built into specifically designed systems in networks such as WHO's neural network (Lindquist *et al*., 2000; Goldstein *et al*., 2002; Gould, 2003; Lee and Abbott, 2003; Wilson *et al*., 2003).

It should be emphasised that these data-mining techniques are specialist tools which offer enormous investigative potential in pharmacovigilance, whatever its area of speciality. However, it is an area where expertise is required for execution, analysis and interpretation, and on occasions it may be misleading and different commercial applications may give divergent results (Lilienfeld, 2004; Hauben *et al*., 2007).

Data mining can have specific uses in identifying signals and emerging signals. One approach is the use of the EBGM or Empirical Bayesian

Geometric Mean which involves a technique known as Bayesian shrinkage (see also Chapter 12). This involves a prior knowledge combined in a prior distribution with the actual data generated from adverse event reporting. The EBGM is a measure of association between a drug and adverse drug reactions and it is a useful tool in signal analysis (Hauben *et al*., 2005; Szarfman *et al*., 2006; Robinson *et al*., 2008). However, it is not without problems, especially where co-administration of drugs might lead to confusion over causality (Hauben *et al*., 2005) and the phenomenon of 'phantom ships'. The latter is a situation where a credible association between an adverse event and drug administration can be shown to be erroneous. Examples in human pharmacovigilance include diazepam and cleft lip/palate, levodopa and malignant melanoma, and simvastatin and cataract. Such associations can be avoided or their frequency reduced by combining statistical methodologies with clinical review, epidemiological evidence and pharmacological and toxicological data (Trontell, 2004; Hauben *et al*., 2006a, b).

Benefit : risk analysis

Benefit : risk analysis, under one name or another, is an integral part of human and veterinary pharmacovigilance. In the European Union, the term appears in several places in the veterinary legislation. For example, in Directive 2001/82/EC as amended by Directive 2004/28/EC, the preamble requires that the assessment of safety, quality and efficacy should allow for the benefit : risk balance to be evaluated prior to a marketing authorisation being granted, and 'at any other time the competent authority deems this appropriate'. Article 26 allows for competent authorities to request data permitting the 'continuous assessment of the risk-benefit balance', while Article 28, which deals with renewal of marketing authorisations, requires a 're-evaluation of the risk-benefit balance'. More specific to pharmacovigilance, at least in legislative terms, Article 74 requires marketing authorisation holders to provide data for the 'evaluation of the benefits and risks', including any information generated from post-marketing surveillance studies, while Article 75 demands that periodic safety update reports 'shall include a scientific evaluation of the risk-benefit balance of the veterinary medicinal product'. Finally, Articles 83 and 84 make it clear that European Union member states must withdraw, suspend, revoke or vary marketing authorisations if 'the risk-benefit assessment of the veterinary medicinal product is, under the authorised terms of use, unfavourable', particularly in terms of animal welfare and consumer safety. These requirements are mirrored in those of Regulation (EC) No. 726/2004 governing the operation of the European Medicines Agency and the centralised procedure. So what exactly does benefit : risk analysis or benefit : risk balance mean and does it imply a quantitative aspect?

Clearly, the term indicates or suggests a comparison of the risks of the use of the medicine with its therapeutic or other clinical benefits. However, there are difficulties in assessing therapeutic benefit, and even greater ones in comparing risks and benefits, or at least in establishing a balance (Miller, 1993; Edwards and Hugman, 1997; Simon, 2002; Holden, 2003; Hirst *et al*., 2006; Califf, 2007). If there are difficulties in assessing benefit, then, clearly, this makes benefit : risk assessment problematic.

These issues have been debated for some time in the field of human medicines, where it is recognised that this has a multifactorial dimension. For example, it is difficult to express benefits quantitatively, or to compare them with the benefits of other medicines or treatments (Edwards and Hugman, 1997; Simon, 2002; Breckenridge, 2003; Califf, 2007). Where quantitative data are available, for example from clinical trials, different audiences are likely to interpret this information differently. This may be problematic when one explanation is given by a drug manufacturer and another by a regulatory authority. It also gives rise to confusion and distrust when industry and government agencies attempt to convince

the public that the benefits of a drug far outweigh any risks, and while this may be comprehended, at least in part, for some classes of drug, such as the side effects of many antineoplastic agents used in oncology, it is less well understood for drugs intended to treat less serious conditions. This becomes even more critical if an effective alternative treatment is already available, especially if this is seen to have, or perceived to have, a better safety profile.

Adverse drug reaction reporting in human pharmacovigilance can be influenced by a number of factors including personal attitudes, reimbursement policies and prior knowledge of adverse drug reactions (Bateman *et al*., 1992; Miller, 1993; Belton *et al*., 1995; Cosentino *et al*., 1997, 1999, 2001; Edwards and Hugman, 1997; Bouvy and Egberts, 2000; Simon, 2002; Califf, 2007). There are also differences in perception of risk among health professionals and between health professionals and the public (Sweis and Wong, 2000; Bongard *et al*., 2002; Abraham, 2003). There are no reasons to suspect that veterinary pharmacovigilance is unaffected, particularly as some of these aspects can best be ascribed to human nature.

If it is difficult to identify benefits and risk, then clearly, assessing a benefit : risk balance is even more problematic. However, there have been a number of attempts in the human medicine field. One of these uses standard comparisons of epidemiological indices, such as standard mortality ratios (SMRs) for fatal outcomes or standard incidence ratios for others (Oscar and Lapeyre-Mestre, 2002). Another, compares the number needed to treat (NNT, the inverse of the absolute risk reduction brought about by treatment with a medicinal product) with the number needed to harm (NNH, a function of the number of patients with adverse reactions in the treated *and* untreated groups) (Holden, 2003). Both methods have the advantage of giving a quantitative description of the overall adverse reaction outcome as a function of the number treated and the number benefiting from treatment. However, both suffer from not considering either the nature of the adverse reaction – was it serious or otherwise, or what the patient (or patient owner) might be prepared to endure to ensure a favourable outcome for the disease.

Attempts have been made to overcome this in human pharmacovigilance, for example by taking into account patient preferences, but this is clearly not an option for veterinary patients, although choice can be made, particularly for companion animals, by the owner. However, even then, these are difficult to incorporate into calculations or to reflect in quantitative estimations and this is further complicated by the need for this information to be reviewed by the regulatory authorities.

Other methods developed for use in human pharmacovigilance have many of the same shortcomings, as demonstrated by the report of the Council for International Organizations of Medical Sciences (CIOMS, 1998). This document recognises many of the problems associated with benefit : risk analysis and, although methods recommended for analysis can relatively easily be extended to the veterinary arena, they carry so many shortcomings that one has to question whether this is worthwhile.

For most purposes, benefit : risk analysis is quantitative, subjective and narrative in nature and requires the nature of the disease and the availability and risks (and benefits) of other therapies to be considered (Girard, 1988). Quantitative approaches to benefit : risk assessment are in their infancy with human medicines and rely on some form of decision analysis (Hughes *et al*., 2007; Mussen *et al*., 2007a, b). In view of their experimental nature, these approaches are currently not appropriate to veterinary medicinal products but should be borne in mind for future development.

For new medicines, risk management plans should be considered and introduced where thought necessary, particularly where labelling and product information may not fully reflect all the associated hazards, to allow regulatory authorities and manufacturers to monitor and, where necessary, to minimise risks (Haas, 2004; Hirst *et al*., 2006; Bull, 2007; Raine, 2007).

Conclusions

It is a fact of life that the majority of medicines, veterinary and human, are associated with a range of adverse reactions, which may be minor and common or rare and serious (Meyboom and Egberts, 1999). Some may have an aspect of both. Developing a mathematical ranking approach may ultimately prove to be of limited utility, if it has any value whatsoever. The overall safety assessment will depend on:

- the nature, severity and frequency of adverse reactions;
- the population of animals affected (all, neonates, geriatric, those with renal, hepatic or other disease, species, breed, gender);
- the disease being treated (trivial, minor, severe, life-threatening);
- the availability of other pharmacological or non-pharmacological (e.g. surgery, radiotherapy) interventions;
- the development of disease resistance;
- from an owner's point of view, the costs (financial and, in many cases, emotional) of further and future treatments.

When the latter becomes an overwhelming factor, euthanasia may become the only viable option, especially for animals with severe or chronic disease states such as cancer and osteoarthritis.

Much of what will be tolerated by way of adverse reactions will depend on the attitude and emotions of the owner, or, in the case of food animals, on their current and future economic value versus the costs of treatment.

To a large extent this brings the argument back to the crude rate or to variations of the crude rate discussed earlier. Although the crude rate is a measure of incidence, with all of its drawbacks and limitations, most being due to poor reporting rates, it is also an approximate measure of risk or a proxy for risk assessment. If the risk defined by clinical trials is found to significantly increase with subsequent marketing and pharmacovigilance activities, and notably with the submission of periodic safety update reports, either in terms of frequency of known and expected adverse reactions or through the appearance of new ones, then one must assume that the risk has shifted and indeed increased. Of course this highlights a further problem in not being able to quantify benefit, for just as the risk may have increased, so might efficacy have increased over and above that suggested by the original (and usually limited) clinical trials. So, for most practical purposes, a statement of the type 'the incidence and severity of adverse reactions has not increased since the marketing authorisation/licence/approval was granted (or since the last periodic safety update report was submitted)' may be sufficient along with a qualitative narrative description of benefits and risks as recently published for some human biological products (Imperato *et al*., 2004).

These conclusions support the view that determination of the crude rate (or one of its variants) is the most important quantitative determination currently employed in veterinary pharmacovigilance, and this can be important as an approximate determinant of benefit : risk, despite the difficulties involved in estimating benefit quantitatively. More complex approaches in pharmacovigilance, including data mining, may have greater applicability in the future, but currently there is no overwhelming need.

As with human medicines, perhaps the most important future area for focus with regard to benefits and risks is convincing the patients' owners, whether these are members of the pet-owning public or farmers and breeders, of the benefits of the medicines used, and of the potential hazards and the associated risks related to their use (Breckenridge, 2003).

The European Medicines Agency (EMEA) has recently addressed the issue of benefit and risk through a draft guideline issued for consultation in September 2007 for adoption during 2008 (European Medicines Agency, 2007). This will eventually form part of Volume 9B (see Chapter 2). The EMEA document envisages that benefit : risk assessment has an almost cyclical process featuring hazard evaluation and risk assessment

balanced against the benefit of the drug in question. Although the document offers useful advice on all areas associated with this somewhat complex issue, it makes no mention of quantitative approaches; the approach is very much qualitative and narrative.

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29 Veterinary adverse reactions and crisis management

K.N. Woodward

Introduction

The excitement and expectancy that accompany and follow the launch of a newly authorised medicinal product are wholly understandable in view of the effort in terms of financial investment and hours of research, regulatory and marketing endeavour that has led up to this position. Marketing plans have been devised and an all-out effort is made to penetrate the market place and gain market share from competitor products. There is nothing worse at this stage soon after launch than an event or events that will detract from the efforts of gaining sales and there is probably nothing worse for a new product in this respect than a steadily building catalogue of adverse drug reactions.

Following the launch of a new veterinary medicinal product, pharmacovigilance feedback, in the form of spontaneous reports, is brought to the attention of both the regulatory authorities and the sponsor company. This information will reveal a number of issues. It will usually confirm those adverse drug reactions that are already known – expected or labelled adverse drug reactions – and it may reveal new ones arising from misuse or abuse of the product. It may also identify other adverse drug reactions, for example those associated with overdose or from dosing durations exceeding those recommended in the product literature, for example from off-label use. Included in this category will be adverse reactions arising when animals not indicated on the product label are treated with the product. In other words, cross-species differences in sensitivity or toxicity may become evident as a result of off-label use.

However, there will frequently be reports of unexpected, unlabelled reactions or cases where the severity of an expected reaction is more severe than that expected. These may arise because they are relatively rare and were not seen in clinical trials because of the small exposed populations normally employed. Or they may arise because the clinical trial populations are not wholly representative of the real animal patient being treated. For example, because of the way that patients are selected for clinical trials, they may well be relatively young and healthy apart from the disease which is the subject of the trial.

Once authorised, the drug may be used in a more diverse population including neonates and juveniles, elderly animals, breeding or pregnant animals (which may have been excluded from the trials), those with other medical conditions including those that might alter the

pharmacokinetics (and so possibly alter the toxicological behaviour) of the drug, e.g. hepatic and renal disease or age-related organ insufficiency, and those being given other medications which could lead to drug–drug interactions. This latter aspect may become even more serious when the attending veterinarian is unaware of such treatments and particularly so if the animal owner is also using unlicensed drugs or, for example, complementary medicines such as herbal remedies.

These 'new' adverse drug reactions might not be serious or of sufficient frequency to be an animal welfare concern. They might eventually lead to minor changes and amendments to the Summary of Product Characteristics (SPC), product literature and label if they are of sufficient concern to the company or to regulatory authorities. However, it must be recognised that any new adverse drug reactions could be serious or even life-threatening, and possibly of sufficient frequency to cause major animal health concerns. In these cases regulatory action can be anticipated and indeed expected. In fact, the drug sponsor must be prepared to initiate regulatory action by taking its own regulatory actions.

Historical experience with a number of human pharmaceutical products has demonstrated, sometimes disastrously, that expected adverse drug reactions may be much more severe than those noted in clinical trials, or their frequency might be much greater than expected, or unexpected reactions may occur which are frequent, severe or irreversible. There are a number of examples of this, including temofloxacin, fenfluramine/dexafluramine, terfenadine, seldane, benoxaprofen and zimeldine (Gad and Chengelis, 2001; Wysowski and Swartz, 2005; Cranney *et al*., 2007; Moore *et al*., 2007; Peck, 2007). Many of these episodes have resulted in harm to patients, withdrawal of the drug, and loss of revenue, adverse publicity and long-lasting harm from lack of trust for the companies involved. They can and do have major adverse consequences for the stock price and for investors. Some of these effects could have been prevented or ameliorated had the companies involved taken appropriate steps at an early stage – in other words, if they had had an appropriate crisis management plan in place. A crisis in this context may be defined as a damaging episode or critical situation arising from adverse drug events; it is a time of great instability and insecurity for all concerned.

Case study: rofecoxib (Vioxx®)

Cyclo-oxygenase (COX) is an enzyme essential for the formation of prostaglandins. These substances are responsible for induction of inflammatory processes, and so inhibition of COX should and does produce anti-inflammatory effects. The major anti-inflammatory agents are the non-steroidal anti-inflammatory drugs or NSAIDs. These drugs have been used for the treatment of arthritis and other musculoskeletal disorders in humans and in animals for decades.

One of the major adverse reactions associated with the use of these substances is the production of adverse gastric effects ranging from irritation to ulceration. In fact, classical NSAIDs rank among the highest causes of adverse drug reactions in humans, and upper gastrointestinal effects (bleeding, ulceration and perforation) due to aspirin, the archetypal NSAID, is one of the major causes of drug-related hospital admissions (Kore, 1990; Laporte *et al*., 1991; Martin *et al*., 2002; Pirmohamed *et al*., 2004). Despite this, these drugs do not rank highly as causes of fatal adverse drug events (Chyka, 2000; Buajordet *et al*., 2001).

COX was shown to exist as two isozymes or isoforms. COX-1 is the constitutive form while COX-2 is an inducible form expressed at sites of inflammation. It was believed that inhibition of COX-1 led to the adverse gastrointestinal effects of NSAIDs, while inhibition of COX-2 produced the desired therapeutic effects. As most NSAIDs inhibited both isoforms, the therapeutic anti-inflammatory benefits were compromised by the adverse effects (Hawkey, 1999; Bertolini *et al*., 2001; Fitzgerald and Patrono, 2001; Mardini and Fitzgerald, 2001). It therefore made therapeutic and commercial sense to identify and develop

NSAIDs that selectively inhibited COX-2 while sparing COX-1. Such drugs should have maximum therapeutic effects with minimal or no ulcerogenic effects (Hawkey, 1999; Vane *et al*., 1998; Bertolini *et al*., 2001; Hawkey and Fortun, 2005).

In 1999, the first two COX-2 selective drugs, rofecoxib and celecoxib, were launched onto the US market, followed by introductions elsewhere in the world. Rofecoxib (Vioxx®) became a best-selling medicinal product for the treatment of arthritis and other inflammatory conditions, with global sales of approximately US $2.5 billion in 2003 (Topol, 2004; Carné and Cruz, 2005). Then, in September 2004, the manufacturer, Merck, announced the withdrawal of the drug (Sibbald, 2004; Singh, 2004; Thompson, 2004). On the same date, 30 September, the US Food and Drug Administration (FDA) also issued a press statement, or more specifically a Public Health Advisory notice, relating to this voluntary withdrawal (FDA, 2004).

The first evidence of adverse effects associated with the administration of Vioxx came from the Vioxx GI Outcomes Research (VIGOR) trial in 2000 which compared rofecoxib with naproxen in a relatively large group of patients; there was a five-fold risk in the incidence of myocardial infarction, some with fatal consequences, in rofecoxib patients compared with those receiving naproxen (Bombardier *et al*., 2000). Other studies have since supported these findings (Mukherjee *et al*., 2001; Ray *et al*., 2002; Campbell and Sneed, 2004; Clark *et al*., 2004; Jüni *et al*., 2004; Solomon *et al*., 2004; SoRelle, 2004; Graham *et al*., 2005; Hudson *et al*., 2005; Lévesque *et al*., 2005; Gislason *et al*., 2006; Hernandez-Diaz *et al*., 2006; Huang *et al*., 2006; Afilalo *et al*., 2007; Brophy, 2007; Brownstein *et al*., 2007; Chen and Ashcroft, 2007; Rahme and Nedjar, 2007; Salzberg and Weir, 2007; Scott *et al*., 2007; Süleyman *et al*., 2007; Zarraga and Schwarz, 2007), while the claimed gastrointestinal safety profile is possibly open to dispute (Hippisley-Cox *et al*., 2005).

It is thought that the cardiovascular effects of rofecoxib are attributable to increased thrombogenicity due to imbalanced prostaglandin production by selective COX-2 inhibitors (Bannwarth, 2005; Burnier, 2005). With a remarkable degree of prescience, the cardiovascular adverse effects of COX-2 inhibitors, and potential nephrotoxic effects (they have the potential for nephrotoxicity in salt-depleted patients and those with renal insufficiency (Rossat *et al*., 1999; Swan *et al*., 2000)), had been predicted some years before (Hawkey, 1999; Perazella and Eras, 2000; Bertolini *et al*., 2001; Rainsford, 2005).

The aftermath of the withdrawal of Vioxx was nothing short of disastrous for the company, which was seen to have reacted too late to the potential adverse effects of the drug, despite signals becoming evident as early as 2000 (Berenson *et al*., 2004; Bannwarth, 2005). Subsequent changes to the label were considered to be insufficient to protect patients. Allegations were made that Merck 'put profits before patients' safety' (Oberholzer-Gee and Inamdar, 2004). Investors reacted harshly, with a 27% reduction in the company's share value and a loss of $25 billion on the capitalisation value (Arellano, 2005), at the time raising issues over its future (Anonymous, 2004; Simons and Stipp, 2004).

Equally damaging were the allegations of collusion between drug companies and regulatory agencies, along with claims of belated regulatory activity (Anonymous, 2005a; Berenson, 2005; Carpenter and Ting, 2005; Eisenberg, 2005). The whole episode served to raise issues of lack of trust in both pharmaceutical companies and the regulatory authorities and provoked questions relating to conflicts of interest (DeMaria, 2004; Fontanarosa *et al*., 2004; Horton, 2004; Karha and Topol, 2004; Lenzer, 2004a, b; Mudur, 2004; Topol, 2004; Wolfe, 2004; Abramson and Starfield, 2005; Allen and Henderson, 2005; Alpert *et al*., 2005; Carné and Cruz, 2005; Cohen, 2005; Epstein, 2005; Gorelick, 2005; Khamsi, 2005; Marris, 2005; Okie, 2005; Smith, 2005; Strom, 2005; Wadman, 2005; Cahana and Mauron, 2006; Kenny, 2007; Philipson *et al*., 2007) and the trustworthiness of the medical literature (Armstrong, 2006; Dobson, 2006; Smith, 2006).

Such elements have been raised before with other drug adverse reactions (Psaty *et al*., 2004).

As has been the case several times in the past, this gave rise to demands for regulatory reform, greater regulatory independence and more openness in regulatory procedures and pharmacovigilance reporting (Dieppe *et al*., 2004; Lenzer, 2004c; Anonymous, 2005a; Carpenter and Ting, 2005; Fielder, 2005; Garattini, 2005; Greener, 2005; Marcus, 2005; Pollard, 2005; Waller *et al*., 2005; Waxman, 2005), but greater regulatory conservatism is expected (Couzin, 2005; Zwillich, 2005).

The episode also raised questions on striking the most reasonable balance between risks and benefits (Adams, 2004; Editorial, 2004; Mamdani, 2004; Anonymous, 2005b; Bobadilla *et al*., 2005; Edwards, 2005; Klotz, 2005; Park, 2005; Urquhart, 2005) and highlighted the difficulties in recalling products once on the market (Becker, 2004; Masters and Kaufman, 2004; Jain *et al*., 2005). This is particularly ironic in this case where the claimed benefits were greater and the risks supposedly lower than was the case with earlier NSAIDs.

The Vioxx affair also raised issues of whether its effects were specific and compound-related or if they were class effects (Couzin, 2004; Bannwarth, 2005; Burnier 2005; Berenbaum, 2005; Curkovic, 2005; Drazen, 2005; Frantz, 2005; Laible, 2005; Maxwell and Webb, 2005; Sooriakumaran, 2006; Hudson *et al*., 2007).

Regardless of the scientific outcomes of such discussions, they can serve to incriminate or otherwise tarnish the reputations of other related compounds and manufacturers, regardless of their biological properties and the risks involved. Moreover, removal of drugs from the market can result in therapeutic uncertainty for professionals and for patients (Hedner and Himmelmann, 2004; Bannwarth; 2005; Dalby, 2005; Fortun and Hawkey, 2005; Klasser and Epstein, 2005; Thiebaud *et al*., 2006; Sun *et al*., 2007). The threat of litigation can certainly provide uncertainty, stress and financial threats for affected companies and for their shareholders (Dyer, 2004; Green, 2005; Kondro, 2004; Lawler, 2005; Parloff, 2005; Tanne, 2005, 2006a, b; Van Way, 2005; Williams, 2005; Sibbald, 2006; Houlton, 2007), and generally raise levels of concern while questioning levels of trust (Freudenberg and Galea, 2008; Ross *et al*., 2008).

In short, the sequelae arising from the Vioxx affair were:

- adverse effects, including fatalities in patients;
- a public and publicised withdrawal of the drug, with concomitant concerns among patients and health professionals;
- a perception that the company waited too long before taking actions – 'profits before patients';
- difficulties in removing the drug from the market;
- a major fall in the capitalisation of the company;
- allegations of collusion between pharmaceutical companies and regulatory authorities;
- perception of incompetence on behalf of the company and regulators;
- greater conservatism in the future with regard to regulatory assessments and decisions;
- loss of trust in the company and in the regulatory process;
- spill-over effect to other drugs (class-effects?);
- threat of litigation.

Preliminaries

Clearly, what may be regarded by a drug producer as a developing and potentially serious issue but one that sooner or later will become a manageable problem may be regarded by the regulatory authorities as a crisis. In other words, the company involved may be presented with a 'crisis' by the authorities (or by the media or the public). In view of the speed and reach of the internet, and other electronic media, bad news travels faster than ever before and reaches a much wider audience than, for example, veterinary practitioners. A company needs to be in a position to identify a potential crisis while it is developing and to take charge of it – once it is

established it is probably too late to avoid negative responses from stakeholders such as the pet-owning public, farmers and veterinarians. The only beneficiaries under these circumstances will be competitors who will use such adverse publicity efficiently and effectively for their own benefit and for the detriment of the afflicted company. To prevent this, systems and documentation must be operating efficiently and effectively, and be thoroughly up to date.

Serious adverse reactions with human medicines are relatively frequent and fatalities are not uncommon (Chyka, 2000; Buajordet *et al*., 2001; Lacoste-Roussillon *et al*., 2001; Lexchin, 2005). While no one would readily suggest that adverse reactions with a veterinary medicinal product would ever have the impact of a Vioxx-type episode, they may damage sales of the product and could tarnish a company's hard-won reputation. Adequate steps must therefore be taken to protect not only the company, but also its numerous stakeholders, including the animal patients being treated.

Prior to product authorisation

Prior to authorisation, certain steps should be taken to ensure that information held by the drug sponsor, some of which is released to the public and veterinary professionals is the most current, as outlined in *Table 29.1*.

Immediately post-authorisation/product launch

Spontaneous reports generated on a newly launched product will generate their own 'awareness' as drug reaction signals begin to arise within the relevant departments of a company. However, to avoid being overtaken and overwhelmed by developing events, and thus to avoid any crisis, certain steps need to be taken, as outlined in the following paragraphs dealing with crisis management and associated issues.

Crisis management

In the event of a crisis arising from developments in pharmacovigilance, particularly in the event of serious unexpected adverse reactions, or when the frequency or severity is significantly greater than expected, crisis management serves a number of purposes:

- It protects the exposed animal patient population.
- It protects patient owners and animal healthcare workers including veterinarians, veterinary nurses, farmers and pet owners from unwarranted concerns and misinformation.
- It provides information to patient owners, veterinarians and regulators.
- It protects the company by complying with local and wider regulatory and legal requirements.
- It protects the business and value of the company.
- It protects the image of the company as a responsible and ethical business.
- It respects the obligations of the company to its shareholders and meets its obligations as a public company.
- It ensures that employees act with due diligence and in an ethical manner.
- It manages communications effectively in a timely manner, with animal owners, prescribers, the public, the media and other stakeholders.
- It assesses, manages and brings an end to the crisis.
- It records and assesses all actions taken, documentation, records, etc., for regulatory and future learning and training purposes.

A crisis may arise at a specific time for a number of reasons, as was evident from the Vioxx example described above, for example:

- emerging warning signals from spontaneous reporting, causing concern to regulatory authorities;
- publications in veterinary journals, farming publications or specialist magazines (e.g.

Table 29.1 Key company information.

Information	*Check*	*Special precautions*
Summary of Product Characteristics (SPC)	Check that the SPC released is the final version as agreed with the authorities. It should constantly be reviewed to take account of all subsequent variations and changes to the original	
Information leaflet	Any information leaflet included with the product should be, indeed must be, compliant with the agreed and current SPC	
Label	Check that the version sent to the factory/printers is the final version based on agreed mock-ups supplied to the regulatory authorities and in accordance with the current SPC	Check that label is compliant, i.e. that manufacturing is using the label agreed with the regulatory authorities, and not an earlier version
Safety information	This must be updated in line with any recommendations made by authorities at the time of authorisation, or in the light of the results of new studies or new publications	Pay particular attention to those areas that define expectedness (and thus unexpectedness) of adverse drug reactions, and the frequency and severity of expected reactions, where possible, in specific patient or species groups
Drug safety or corresponding R&D department	This department must be kept thoroughly informed of major regulatory steps including decisions to grant marketing authorisations, actual issuing of marketing authorisations and date(s) of product launch(es) and specific geographic markets affected	
Marketing departments	Those responsible for marketing the product, and notably those with direct client contact, for example with veterinarians and farmers, should be thoroughly trained in the use of the product, its benefits and possible side effects. Specifically, they should be fully acquainted with procedures to handle product complaints and specifically adverse drug reactions	
Technical services	The technical services personnel, who normally may be expected to deal with the majority of products complaints, including adverse drug reactions, must be advised of the drug launch and be fully acquainted with its product profile including its pharmacology, toxicology, its therapeutic spectrum, any known drug–drug interactions and its adverse drug reactions	Technical services personnel must be fully acquainted with and trained in the use of the company's pharmacovigilance systems and all of its relevant standard operating procedures with respect to adverse drug reaction reporting and the relevant regulatory requirements

those aimed at dog or horse owners) suggesting an emerging problem;
- a 'bombshell' publication (*Report of 99 Cattle Deaths After Using Our Drug*);
- internet exchanges identifying the product concerned as a 'problem product';
- a combination of all of these, particularly the first two bullet points;
- competitor adverse comments, notably to veterinarians or farmers or other client groups.

Crisis management team

Having a crisis management team in place as a more or less permanent fixture, which can meet as and when needed, should allow a product sponsor to react to what may otherwise become a crisis in a rapid, orderly and planned manner. Members of the team should have thorough knowledge of the product at all stages of its development, including the post-marketing period, and be able to analyse pharmacovigilance data and react to it accordingly when this is appropriate. This of course raises the crucial issue of who should be on such a team. This will depend on the nature of the product and the structure of the company concerned. Some ideas are given in *Table 29.2*, but this should not be regarded as the only possible solution. There is no reason, for example, why several virtual teams cannot exist side by side to shepherd several products. The team should be empowered with decision making and should be composed of authoritative and able individuals. In fact the *virtuoso* team concept could well apply to the crisis management team (Fischer and Boynton, 2005).

The kind of expertise available to service a group of this nature will depend on the structure and size of the company. For example, larger companies will have greater resources available and may be able to draw on expertise or experience from a human pharmaceuticals team. Smaller companies will need to be more selective than this, and possibly make more use of consultant expertise, including local veterinarians or those drawn from academia. They may also need to consider the benefits of employing professional media experts to deal with questions and reactions. Larger companies will probably have their own in-house expertise as well as rules and procedures to be followed in dealing with press and media representatives.

The major functions of the Crisis Management Team should be to:

- identify the major issues arising in what could well develop into a pharmacovigilance-based crisis, and put these into context;
- assess the risks and benefits of the drug/product;
- identify and analyse the possible options;
- select a strategy based on the above;
- implement the strategy;
- evaluate the results of that strategy;
- engage all the affected partners and stakeholders.

Moreover, this should not be regarded as a once-only exercise but rather the operation of a feedback loop, at least until the crisis is avoided or, if it develops, until it ebbs.

This is depicted diagrammatically in *Figure 29.1* and the functions are discussed in more detail in *Table 29.3*.

In considering these steps, some specific questions should be asked and some crucial points addressed. *Figure 29.1* shows that arrows may point in both directions, suggesting that some aspects may need to be reconsidered and readdressed in the light of developments, discussions or preliminary conclusions. Some further aspects of the steps in *Figure 29.1* and *Table 29.3* are considered in *Table 29.4*.

Concluding remarks

These notes and guidance are intended to outline the strategy for managing a developing crisis as a result of a pharmacovigilance issue. The main points of the approach can be summarised as follows:

Table 29.2 Possible key members of the crisis management team.

*Member**	*Key role*
Chair (e.g. Head of R&D, Head of Drug Safety)	To chair the team, co-ordinate decisions, inform senior management of actions taken/needed to be taken, and to provide lead in issues related to pharmacoepidemiology
Preclinical safety/clinical development	To provide expertise on preclinical safety (pharmacology, pharmacokinetics and animal toxicology)
Clinical pharmacology/ toxicology	To provide input in areas of clinical pharmacology, human and animal toxicology
Clinical trials	Expertise in clinical trials with the affected product, including adverse events
Legal department	To advise on legal issues, obligations and dealing with the regulatory authorities
Regulatory affairs department	Expertise on general regulatory issues and obligations; knowledge of the dossier in the regions where the affected product is registered/ authorised. Knowledge of labelling, SPC and issues raised by regulatory authorities during the authorisation process
Marketing	Input on marketing strategies used (including targeting of patient or physician groups)
General manager/country or regional manager	Overall responsibility for marketing strategy; legal responsibility for corporate liability
Finance	Knowledge of sales, input on financial impact of product loss – also (possibly) legal responsibility as registered officer of company
Media team/consultant	To provide input to media strategy issues and to: • conduct daily monitoring of news media (including internet) • advise on presentation • advise on response to media stories • advise on interview techniques for staff likely to have to face the media, especially television interviews • provide general media training for 'front-line' staff, and regular updating of such training • advise on creating an internal communications infrastructure
All or specific appointees	To: • carry forward agreed actions • develop master task list for members of the team or for those identified by the team to take certain actions • co-ordinate document management • review and approve documentation, e.g. letters to authorities, reports • track progress of allocated tasks

*Experts with specific knowledge of the drug/product may be co-opted accordingly, as will those with specific expertise, e.g. pathologist, microbiologist, veterinarian, individuals involved in collating spontaneous report data for a specific product, those directly responsible for clinical trials with a specific product or therapeutic class, as well as outside experts with either a specific expertise in the drug or type of drug (veterinary cardiologist, clinical oncologist, vaccines expert) or experience of using specific drug/product or drug/product classes, etc.

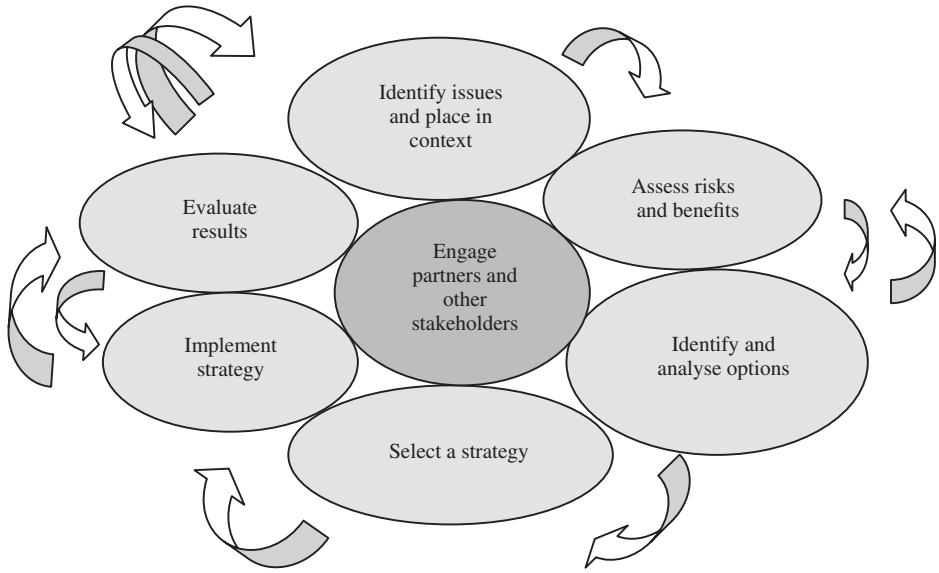

Fig. 29.1 Crisis management team functions.

Table 29.3 Potential major functions of crisis management team.

Function	*Possible points for discussion and elucidation*
Identify issues and put into context	Identify a developing scenario – was an adverse reaction(s) expected? Nature of reaction – more frequent than expected, more severe, unexpected? Any evidence to support/negate from laboratory animal studies, target animal safety tests, patient group(s) affected, possible mechanisms, resulting from drug–drug interactions or breed- or species-specific phenomena, etc.
Assess benefits and risks	Assess: • benefits of drug • known risks of drug • emerging risks • comparison with other alternative marketed products or other therapies
Identify and analyse options	To include: • informing or discussing issues with regulatory authorities • suggesting remedies, e.g. reduce dose or administration frequency, exclude patient groups, contraindications, warnings (on SPC, label, information leaflet) • seeking advice from regulatory authorities and their expert bodies (CVMP in the EU) • suggesting further studies to rule out the effect (identify causality) or elucidate mechanism • voluntary withdrawal or other restrictions • identifying affected partners and stakeholders and informing accordingly (Dear Dr Letter, note in general (e.g. *Veterinary Record* and/or specialist (e.g. farming or specialist medical press, news release to media) or medical press if adverse reaction is as a result of inadvertent human exposure). Identify if possible suitable treatments • establishing decision points – when to take actions, depending on the outcome of investigations, scientific and regulatory discussions and negotiations

Table 29.3 *Continued*

Function	*Possible points for discussion and elucidation*
	• amending key documents such as clinical protocols (for future trials), SPC • producing expert report detailing the issues and possibly refuting any groundless allegations • providing statement(s) to the financial communities to maintain shareholder/stock market confidence • publishing strategy to strengthen confidence (especially for the stock market) that problem, real or otherwise, is being effectively addressed • briefing documents for the company's media representatives • briefing documents for those directly exposed to the drug/product (e.g. sales force, trials co-ordinators, marketing representatives) • question and answer briefs • lessons learned for documentation for a number of audiences (staff training, veterinarians, veterinary nurses)
Select a strategy	May be based on advice from regulatory authorities. In some circumstances, this may be based on urgent action without prior referral to authorities, e.g. if the team considers that the first action should be voluntary suspension or surrender of the authorisation
Implement strategy	Carry out agreed actions. Release of information should be according to agreed schedules, and only after it has been reviewed and agreed by the team. Release must respect the needs of patients, prescribers, the investment community and shareholders – and the regulatory authorities and legislation in force
Evaluate results	Sometime after the crisis has ended (normally within 1 month but no later than 3 months), the team should prepare an 'impact analysis'. This will be presented to senior management and the following will be addressed and should essentially be an enquiry into how the episode was handled: • In retrospect, was there ever a chance of a crisis developing in this instance? • If so, or if a crisis actually began, what was it? What was its essence? • Could it have been foreseen and prevented? • What parts of the plan worked well? • What parts did not work well? • Should the plan be modified? What should be modified? • Were those involved properly trained to deal with the crisis? The following major points must be considered: • How long did the crisis last for and could it have been prevented? • What was the impact on patient welfare? • What was the impact on healthcare professionals such as veterinarians? • What was the impact on sales, the company's image (and possibly share price or investor confidence) and what is the evidence that the crisis management team and plan ameliorated any adverse effects on any of these? • What regulatory changes (to the authorisation, the SPC, the label) were necessary, and were these the only options or even the correct options? Were there any others? • What are the options for the future and for the future of the product?

Table 29.4 Further aspects of the crisis management approach.

Function	*Further considerations*
Benefit:risk assessment 1	• Is the drug administered for a life-threatening condition or for a (relatively) trivial illness? • Is the drug/product curative, preventative or palliative? • What are the benefits and risks of other established treatments? • What are the comparative benefits and risks for the market leader? • What is the comparative/additional/unique benefit of this drug/product over the alternatives?
Benefit:risk assessment 2	• Can the adverse effects of treatment with our product be ameliorated, e.g. with an antagonist? • Has the effect arisen from veterinary ignorance of the disease, the species being treated or the drug or drug class? • Is it related to off-label use? • Is it related to treatment of at-risk groups (elderly, neonatal, paediatric, renal/hepatic insufficiency)? • Has it arisen from overdosing (greater than indicated), prolonged duration (longer than indicated) or too high a frequency of administration? • Are the effects more severe or more frequent than expected (or both)?

Benefit:risk assessment 3	• Is the effect a class effect – has it been reported with one or more drugs in the same class?
	• Has it been reported previously in the same species or patient groups?
	• Could it be a drug–drug interaction and, if so, with what?
	• Is it a recognised syndrome, particularly one of the more severe:
	  ○ aplastic anaemia?
	  ○ other blood dyscrasias?
	  ○ multi-organ failure – autoimmune disease?
	  ○ antibiotic-induced clostridial disease?
	  ○ pancreatitis?
	  ○ and so on!
	• If so, is it frequent enough, taking into account the nature of the condition being treated, and the nature of the treatment (curative versus palliative) to warrant continued availability?

Options	• What level of adverse reaction might be considered appropriate (and by whom)?
	• Is regulatory advice advisable or essential and, if so, now or at some later stage?
	• Should a strategy be developed first or is the situation so serious or seemingly serious that the regulators should be approached immediately?
	• Is it so severe that a voluntary or (temporary) suspension of the authorisation should be considered?
	• Should the authorisation be surrendered?
	• Consider:
	  ○ numbers of patients affected
	  ○ numbers affected/numbers treated
	  ○ type of patients involved – high value (horse), high attachment (companion animal), risk to food chain (food animal)
	  ○ contributing factors
	  ○ actions taken with other drugs causing similar effect
	  ○ label, SPC, posology changes

Information release	• How to control it • Who to and when (specialist journals, the press, radio, e.g. farming programmes) • Media 'witch-hunts' • Competitor campaigns • Expert media advice • Expert legal advice

- Identify the situation as a (developing) crisis.
- Define the objectives in dealing with the crisis.
- Make an assessment of the problem, the benefits and risks of the product, and how to deal with the issues.
- Devise a strategy to deal with the problem, including a communications strategy.
- Devise a communications infrastructure.
- Agree to and see through the actions.
- Evaluate our approach in the aftermath of the crisis and, if necessary, make improvements and learn from our experiences.

There is perhaps one important aspect that is often overlooked – the interaction between marketing and pharmacovigilance. All those involved in an animal health company regardless of where they are employed – R&D, regulatory, manufacturing, legal, etc. – are naturally going to be enthusiastic about the success of their products and especially about the success of newly launched veterinary drugs. Nowhere is this truer than in the marketing departments and sales force. Individuals here will wish to promote the benefits of a company's products but may be reluctant to recognise any adverse effects, and these aspects could be overlooked. Yet these individuals are often best placed to hear firsthand about possible adverse drug reactions *and* are often best placed to make realistic benefit : risk assessments, even if these are not of the quantitative variety required by regulatory authorities.

Thus, involving marketing departments in pharmacovigilance activities, including crisis management, and ensuring their thorough training in pharmacovigilance concepts can only be beneficial to an animal health company. This has certainly been recognised in the human medicine context (Food and Drug Administration, 1999; Edwards, 2004). Furthermore, information may need to be shared with other companies and especially where there are cross-licensing agreements in place (Borner *et al.*, 2006).

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30 The role of veterinary pharmacovigilance in risk analysis and the influence of risk perception on veterinary pharmacovigilance

H.P.A. Illing

Introduction

Public perceptions of risk drive priorities and legislative agendas. Society demands that veterinary products, like all chemicals, be 'safe'. Society also demands that they be effective and of appropriate pharmaceutical quality. These requirements are exercised through legislation (statutory law) concerned with the safety and efficacy of veterinary products, usually introduced *post hoc*, in response to perceived inadequacies in the previous situation, as well as through the law concerning the rights of individuals. Generally legislators and policymakers are concerned with establishing legislative frameworks and regulatory bodies. Agencies, such as the European Medicines Agency (EMEA), the UK Veterinary Medicines Directorate (VMD) and the European Commission (EC), develop detailed requirements, evaluate the evidence and enforce (or oversee the enforcement of) the legislation. These agencies are usually advised by 'expert committees' on the scientific aspects of policymaking and on the acceptability or otherwise of applications for licensing specific chemicals, such as those intended for use as veterinary medicines.

A toxicant is definable as an agent, which, when applied to a biological system, causes a deleterious perturbation to that system. Thus, when that effect is being expressed, the agent involved is toxic. It is no longer 'safe'. If the word 'beneficial' is used in place of 'deleterious', efficacy is being investigated, and the effect is 'desired'. The key element in either definition is a value judgement concerning what constitutes a 'deleterious' or a 'beneficial' effect. In both cases the decision concerning what is 'deleterious' or 'beneficial' is in the eye of the beholder and is a function of circumstance and context. Indeed what may be beneficial to humans may involve extremely deleterious effects (death) to members of another species (e.g. a rat) if it is deemed a pest, and what may be beneficial in ill-health (e.g. reducing blood sugar levels in diabetics) may be deleterious (causing hypoglycaemia) in healthy humans. Thus societal judgements are required, which must be justifiable to the wider public if and when necessary.

When health (to the target species and to humans) is the issue, the aim is to prevent ill-health and to restore health. In order to do this it is necessary to have means of predicting ill-health. This prediction may include information from structure activity relationships, physico-chemical data, testing in vitro and regulatory and other testing in vivo. The bodies concerned with

evaluating and efficacy safety therefore set minimum testing requirements for those who wish to place on the market a chemical substance or to market it for a specified use, e.g. a veterinary medicinal product. Furthermore, for veterinary medicinal products, these bodies evaluate the results of the tests.

Prior to marketing a veterinary medicinal product it has to be tested for safety and efficacy. Safety is concerned with minimising unwanted health and environmental risks; efficacy is concerned with maximising a particular (beneficial) health risk to a specific target. In the case of a veterinary medicinal product, testing may be undertaken in the target species and some monitoring of potentially exposed humans may be possible. However, as only limited populations can be exposed during testing, post-market monitoring is necessary to ensure that safety is maintained. Veterinary pharmacovigilance is pharmacovigilance applied to veterinary medicinal products, i.e. the surveillance of (veterinary) medicinal products after authorisation to ensure their continued safety (EMEA, 2005). It is the gathering of information on adverse reactions that may occur after the administration of medical products. Woodward (2005) indicates that this includes adverse effects in treated animals, exposed humans and the environment.

Veterinary products are chemicals. They may be relatively simple pharmaceutical products or complex macromolecules such as the antigens found in vaccines and the components of other biological products. Thus, the general principles used to analyse the risks associated with chemicals are applicable to the analysis of chemicals intended for a specific use, namely to treat sick animals. In most cases across the globe, veterinary products have their own specific legislation and this generally results in their exemption from the legislation applicable to other categories of chemicals in general (e.g. pesticides and biocides) and particularly those in the EU covered by the REACH Regulation (EU Regulation No. 1907/2006 on the Registration, Evaluation and Authorisation (and Restriction) of Chemicals).

The aim of this chapter is to set out the risk analysis framework within which health effects are evaluated and the role of veterinary pharmacovigilance within that framework. It also identifies influences that risk perception may have on that framework and on the operation of evaluating veterinary products, and hence the need for good communication. The first part of this chapter therefore defines the concepts of hazard and risk and the risk analysis framework. The key documents concerned with risk analysis for the health and environmental effects of chemicals are published by the National Research Council (1983), Royal Society Study Group (1983, 1992), Health and Safety Executive (2001), Organisation for Economic Co-operation and Development (OECD) (2003) and International Programme on Chemical Safety (IPCS) (2004).

Hazard and risk

The key distinction between hazard and risk needs to be defined first. In the OECD (2003)/IPCS (2004) definition:

- **Hazard** is 'the inherent property of an agent or situation having the potential to cause adverse effects when an organism, system or (sub)-population is exposed to that agent'.
- **Risk** is 'the probability of an adverse effect in an organism, system or (sub)population caused under specified circumstances by exposure to that agent'.

Hazard is the intrinsic property of an interaction at the receptor site of the agent and the target, while risk is the probability of that property being expressed.

The risk analysis framework

Risk analysis is a process for controlling systems where an organism, system, (sub)-population or ecosystem could be exposed to a hazard. Risk analysis consists of three components:

- risk assessment;
- risk management;
- risk communication

(OECD, 2003; IPCS, 2004).

In the case of veterinary medicinal products this risk analysis is the process conducted under legislation made at the European Union level leading to the authorisation of veterinary medicinal products, and includes the centralised procedure, the mutual recognition and decentralised procedures and national procedure (EMEA, 2006).

The OECD/IPCS definitions for risk assessment and risk management are given below.

Risk assessment

Risk assessment is:

> 'A process intended to calculate or estimate the risks to a given target organism, system or (sub) population, including the identification of attending uncertainties, following exposure to a particular agent, taking into account the inherent characteristics of the agent of concern as well as the characteristics of the specific target system.
>
> The risk assessment process includes four steps: hazard identification, hazard characterisation (related term: dose-response assessment), exposure assessment and risk characterisation. It is the first component in a risk analysis process.'

Risk management

Risk management is:

> '(A) Decision making process involving considerations of political, social, economic and technical factors with relevant risk assessment information relating to hazard so as to develop, analyse, and compare regulatory and non-regulatory options and to select and implement (an) appropriate regulatory response to that hazard.
>
> Risk management comprises three elements: risk evaluation; emission and exposure control; risk monitoring.'

Two further elements are important when discussing risk. These are risk communication and risk perception.

Risk communication

Risk communication is:

> 'Interactive exchange of information about (health and environmental) risks among risk assessors, managers, news media, interested groups and the general public.'

Risk perception

Risk perception is not defined by the OECD/IPCS. However, the Royal Society Study Group (1983) called perceived risk the combined evaluation that is made by an individual of the likelihood of an adverse event occurring in the future and its likely consequences. In the introduction to the chapter on risk perception in the 1992 report of the Royal Society Study Group, risk perception (from the perspective of the social sciences) involves people's beliefs, attitudes, judgements and feelings, as well as the wider social or cultural values and dispositions that people adopt towards hazards and their benefits.

When aggregated, individual beliefs, attitudes, etc. become the societal beliefs, attitudes, etc. that both drive legislation and drive concerns about decisions taken by public bodies set up to administer and enforce the legislation. For this reason it is essential that those evaluating risks and benefits seek to maintain the confidence ('trust') of the general public in their decisions. This includes communicating the basis of the evaluations as well as the outcomes in a manner that can be understood by society as a whole.

Steps involved in risk assessment and risk management

The definitions given below refer to safety; a parallel set of definitions can be derived for efficacy. These definitions are as follows.

Hazard identification

Hazard identification is:

> 'The identification of the type and nature of adverse effects that an agent has the inherent capacity to cause an organism, system or (sub)-population.'

Hazard characterisation

Hazard characterisation is:

> 'The qualitative, and wherever possible, quantitative description of the inherent properties of an agent or situation having the potential to cause adverse effects. This should, where possible, include a dose-response assessment and its attendant uncertainties.'

Exposure assessment

Exposure assessment is:

> 'Evaluation of the exposure of an organism, system or (sub)-population to an agent (and its derivatives).'

As with pesticides (Illing, 1997), for veterinary products there is likely to be a given use in a specified species and hence only one scenario for efficacy. However, a number of exposure scenarios may have to be investigated for safety reasons. These safety scenarios may include:

- those involved in administration of the veterinary medicinal product;
- those exposed when looking after the animal's welfare whilst it is being treated;
- those possibly exposed to meat and other foods of animal origin, derived from the treated animal;
- humans, other species of animals and plants and microorganisms that might be exposed in or via the environment.

Both efficacy and safety have to be evaluated in the target species.

Risk characterisation

Risk characterisation is:

> 'The qualitative and, whenever possible, quantitative determination, including attendant uncertainties, of the probability of an occurrence of known and potential adverse effects of an agent to a given organism, system or (sub)-population under defined exposure conditions.'

Risk evaluation

Risk evaluation is:

> 'Establishment of a qualitative or quantitative relationship between risks and benefits of exposure to an agent, involving the complex process of determining *the significance of the identified hazards and estimated risks to the system concerned or affected by the exposure*, as well as the significance of the benefits bought about by the agent.' (Author's italics)

In the case of safety (including human health issues arising from administration of a veterinary product to an animal) there are two principal target organisms: the target animal species and humans. As risk evaluation involves assessment of the 'significance of the identified hazards and estimated risks' to 'those (humans) concerned with or affected by the exposure' it involves more than a technical assessment of the toxicological and exposure data. 'Significance to those concerned with or affected by the exposure' implies that the way in which those concerned with or affected by the exposure perceive the risks, and hence the sociological and psychological factors affecting how people perceive risk, become important. Sociological and psychological

opinions concerning risk have to be taken into account when evaluating risks.

Risk monitoring

Risk monitoring is:

> '(The) Process of following up the decisions and actions within risk management in order to ascertain that risk containment or reduction with respect to a particular hazard is assured.'

The essential aim of pharmacovigilance is this monitoring of risks.

Emission and exposure control

Emission and exposure control is the third part of risk management for chemicals. In practice, this applies to veterinary products – during manufacture and use and during disposal. Exposure control can be seen in terms of:

- restricting the sale of the product to certain people, ensuring adequate dosage to the target animal;
- ensuring that human exposure is restricted to safe levels through appropriate precautions when manufacturing and when administering the materials to animals, and through suitable withdrawal periods for food species;
- ensuring that the environment is adequately protected, where necessary by specifying how animals may be housed and their excreta disposed of.

Figure 30.1 illustrates the stages of risk assessment and management and their interrelationships.

For veterinary medicinal products risk management includes (as part of 'emission and exposure control') the setting of conditions for sale and the enforcement of those controls, and (as part of 'risk monitoring') veterinary pharmacovigilance. Although veterinary pharmacovigilance does not contribute significantly to the initial evaluation of the majority of veterinary products, it contributes greatly when re-evaluation is being undertaken after a period of marketing. Indeed, adverse effects identified as part of a pharmacovigilance programme can trigger such a re-evaluation.

The outer box in *Figure 30.1* represents the way in which the question is framed by society – i.e. the legislation within which the risk analysis is

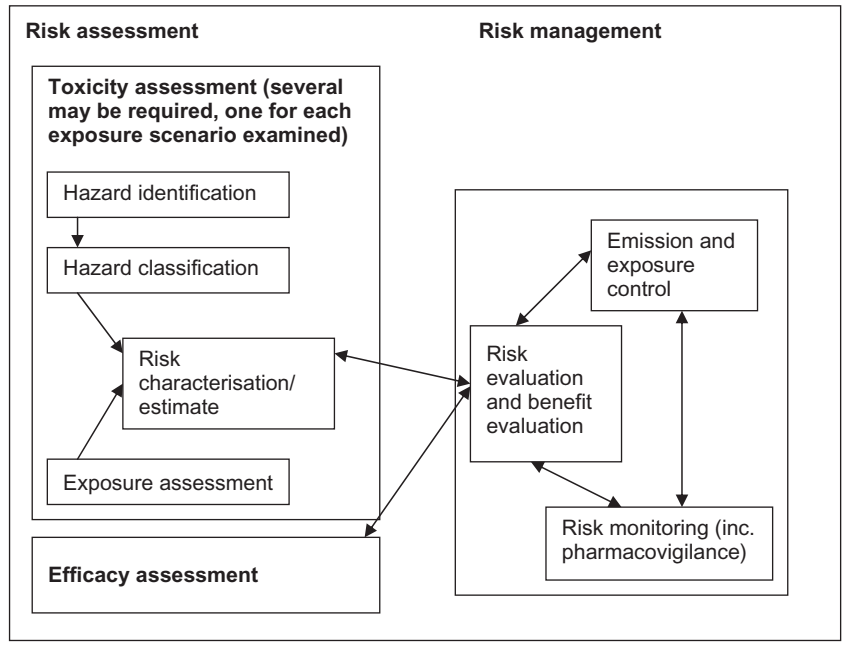

Fig. 30.1 Risk assessment and risk management.

conducted. This is set up by legislative bodies (such as the European Parliament and Council of Ministers) acting on behalf of society. The evaluation of risk (and benefit) also includes societal judgements, taken by the appropriate body set up by the legislators and acting on behalf of society (for the centralised procedure, the EMEA on the advice of the Committee for Medicinal Products for Veterinary Use (CVMP)). As these bodies are established by legislators acting on behalf of society as a whole, they are ultimately responsible to society and their continued existence depends on how society as a whole perceives their activities.

Hence there is a need for openness and transparency in the decision-making process and for multidirectional communication. This communication is to ensure that the procedures used and the risk assessments arrived at are acceptable to society as a whole. It is also to ensure, so far as is reasonably practicable, that the perceptions that society and different groups within society have are taken into account when making decisions concerning risk. On occasions elements within society may find the decisions unacceptable, and it is when acceptability becomes an issue to certain elements in society that campaigning ('pressure') groups form in an attempt to change the decision, the decision-making process or both.

The philosophical framework for risk evaluation

The Royal Society Study Group first put forward a risk evaluation framework in 1983. This framework (the 'tolerability of risk' concept) has been restated and slightly advanced in *Reducing Risks, Protecting People* (Health and Safety Executive, 2001). It was originally developed to handle engineering risk, but it is equally applicable to risks from chemicals, including veterinary products. The Health and Safety Executive (2001) and Illing (2001) have discussed the application of this framework to the evaluation of health risks arising from exposure to chemicals.

The criteria for reaching decisions can be classified according to three 'pure' criteria (Health and Safety Executive, 2001). These are:

- An *equity-based* criterion, which starts from the premise that all individuals have unconditional rights to certain levels of protection. This leads to standards, applicable to all, held to be usually acceptable in normal life. In practice this leads to fixing a limit to represent the maximum level of risk above which no individual can be exposed. If the risk characterisation indicates that the risk is above this limit the risk is held to be unacceptable – whatever the benefits.
- A *utility-based* criterion, which applies to the comparison between incremental benefits of measures to prevent the risk of injury or detriment (for health effects, ill-health) and the cost of the measures. The utility-based criterion compares the relevant benefits (e.g. statistical lives saved, life-years extended, reduced ill-health and better quality of life) obtained by adoption of a particular risk prevention measure with the net cost of introducing it, and requires that a balance be struck between the two. This balance can be deliberately skewed towards benefits by ensuring gross disproportion between costs and benefits.
- A *technology-based* criterion, which essentially reflects the idea that a satisfactory level of risk prevention is attained when 'state of the art' control measures (technological, managerial, organisational) are employed to control risks, whatever the circumstances.

These criteria underlie the regulatory process first outlined by the Royal Society Study Group (1983). The scheme is based on:

- an upper limit of risk which should not be exceeded for any individual ('unacceptable');
- further control, so far as is reasonably practicable, making allowances if possible for aversions to the higher levels of risk or detriment ('tolerable');

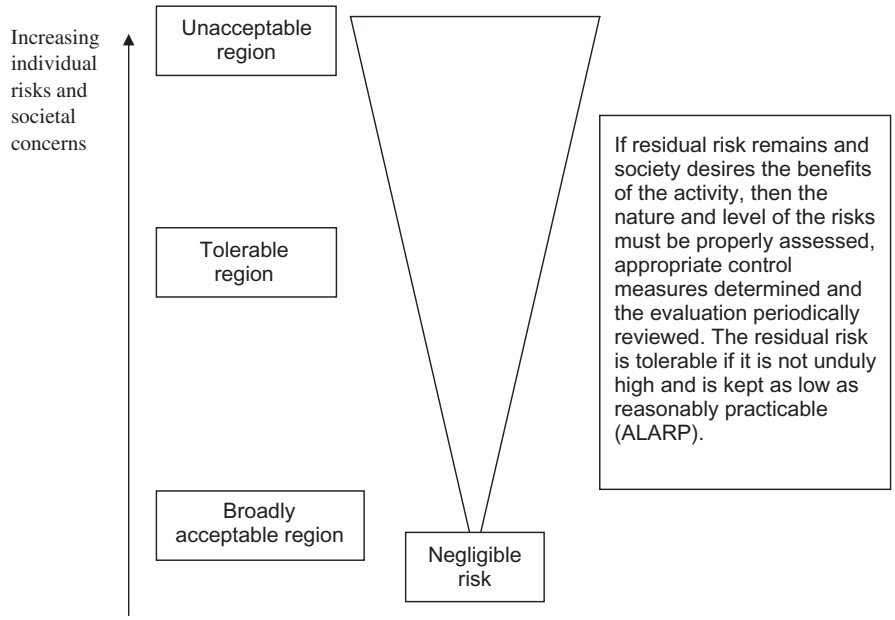

Fig. 30.2 Outline of the Royal Society approach to risk management/ 'tolerability of risk' framework. (Based on Health and Safety Executive, 2001.)

- a cut-off in the deployment of resources below some level of exposure or detriment judged to be trivial ('broadly acceptable').

The scheme is outlined in *Figure 30.2*.

This approach to risk evaluation can be applied to health effects, both to the target species and to incidentally affected species. For many health effects, the risk evaluation is concerned only with determining what constitutes a 'broadly acceptable' risk, and hence with the equity criterion. This is the case if any violation of an equity criterion for 'safe' (the 'broadly acceptable' level of risk), such as a residue level in a foodstuff, is exceeded (MRL violation), thus resulting in its immediate withdrawal from the market. Where possible it is also applied to the indirect risks to the environment and to humans mediated via the environment. In this case, the more likely result is the withdrawal of the veterinary medicinal product, which, for this purpose, is yet another of the many chemicals present in the environment.

However, the current approaches to occupational exposures can make use of the concept of 'tolerable risk' if exposure is not readily kept to levels that are 'safe' in that they represent a 'broadly acceptable' risk. The safety of veterinary medicines in the target species is normally considered on the basis of 'tolerability', making use of a utility criterion and weighing potential risks against probable benefits. The risks and benefits associated with the use of a drug or veterinary medicinal product in general are evaluated by an authorising or licensing body, and the risks and benefits associated with use in a specific patient/animal in particular are further evaluated by the person prescribing the drug.

Risk perception

What risk?

It should be noted that engineers and biologists define risk slightly differently, and much of the discussion concerning 'objective' risk is based on the engineer's definition of risk. This dichotomy is apparent in the Royal Society Study Group report (1983) and is explained in, for example, Illing (2000). In engineering, risk is 'risk of exposure' and any uncertainty in whether a specified health effect will result from a given exposure (level and duration) is 'uncertainty in defining the hazard'. It is based on identifying possible

events associated with the release and dispersion of significant amounts of a toxic substance and/or analysing potential failure mechanisms leading to those exposures. It can also include identification of a geographic area for which, following dispersion, exposure matches or exceeds a harm criterion (a hazard statement – a quantitative statement of the exposure conditions associated with a specified level of harm).

To human health toxicologists, the risk is 'risk of end-effect', and, at least as far as the target organism is concerned, the risk of exposure is assumed. In probability terms 'risk of exposure' is taken as 1, i.e. the exposure occurs following administration of a drug to the target species. The engineer's 'uncertainty in defining the hazard' – the probability that the toxic effect will be seen at a given dose, effectively the probability stated in a dose-response curve – is the biologist's 'risk of end effect'. In the case of toxicity to organisms other than the target, the risk is actually the probability arising from both sources – the probability that an exposure of a given level and duration will take place and the probability that the health effect will result from that exposure.

Societal input

As already explained above, societal input is required at two stages. The first is at the stage of the framing of the risk analysis process, in terms of both what questions to ask and the administrative structures through which they will be asked. The second is at the decision-taking stage, the risk evaluation. The latter is usually undertaken indirectly, with an allowance for societal concerns tacitly included in the uncertainty factors associated with limit setting or with determining whether margins of exposure are adequate (Illing, 1999, 2006). Understanding these inputs requires an understanding of how risk is perceived, as described by social scientists. It is imperfect.

In the 1983 Royal Society Study Group report the risk assessment undertaken by technical and scientific experts was described as 'objective (statistical) risk' and they differentiated it from 'perceived risk'. They were, in effect, dealing with 'objective risk' in terms of risks due to failure rates and risks of premature death due to a nominated cause. They recognised that there may be a perplexing disparity between these two.

The chapter on risk perception in the 1992 report from the Royal Society Study Group states that:

> 'Risk perception involves people's beliefs, attitudes, judgements and feelings, as well as the wider social or cultural values and dispositions that people adopt, towards hazards and their benefits . . . Furthermore the perception of risk is multidimensional, with a particular hazard meaning different things to different people (depending, for example, upon their underlying value systems) and different things in different contexts. In some circumstances, important aspects of risk perception and acceptability involve judgements not just of the physical characteristics and consequences of an activity but also social and organisational trustworthiness of risk management and regulatory institutions. What is clear is that risk perception cannot be reduced to a single subjective co-ordinate of a particular mathematical model of risk, such as the product of probabilities and consequences, because this imposes unduly restrictive assumptions about what is an essentially human and social phenomenon.'

The last part of this statement applies particularly to the engineering approach to risk assessment, with its use of numerical values for 'risk of death' (premature death due to a specified cause) and for failure rates of mechanical failures.

Aven and Kristensen (2005) summarise some of the important general lessons gleaned from risk perception research as:

1. Risk acceptance cannot be based on evaluations of expected values only. A more comprehensive risk picture is required.
2. People are poor assessors of uncertainties, if the reference is an objective true statistical probability.

3. Probability assignments (uncertainty assessments) are influenced by a number of factors.
4. Perception, acceptance and tolerability of risk are influenced by a number of factors, such as dread and knowledge.
5. There are significant individual and group differences in risk perception and risk acceptance.
6. Risk perception and acceptance may be fundamentally related to social judgements of things such as responsibility, blame and trust in risk management and managers.

Jasanoff (1992) suggests that scientists adopt three propositions in developing the technical or actuarial approach to determining the risk criteria representing 'unacceptable', 'tolerable' or 'broadly acceptable' risk, namely:

- Given enough data, experts will generally agree with each other in their risk assessment.
- The only scientific way to think about risk is essentially in actuarial terms.
- Any other way of thinking about risk is possibly wrong, certainly unscientific and perhaps even antiscientific.

She did, however, admit that biological scientists have generally been more willing to recognise the need for qualitative judgements, and hence the possibility of expert disagreements in risk assessment. She also stated that chemical products, in particular, may be rejected not because they are 'unsafe' in any conventional sense, but because the public is insufficiently persuaded that they serve a legitimate social need. The important point is that there is often a serious disjunction between how the technical expert (the risk assessor) sees risk and how the public perceives risk. Biological scientists do, in practice, use the same principles as engineers and actuarial risk assessors. However, they usually employ less easily calculated end points, such as minor ill-health effects, some of which may have been assessed subjectively by questionnaire. They also try to avoid the more complex mathematical models offering numerical solutions. Thus, although the public perception is that science is precise and clear-cut, when it comes to ill-health, the experts are required to take sometimes complex and difficult judgements concerning the interpretation of the data.

Complexity and dependence on belief patterns

Slovic (1999) emphasises the subjective and value-laden nature of risk analysis. Even a relatively simple measure, such as the end point for expressing a risk, can be complex and judgmental (*Table 30.1*). Which of these is the correct measure?

Clearly, the choice of measure will influence how a risk is perceived and evaluated and such choices are inevitable when evaluating veterinary products.

Table 30.1 Ten ways of expressing risk of death (from Illing, 2006). The first nine are based on Slovic (1999). In reality, once born, death is inevitable. Thus all except the last two ways measure the occurrence of premature death due to the substance, usually deaths that occur within a short time of the exposure. The last two are estimates of how premature that death may be.

1.	Per million people (employed in the industry)
2.	Per million people within a given distance from the source
3.	Per unit of concentration
4.	Per site or industrial plant
5.	Per tonne of toxic substance released to air
6.	Per tonne of toxic substance released to air and absorbed by people
7.	Per tonne of substance produced (placed on the market)
8.	Per million pounds (sterling) of substance produced
9.	Loss of life expectancy associated with exposure to hazard
10.	Loss of quality-assessed life years of expected life

There are also other sociological aspects of risk that affect risk analysis. Anthropologists and cultural sociologists have suggested that social responses to risk are determined by prototypes of cultural belief patterns – clusters of related convictions and perceptions of reality. Renn (1998) summarises four such prototypes:

- *Entrepreneurs* – those who perceive risk taking as an opportunity to succeed in their personal goals and are less concerned about equity issues and wish government to refrain from extensive regulation and risk management efforts.
- *Egalitarians* – those who emphasise cooperation and equality rather than competition and freedom, focus on long-term effects of human activities, are more likely to abandon an activity (even if perceived as personally beneficial) than to take chances and are particularly concerned with equity.
- *Bureaucrats* – those who rely on rules and procedures to cope with uncertainty and believe that, as long as risks are managed by a capable institution and coping strategies have been provided for all eventualities, there is no need to worry about risks.
- *Atomised* or *stratified individuals* – those who principally believe in hierarchy, but miss group identity and a system of social bonding; these people only trust themselves, are often confused about risk issues and take high personal risks while opposing any risk they feel is being imposed on them, and see life as a lottery.

Aven and Kristensen (2005) quote a fifth:

- *Autonomous individuals (hermits)* – those who believe that risks are acceptable as long as they do not involve the coercion of others.

These cultural prototypes have different 'world-views'. Slovic (1999) identifies five world-views:

- Fatalism ('I feel I have very little control over risks to my health').
- Hierarchy ('Decisions about health risks should be left to the experts').
- Individualism ('In a fair system, people with more ability should earn more').
- Egalitarianism ('If people were treated more equally, we would have fewer problems').
- Technological enthusiasm ('A high technology society is important for improving our health and social well-being').

He also suggests that world-view is strongly linked to the public perception of risks.

The most obvious inference from this is that there is no common position concerning what constitutes a 'broadly acceptable' risk or 'safety'. What constitutes a 'broadly acceptable' risk varies with cultural prototype and world-view.

Factors influencing public perception of risk

Factors that can influence the public perception of what constitutes a 'broadly acceptable' risk have been enumerated repeatedly since first being stated by Otway and von Winterfeldt (1982; *Table 30.2*).

Equity of the consequences of the risk implies that the risk of harm and the benefits (rewards) are distributed fairly, either because the same people are affected or because those facing the risk of harm are properly compensated. Control of the risks refers to the perceived level of control that people feel that they have over the risk. The individual may feel the risk is voluntary and under his/her control, or that the risk is involuntary, having been imposed by a process completely outside his/her control. These factors are important when determining what a 'broadly acceptable' risk is. For example, personal control of the risk is very limited when dealing with the imposed risks associated with air quality standards, but considerable when associated with activities where there is considerable choice, such as whether to use personal care products and which product to use.

Table 30.2 General (negative) attributes of hazard that influence risk perception (description from Otway and von Winterfeldt (1982) as given by the Royal Society Study Group (1992); comments mainly from Illing (1999)).

Description	*Comment*
Involuntary exposure to a risk	Occupational exposures not involuntary (can opt to work elsewhere or not work); food/environmental exposure is often considered involuntary
Lack of personal control over outcomes	Some personal control at work; some choice in diet although labelling (or a lack of) may not make avoidance of a particular food chemical (veterinary product) easy
Uncertainty about probabilities or consequences of exposure	Information can lessen these uncertainties
Lack of personal experience with the risk (fear of the unknown)	'Dread'
Difficulty in imagining risk exposure	
Effects of exposure delayed over time	For example, carcinogenicity
Genetic effects of exposure (threatens future generations)	Hence great care needed when evaluating mutagenicity and reproductive toxicity
Infrequent but catastrophic events ('kill size')	
Benefits not highly visible	In the eyes of some elements in society, this applies to environmental/food exposure (hence 'organic' food production)
Benefits go to others	Could apply to workplace, but can be compensated for monetarily
Accidents caused by human failure rather than natural causes	Also human ill-health

Types of scientific advice (e.g. recommendations concerning acceptability of risks relating to use of veterinary products) being offered

Stilgoe *et al*. (2006) claim that a change in the role of scientific advice is taking place, from 'advice as absolute' (type 1) to 'advice as contingent' (type 2), and identify the characteristics of the ideal types this presents (given in *Table 30.3*).

Type 1 advice (essentially *'ex cathedra'* statements) was typical of the advice given up to and including the early 1990s.

Outside of science, the recognition that science, like other areas of knowledge, is rarely absolute and binary (yes/no or safe/unsafe) is still limited. Statistically it is possible to define a binary system for analysing the success or otherwise of a licensing process for veterinary medicinal products in terms of type 1 and type 2 statistical errors, i.e. licence, marketing authorisation or approval granted to a human or veterinary medicinal product that is, in the end, less than satisfactory

Table 30.3 The role of scientific advice: idealised types (from Stilgoe *et al*., 2006).

Type 1	*Type 2*
Advice is valued as objective and absolute	Advice is valued as offering a range of perspectives and options
Scientific advisers lend both advice and authority to policy	Advisors are conduits to accessing information and debate
Advice is separate and unaffected by politics	Advice is diverse and conditional

(type 1) or good medicines or veterinary medicinal products that are lost to society because they are not licensed (type 2).

However, this is rarely obvious when a decision on whether to license, authorise or approve a product is being taken. There is a 'grey' area of uncertainty within the decision taking. This includes known uncertainties (e.g. uncertainties associated with inter-species and inter-individual variation) and unknown uncertainties (which are unlikely to be predicted and may only be discovered through careful post-marketing monitoring or pharmacoepidemiology). Pharmacovigilance is specifically intended to deal with the cases where the unknown or inadequately characterised deleterious effect becomes apparent post marketing.

Even when the uncertainties are adequately monitored, the apparent 'failures' (including products removed from the market as a result of good pharmacovigilance) may become publicised and are all too easily taken to represent system failures rather than the consequences of dealing with uncertainties.

Jasanoff (2007) points out that uncertainty, ignorance and indeterminacy are always present and suggests that:

> '. . . researchers and policy makers (perhaps better read as 'scientists and the general public') need ways for accommodating the partiality of scientific knowledge and for acting under the inevitable uncertainty it holds'.

She also pleads for humility, concerning both the limits of scientific knowledge and when to stop turning to science to solve problems. The proposition put forward by Jasanoff (2007) implies a plea to recognise that the recommendations of scientific advisory committees comprise type 2 advice rather than type 1 advice. She also suggests that there is a role for 'lay' members of scientific advisory committees to bring different perspectives that challenge the implicit assumptions of scientists and thus allow the uncertainties in the decisions taken to become more apparent.

The type 1 approach, with its assumed certainty, has become less and less acceptable with the passage of time (see, for example, Illing, 2001, p.16). If the type 1 approach is persisted with, differences between the technical risk assessors' and the public's perception of the adequacy of the risk analysis are exaggerated. Differences may also be exacerbated by the remoteness (or perceived remoteness) of the body taking the decision from those affected by the decision.

Risk evaluation

The organisational structures of risk evaluation

In the case of the EMEA, its Management Board, which in effect sets the framework at the behest of the EU Parliament and Council of Ministers, includes nominees from the European Parliament, the European Commission and national governments and representatives of relevant organisations, including a representative of veterinarians' organisations. The risk evaluations that lead to Agency decisions concerning acceptability are based on recommendations from the CVMP, which is composed of national experts. The societal input to the framework is therefore clear. However, the input to risk evaluations is indirect and has to be exercised through the Management Board.

When national decisions are taken in the UK, for example, the advice from the UK Veterinary Products Committee is from a body that includes 'lay members', as well as members appointed from those affected by the decisions, such as farmers.

In the EU the CVMP consists of technical experts from the national governments and other institutions making risk evaluation recommendations on behalf of society as a whole. There is no direct stakeholder input to the committee. This lack of stakeholder input to the risk evaluations by the CVMP means that the technical experts are taking societal decisions. This is true across the EU in other regulatory areas where other EU bodies consisting of technical experts are still making risk evaluation

recommendations on behalf of society as a whole without direct societal input.

Influence of risk perception on risk evaluation

The type 2 approach of Stilgoe *et al*. (2006) has been implemented at both EU and UK levels, at least in part. There have been moves towards openness and transparency, with publication (or making available on the internet) of information on Committee membership, the advice that is offered by the Committee and (when commercial confidentiality allows) either the underlying material on which that advice is based or, at least, a summary of that material.

Wider recognition of the need for 'stakeholder' involvement in risk evaluation has originated with those concerned with risks to the environment, is relatively recent and is largely Anglo-Saxon in origin (Presidential/Congressional Commission on Risk Assessment and Risk Management, 1997; Royal Commission on Environmental Pollution, 1998). Aven and Kristensen (2005) include this in their 'comprehensive approach' to risk evaluation. They say that a broad perspective is required which typically includes:

- observational data;
- risk analysis descriptions;
- perceived risk information;
- judgements made by people with special competence;
- expert groups;
- groups of representatives from the various interested parties to build trust and consensus.

I would argue that 'observational data' include the information made available, initially in the form of the experimental data included in the risk characterisation and subsequently by incorporating risk monitoring data (including pharmacovigilance data) into the iteration of the risk evaluation. The risk analysis descriptions are the summaries of the data and the margins of exposure. I have argued that perceived risk information (in the form of societal judgements) is incorporated in the uncertainty factors used to determine the acceptable doses in toxicological risk evaluations (Illing, 1999). Judgements made by people with special competence and expert groups (including those judgements given in advice to and by scientific committees) may be either part of the risk characterisation, if purely associated with technical issues, or part of the risk evaluation if dealing also with societal issues. 'Groups of representatives from the various interested parties to build trust and consensus' are stakeholders, and their presence is intended to try to ensure that the ways in which risks are perceived by the general public are taken into account in the risk evaluation and to act as a conduit in communication between the experts and the general public.

In the occupational field, stakeholder participation in the UK can be traced back to the Robens report of 1972 (Anonymous, 1972; Simpson, 1973) and the Health and Safety at Work Etc. Act 1974 (Health and Safety Executive, 2004). It now includes 'lay' members of expert scientific advisory committees, including the UK VPC.

Risk evaluation is an iterative operation and, like science generally, it changes with new knowledge and new interpretations of existing knowledge. It also changes with new public perceptions concerning the type of risk being evaluated. Clearly scientific advisory committees have to reflect the probable views of experts generally, and so *can* act as conduits to accessing information and the scientific debate on the issues associated with toxicity and efficacy. They also have to reflect how society views the risks posed. Finally, and as already stated, advice is conditional and depends on the questions being asked, on how the questions are framed and on how society sees 'acceptable risk'/'safety'.

Conclusions to risk perception

There is a difference between 'objective' and 'perceived' risk that needs bridging. An

understanding of how risks are perceived and communicated is required. Transparency and openness when evaluating and managing risks are attempts to involve and inform those concerned with or affected by decisions. It is essential that communication between all those involved in the risk assessment and management process takes place.

Whether stakeholder involvement with the scientific advisory committees is essential is a matter that can be debated further. Aven and Kristensen's views (2005) can be interpreted as an indication that stakeholders should be involved if the risk evaluation is to be comprehensive in its approach. Stilgoe and coworkers (2006) point out that the inclusion of 'lay' members should encourage the challenging of implicit assumptions made by scientists. This should include the implicit assumptions by scientists concerning societal judgements (perceived risks), and hence help to achieve some convergence between 'objective' and 'perceived' risk.

A potentially serious problem with the internationalisation of standard setting is remoteness or a perception of remoteness. The driving force to internationalism is the removal of barriers to trade which is seen by most observers as a benefit. With internationalisation comes a 'lowest common denominator' approach, and hence less ability to tailor the decisions to specific local circumstances. For example, health effects can be seen in terms of extension of life expectancy or improvement in quality of life. Where baselines are low, as occurs in some less developed countries, these improvements can be effected readily. However, they then may unmask illnesses that are thought to require long development times, such as cancers. They may also unmask and render more difficult to cope with effects on reproduction, as might occur with a reproductive toxicant, due to lower birth rates and much lower perinatal mortality. Imposition of standards appropriate to advanced economies may be inappropriate for less developed countries.

Remoteness also gives greater opportunity for 'pressure groups' to gather individuals from cultural prototypes that are dissatisfied in order to exert influence on decisions concerning risk. 'Pressure group' influence is likely to be greatest when 'control' over or assent to decision taking is least obvious and at its most remote. Thus there are risks associated with internationalisation.

Risk communication

As indicated above, it is essential that communication takes place between all those involved in the risk assessment and management processes. Communication is essential to the development and maintenance of trust. Trust has to be developed in both the process and the organisations carrying out the process. This applies in terms of both the framing of the structure of the organisation set up to undertake the work and the way in which the work is undertaken. It also involves the investigation of incidents in order to ensure that appropriate organisational structures are in place, both to make the judgements concerning the acceptability of the risk and to react appropriately when incidents occur.

Slovic (1999) states:

> 'Like perception, trust correlates with gender, race, worldviews and effect.'

and:

> 'Trust is fragile. It is typically created slowly, but it can be destroyed in an instant – by a single mishap or mistake. Thus, once trust is lost, it may take a long time to rebuild to its former state. In some instances, lost trust may never be regained.'

He also states:

- Negative (trust destroying) events are more visible or noticeable than positive (trust building) events.
- When events are well-defined and do come to our attention, negative (trust destroying) events carry much greater weight than positive events.

- Sources of bad (trust destroying) news tend to be seen as more credible than sources of good news.
- Distrust, once initiated, tends to reinforce and perpetuate further distrust.

Kasperson *et al*. (1988) proposed a model, 'the social amplification of risk', in order to address why some relatively minor risks or risk events elicit strong public concern, yet some major risks/risk events pass relatively unnoticed. The claim is that amplification of risks occurs at two stages: transfer of information about the risk, and in the response mechanisms of society. Signals about risk are processed by the individual and social amplification stations, including the scientist who communicates the risk assessment, the news media, cultural groups and interpersonal networks. The amplification leads to behavioural responses which, in turn, lead to secondary impacts.

Just as individuals give greater weight and attention to negative events, so do the news media elements. Much of what the media report is bad (trust destroying) news. In addition, witness the social phenomenon of the rise of powerful special interest groups, well funded (often by a fearful public) and sophisticated in using their own experts and the media to communicate their concerns and their distrust to the public to influence risk policy debates and decisions. An essential part of the building of trust and hence breaking the cycle of social amplification is improving means of public participation within the deliberative process. It includes mediation, oversight committees and 'stakeholder' involvement, as well as openness in decision taking and the use of appropriate and appropriately trained spokespersons.

Some specifics relating to veterinary pharmacovigilance

What may be detected?

The adverse effects detected by veterinary pharmacovigilance programmes may be:

- known effects seen with greater intensity or frequency than expected from initial risk evaluation;
- effects known about but not quantifiable (known unknowns) in the initial risk assessment and hence either considered for monitoring or disregarded in the risk evaluation;
- effects that were not known about and were not (or even could not be) detected or predicted in the initial risk assessment (unknown unknowns), in which case they could not be considered in the initial risk evaluation.

What is an adverse event from the point of view of recording it?

An adverse reaction is:

> 'A reaction which is harmful and unintended and which occurs at a dose normally used in animals for prophylaxis, diagnosis or treatment of disease or the modification of physiological functions'.

These adverse reactions include:

- Serious adverse reactions:

  'An adverse reaction which results in death, is life threatening, results in significant disability or incapacity, is a congenital anomaly/birth defect, or which results in permanent or prolonged signs in the animals treated'

- Unexpected adverse reactions:

  'An adverse reaction, the nature, severity or outcome (of which) is not consistent with the summary of product characteristics' and

- Human adverse reactions:

  'A reaction which is noxious and unintended and which occurs in a human being following exposure to a veterinary medicine'
  (EMEA, 2006).

These definitions are very general and, although 'severe' is defined, 'adverse' is not. The definition

of unexpected adverse reaction identifies that the monitoring being undertaken includes monitoring for 'unknown unknowns', effects that the initial risk assessment on which the decision to authorise was taken did not identify because it was not capable of identifying them.

It is notable that, in the UK, the veterinary pharmacovigilance scheme was known as the 'Suspected Adverse Reactions Surveillance Scheme' (Woodward, 2005). The EMEA (2006) stated that the new provisions being implemented encouraged the reporting, especially of those suspected adverse reactions that are 'serious and unexpected in animals, and those occurring in human beings'. In other words, reported adverse reactions are only 'suspected' until they have been evaluated and causality has been established.

In any reporting scheme, the reporting implies that at least someone has perceived the ill-health as an adverse reaction, and there is clear room for a difference in perception, both of the meaning of 'adverse' and of the question of causality, between the reporter and the evaluator. It also implies that the responsibility for providing consistency of what constitutes 'the final decision' lies with the evaluator. Clearly this leaves scope for reporter dissatisfaction with the evaluation.

What frequency of adverse event merits management?

Determination of what frequency of adverse event is associated with a 'broadly acceptable' risk also matters. For a serious, life-threatening effect or death (actual foreshortening of life), only low frequencies of occurrence are acceptable. For minor ill-health a more frequent occurrence may be acceptable. However, there is no consensus about what frequencies of sub-lethal effects are 'broadly acceptable' and it usually requires commissioning of morbidity and mortality studies (epidemiology or pharmacoepidemiology) to determine the frequencies of ill-health or death occurring due to the exposure. It is likely that the frequencies considered 'broadly acceptable' (or 'tolerable') will differ according to the group (the target species, collaterally damaged animal/plant species, occupationally exposed humans, food-exposed general public, environmentally exposed general public) and how the members of that group perceive risk generally and, in the case of the food-exposed general public, food-borne risk in particular.

The perception of what constitutes a 'broadly acceptable' and a 'tolerable' risk will vary according to the type of exposure claimed to cause the effect, the seriousness of the adverse event and its frequency, as viewed technically and as perceived by each particular potentially exposed group, along with the perceived benefits, as well as the perception of who derives those benefits (animal, owner, farmer).

How is the decision taken?

In general, when a member state considers, on the basis of pharmacovigilance data, that a marketing authorisation needs to be suspended, withdrawn or varied to restrict the indications or availability, amend the posology, add a contra-indication or add a new precautionary measure, the issue is considered by the appropriate advisory or regulatory body (the CVMP for centralised procedure products (EMEA, 2006) and national authorities and their advisors for products registered through the mutual recognition, decentralised or national procedures). This implies that these are acting as final evaluators. Member states raise the question and the CVMP or national advisory bodies provide an opinion, which is then implemented (or otherwise) through the EMEA and European Commission, or through national regulatory bodies (as appropriate). These organisations need to take into account the perceptions of the different interest groups when reaching their conclusions.

The potential for differences in the technical evaluation and the perception of adverse events leads to a need for a speedy and effective evaluation process. It is easier to implement appropriate management measures in an orderly fashion

before public perceptions have become inflamed through loss of trust instead of having to overrespond in a situation in which trust has already been lost and, possibly, litigation entered into. When compared with what happened before the implementation of pharmacovigilance schemes, clearly pharmacovigilance schemes have had and will continue to have a major role to play in ensuring that speedy and effective evaluation occurs.

Conclusions

Veterinary medicinal products are chemicals, sometimes complex chemicals, intended for specific medicinal uses in animals or in groups of animals. They are subjected to risk analysis, both initially, before marketing and, post marketing, through monitoring either periodically or when suspicions are aroused concerning the validity of the risk evaluation. The risk evaluation (and re-evaluation) has to take into account both the 'objective' (expert opinion) risk and the way in which society, and the relevant stakeholders, perceive the risks. The perception of the validity of the judgements made on society's behalf concerning the risks depends on trust. Consequently, structures for risk decision taking must understand societal concerns and take them into consideration when making judgements. Multidirectional risk communication is essential if the process and the outcomes are to be accepted. Risk decisions must be timely if trust is not to be lost. Veterinary pharmacovigilance schemes are a key element in ensuring that re-evaluation decisions are timely and that trust is engendered.

Embedded in these schemes are societal judgements concerning what constitutes 'adverse' under different exposure circumstances. Also embedded are decisions concerning what are the (low) maximum frequencies and seriousness of effects deemed 'broadly acceptable' (or 'tolerable') by society and those affected ('stakeholders') under different exposure circumstances. Different groups within society (and within those considered 'stakeholders') may have different views of the world, and hence different perceptions of the risk and of its acceptability, and they may form campaigning organisations (pressure groups) to further their opinions and achieve their goals. These different perceptions, if allowed to persist and gain widespread public credence, can also lead to litigation (worker versus employer or owner versus drug company or veterinarian, for example), 'defensive medicine' (protecting the practitioner from litigation rather than seeking to benefit the individual, the animal and society), and a desire to introduce new statutory measures.

Good and careful communication (to and from a well-structured and capably organised system for evaluating the risks) is the most appropriate way of seeking convergence of the expert view and the public's perception, and hence obtaining trust in the process by which the risks arising from veterinary products are regulated. By minimising the divergence of perception, the likelihood of ensuing litigation is also minimised.

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31 The role of quality assurance in veterinary pharmacovigilance

R. Visanji and H. Politis-Norton

Introduction

Quality assurance (QA) is described as the formal methodology or programme for the systematic monitoring and evaluation of the various aspects of a project, service or facility to ensure that standards of quality are being met (Merriam-Webster Online, 2008; http://www.m-w.com).

Pharmacovigilance is an important tool for monitoring and increasing overall drug safety as it assures continuous surveillance of veterinary medicinal products under field conditions after the granting of marketing authorisations, licences or approvals.

In the EU, veterinary product marketing authorisation holders (MAHs) have the legal responsibility to ensure that there is an appropriate system of pharmacovigilance in place to ensure that adverse events (AE) reported with authorised veterinary medicinal products, both in the EU and in third countries, are processed in accordance with requirements as per authorisation type and product type, e.g. vaccine or pharmaceutical (Anonymous, 2001a, b, 2004). Additionally there is the expectation that appropriate action can and will be taken to prevent overall system failure.

Therefore an MAH's QA pharmacovigilance programme should at a minimum:

- perform a formal review of internal and external (e.g. subcontractors) pharmacovigilance systems and associated processes to ensure that these meet international and local regulatory requirements as well as company set standards and policies;
- allow for problem identification and implementation of Corrective Actions and Preventative Actions (CAPA), with subsequent follow-up;
- facilitate during Competent Authority/Regulatory Authority pharmacovigilance inspections (see section headed 'Pharmacovigilance inspections');
- establish and/or improve levels of pharmacovigilance compliance between internal challenges and the external changing industry, environmental and regulatory landscape (see *Figure 31.1*).

QA pharmacovigilance audit programmes

QA is responsible for the identification, execution and reassessment of the pharmacovigilance audit programme. A quality audit is defined as the

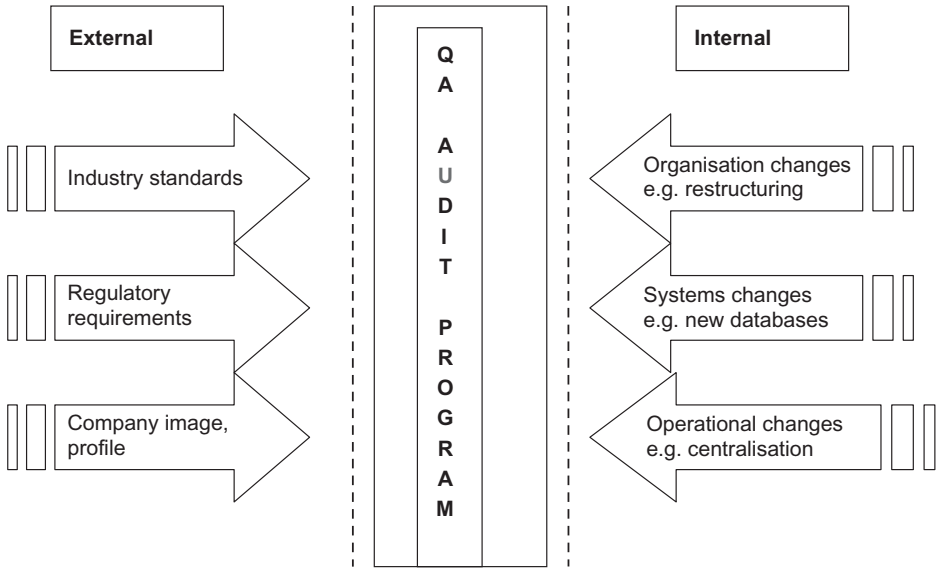

Fig. 31.1 Challenges faced by the QA pharmacovigilance audit programme.

'. . . systematic and *independent* examination to determine whether quality activities and related results comply with planned arrangements and whether these arrangements are implemented effectively and are suitable to achieve objectives' (International Standards Organisation (ISO), 1990).

Identification

The audit programme is developed:

1. by identification of the audit universe; and
2. by performing a risk analysis.

The following are examples of what constitutes a typical pharmacovigilance audit universe:

- Headquarter and subsidiary MAH departments responsible for pharmacovigilance activities.
- Other headquarter and subsidiary MAH departments through which initial and follow-up information of AEs could be received, e.g. clinical, medical/technical information, customer service, marketing and sales (medical representatives), manufacturing quality assurance.
- External sources of AEs, e.g. subcontractors, contractual partners, vendors (e.g. call-centres), Clinical Research Organisations (CROs).

Once the audit universe has been described the frequency of audits needs to be identified. Generally this can be accomplished through a risk analysis. A strategic, risk-based audit programme assists in prioritising audit activities consistent with the organisational goals and available resources. Risk is described as the combination of the probability of occurrence of harm and the severity of that harm (ISO, 1999). For the purposes of the QA pharmacovigilance audit programme, 'risk' could be evaluated against:

- the regulatory environment, in particular the increase in the number of Competent/Regulatory Authority Pharmacovigilance inspections;
- operational changes to ensure optimal efficiency and effective allocation of resources;
- changes in internal and external standards, e.g. regulatory legislative requirements;
- organisational changes, e.g. following a merger or acquisition;

- business-specific risk, in particular to protect and enhance company assets and company image.

Execution

As soon as the audit programme has been developed and agreed with senior management it is formally announced to the organisation, in particular to those groups who will be audited. The announcement will include proposed audit dates and number of auditors per audit. Each audit/audit team will have an allocated audit team leader who acts as a contact point and is responsible for the audit planning, supervision and reporting.

Most audits generally comprise four distinct phases, namely:

- Phase 1: Off-site pre-audit preparations
- Phase 2: On-site audit conduct
- Phase 3: Off-site post-audit activities
- Phase 4: Post-audit verification of completed actions/commitments

Phase 1: Off-site pre-audit preparations

- Creating a plan to document how the audit will be conducted.
- Developing audit work documents, e.g. checklists, questionnaires and data sampling criteria.
- Defining the audit agenda, objectives and scope and identifying the type of information to be requested from the auditees, e.g. Standard Operating Procedures (SOPs), Departmental/Organisational Organograms, applicable legislation, adverse reaction reports, periodic safety update reports, etc.
- Communication of the audit agenda to the auditee and requests for information.

The audits may focus on a particular product, company system or safety monitoring process, or they may extend to the entire local or even global pharmacovigilance system. The scope of veterinary pharmacovigilance is much broader than for human medicinal products, at least in the EU, and may include not only AEs in animals but also lack of efficacy, AEs following off-label use, AEs in humans as users or consumers, validity of withdrawal periods for drug residues in tissues of food-producing animals (e.g. eggs, milk, meat), maximum residue limit (MRL) violations and adverse environmental effects including ecotoxicity issues. For veterinary medicinal products, all suspected adverse drug reactions in humans should be considered as a serious adverse reaction and processed through the company's expedited reporting process, even if the symptoms resulting from exposure are minor and transient. These layers of complexity should carefully be considered when defining the scope of quality audits.

Phase 2: On-site audit conduct

- Extensive and/or repeat face-to-face interviews.
- Documentation review to validate information provided during interviews.
- Evaluation of how safety information flows in and out of the pharmacovigilance system and associated processes.
- Review of the presence of and adherence to Standard Operating Procedures (SOPs) or company policies describing pharmacovigilance processes/system.
- Presence of clearly defined personnel roles and responsibilities.
- Compliance with local and/or international regulatory requirements, as appropriate.
- In the EU, assessment of the role of the Qualified Person for Pharmacovigilance (QPPV), his or her activities and documentation (job description, curriculum vitae) and back-up procedure[1] in the QPPV's absence.

[1] Article 74 of Directive 2001/82/EC requires the holder of each marketing authorisation to 'have permanently and continuously at his disposal an appropriately qualified person responsible for Pharmacovigilance'.

- The collection, collation, processing, quality control, causality coding, classification and veterinary review of AEs, and in particular:
  - Presence and/or effectiveness of AE procedure to ensure notification of outcome information, identification of duplicate reports, quality control checks of processed data against source information, codification, expedited reporting (compliance metrics), electronic reporting as required by local or international regulatory requirements and reconciliation with other MAH AE sources, e.g. medical/technical information, clinical research and manufacturing quality assurance.
  - Presence and/or effectiveness of follow-up procedures are important as, for example, a violation of the withdrawal time (detected by the presence of violative residues of the product in meat or milk, etc.) would be considered an adverse event and these are frequently reported to the MAH directly from the field and frequently require follow-up. Follow-up of adverse reactions in animals with the responsible veterinarian; follow-up of adverse reactions in humans with attending physicians.
  - Management of various AE types, i.e. spontaneous, clinical trials, literature, product quality complaints associated with AEs and detrimental effects of environmental 'contamination' due to animal health products being administered in feed or water and the potential impact of medicated feed or water being discarded into the environment, from animal waste or from contaminated litter, or from direct environmental contamination, e.g. with organophosphorus or synthetic pyrethroid sheep dips.
  - Management of AEs from external sources, i.e. animal health professionals (veterinarians), animal owners, licensing partners, competent authorities, literature.
  - Management of AEs from internal sources, i.e. company personnel such as medical/veterinary representatives, medical/technical information, manufacturing quality assurance and clinical veterinary representatives.
- Reporting of Serious Adverse Drug Reactions (SARs) to Competent/Regulatory Authorities in expedited and/or aggregate report format as per local or international regulatory requirements.
- Aggregate reports – e.g. European Union PSURs/USFDA periodic drug experience reports are assessed as to whether they are in the correct format, of good quality and the level of compliance (non-submission, on time submission) as per local and/or international regulatory requirements.
- Signal detection and review of safety information. Auditors will review not only compliance with these requirements but also that data trending is sufficiently robust to ensure that any applicable label changes (e.g. disposal instructions) are implemented in a timely manner.
- Whether benefit:risk assessments are conducted/adequately conducted and that mechanisms are in place to ensure subsequent notification to Competent Authorities and animal health professionals of changes in benefit:risk balance.
- If there are procedures in place, to manage all responses to requests for information from Competent/Regulatory Authorities and meeting commitments associated with marketing authorisations/licences/approvals.
- How product recalls, urgent safety restrictions and safety variations are managed.
- Management of databases or recording systems, e.g. back-up of data, security, deletion of individual AE reports, validation and business continuity plans.
- How the users of databases or other recording systems are supported, e.g. user training, database and/or coding manuals, 24-hour helpdesk support.
- Whether a procedure(s) is/are in place to ensure personnel training, which is usually based on role and responsibilities with regards

to AE processing, and tracked and/or otherwise documented. Training could be *internal*, e.g. SOP, on-the-job training, or *external*, e.g. university courses, conferences, seminars.
- That there is a policy in place to ensure secure and timely archiving and correct retention of AE reports, aggregated reports, source documentation and other essential information.

Phase 3: Off-site post-audit activities

Off-site post-audit activities include the draft/final audit report and auditee response in the format of a CAPA Plan. The CAPA Plan comprises discrete identified actions/commitments allocated to responsible persons with realistic targets due and/or end dates. It is the responsibility of the auditees to notify the audit team leader of completed commitments and reasons for delays or extensions.

Phase 4: Post-audit verification of completed actions/commitments

Post-audit verification of completed actions/commitments are described in the auditee CAPA Plan. This activity can be accomplished in the format of separate verification audits or alternatively included in the objectives/scopes of future re-audits.

Reassessment

An ongoing critical review is essential to ensure that standards are consistently met and where failings have been identified or recognised the necessary corrective and preventative action is taken.

A good quality system is a dynamic quality system where needs for improvements are identified in a timely manner and corresponding actions are implemented also in a timely manner. Therefore more and more companies conduct shorter audits focusing on a more limited scope and thus enabling the rapid communication of audit findings and rapid implementation of the findings.

The audit programme should be monitored and reviewed on an ongoing basis to ensure that:

- Desired results are being achieved.
- Changes and challenges within the internal and external audit universe are being met.
- The independence of the auditing group and/or programme has not been compromised.
- There is a consistent audit methodology across areas being or likely to be audited.
- Auditors are evaluated and trained accordingly.

Pharmacovigilance inspections

Competent/Regulatory Authorities can be obligated by local and/or regional regulations to perform routine pharmacovigilance inspections (Anonymous, 2001a, 2004, Article 20 and Article 44(1) respectively; see Chapter 9). The scope of these inspections can include a variety of departments, for example:

- pharmacovigilance;
- medical/technical information;
- regulatory affairs;
- information technology;
- veterinary clinical research;
- quality assurance/auditing departments;
- manufacturing;
- contractual partners;
- subcontractors.

Previously the majority of the inspections were generally related to submission of new product applications. The scope of the inspections would be limited to the activities performed during the development of the new drugs. The main focus of inspections was to verify that data submitted for approval were accurate and complete.

The focus has now changed and inspections are moving away from data to the processes involved in their generation. The effects of a veterinary medicinal product does not end with

registration and new risks will be identified once the product reaches the market and these should be identified and communicated to relevant authorities in a timely manner. Therefore vigorous pharmacovigilance systems are essential, as are regulatory inspections to verify that companies act responsibly.

Quality assurance role

QA plays an important role in both inspection preparedness and facilitation during the inspection. Inspection preparedness may include but is not limited to:

- notifying senior management of announced inspection;
- establishing and maintaining relevant points of contact in the Competent/Regulatory Authority once notified of pending inspection;
- identifying internal inspection task groups;
- identifying incomplete commitments from previous internal audits or external inspections;
- writing up an inspection plan, assigning roles and responsibilities before, during and after inspection;
- training personnel to be interviewed;
- 'mocking' inspection exercises;
- preparing documentation and associated quality control checks;
- facilitating during the inspection, e.g. documentation control, scribing and the chaperoning of inspectors.

Conclusions

Pharmacovigilance audits and QA activities play an important role in the operation of company pharmacovigilance undertakings. They ensure regulatory compliance and play a part in the smooth running of systems, while helping to ensure data integrity. They have a crucial role to play in preparing and indeed being prepared for regulatory pharmacovigilance inspections. QA and its associated activities should be regarded as integral and invaluable tools in the operation of any pharmacovigilance system.

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32 Concordance between results from animal toxicology studies and adverse reactions in animals

K.N. Woodward

Introduction

Drugs, including veterinary drugs and their components, are tested in toxicology studies to fulfil regulatory requirements and to ensure that they comply with various safety requirements including consumer and user safety. They are also often investigated in supplementary studies so that mechanistic aspects of their biological activities may be better understood (Ritchie, 1991; Woodward, 1991a, 1992a, 1997, 2000, 2004; Paige *et al*., 1997; Paige, 1998; Friedlander *et al*., 1999; Dayan, 2000; Gad and Chengelis, 2001). Although these studies are conducted for predictive purposes for human health, they may also have utility in predicting the likelihood of adverse drug reactions occurring in the treated animal patients.

Unfortunately, most of the toxicology data generated in support of veterinary medicines remains confidential for commercial reasons. The same applies to much of the pharmacovigilance data. Hence, comparisons are difficult, and where information is available from the regulatory authorities, it is often too brief to be used for purposes of analytical study. Exceptions to this are the reports and toxicology monographs published by the Joint FAO/WHO Expert Committee on Food Additives (see Woodward, 2005). Along with other limited toxicology data in the public domain, these can be used with pharmacovigilance data for comparative purposes. However, it does mean that these comparisons are limited to a restricted number of substances.

This chapter examines the publicly available data on a number of veterinary drugs in order to gain some insight into the relationships between preclinical toxicity and pharmacovigilance data and to establish whether or not laboratory toxicology data are suitable for predicting adverse drug reactions in animals.

Selected drugs

Benzimidazole anthelmintic drugs

There are a number of drugs in the benzimidazole group which are currently used in veterinary medicine as anthelmintic agents. They include thiabendazole, albendazole, fenbendazole, flubendazole, mebendazole, oxfendazole, oxibendazole, triclabendazole and albendazole sulphoxide.

Febantel and netobimin are two prodrugs which are converted to fenbendazole and

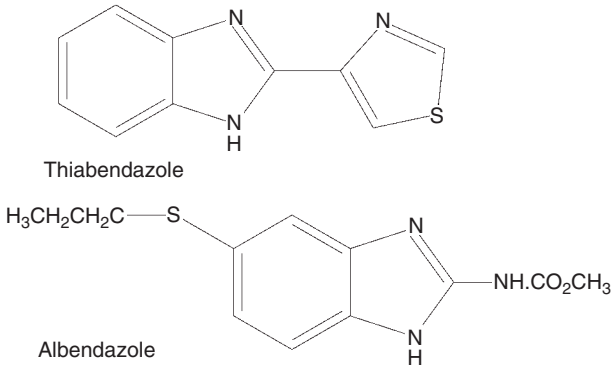

Fig. 32.1 Thiabendazole and albendazole.

albendazole respectively in vivo following administration (McKellar and Scott, 1990). These drugs are used to treat a number of parasitic infections in livestock, companion animals and birds (Brander, 1982; McKellar and Scott, 1990; Reinemeyer and Courtney, 2001), while albendazole has been used in human therapy (Basuroy *et al*., 2008; Kayaoglu, 2008; Rajshekhar, 2008).

The chemical structures of the benzimidazoles are typified by thiabendazole, one of the first of the group to be introduced, albendazole (McKellar and Scott, 1990) (*Figure 32.1*), albendazole oxide, oxfendazole, fenbendazole, oxibendazole and the prodrugs febantel and netobimin. Others in the group are rarely or no longer used including parbendazole, cambendazole and luxabendazole (McKellar and Scott, 1990).

The benzimidazoles are thought to exert their anthelmintic effects by binding to tubulin and thus acting as mitotic spindle poisons by disrupting cell division; they may also disrupt cellular bioenergetics by disrupting proton transfer across cell membranes (Delatour and Richard, 1976; Davidse, 1977; Sharma and Abuzar, 1983; McKellar and Scott, 1990; McCracken and Stillwell, 1991; Ramirex *et al*., 2007).

Studies in animals have suggested that some of the benzimidazoles may be teratogenic. Parbendazole produced evidence of teratogenicity is rats and sheep (Di Cuollo *et al*., 1974), while albendazole may be teratogenic in rats under certain conditions (Delatour *et al*., 1982). Oxfendazole, cambendazole, mebendazole and oxfendazole are embryotoxic in rats (Delatour *et al*., 1974, 1982, 1984; Delatour, 1983; El-Makawy *et al*., 2006). Doses of albendazole up to and including 10 mg/kg body weight per day through days 6–15 of gestation had no effects on the development of rats, but doses of 20 or 30 mg/kg body weight per day resulted in fetal deaths, reduced ossification and malformations (Teruel *et al*., 2003). In rats and sheep, albendazole and its metabolites are able to cross the placenta and reach the fetus (Capece *et al*., 2002, 2003); doses of 10 or 14 mg/kg body weight per day on day 10 of gestation resulted in skeletal anomalies in rats. The prodrug netobimin resulted in skeletal and vascular malformations when given to pregnant sheep on day 17 of gestation (Navarro *et al*., 1998) and resulted in resorptions and an increased incidence of skeletal malformations in rats (Navarro *et al*., 1999). Fenbendazole has not produced embryotoxic effects in a number of species tested (Booze and Oehme, 1982). Thus, there is some evidence that *some* benzimidazoles may induce embryotoxicity and birth defects at higher doses (in excess of 10 mg/kg body weight) when given during sensitive stages of pregnancy.

When given therapeutically to non-pregnant animals, benzimidazoles produce few adverse effects, even when given in doses several times in excess of those recommended. Thus, fenbendazole produced no adverse effects in cats when given at up to 5 times the recommended dose and 3 times the recommended duration of dosing (Schwartz *et al*., 2000). No toxic effects were seen in cattle given 2,000 mg/kg body weight fenbendazole (the therapeutic dose is around 5 mg/kg body weight) (Muser and Paul, 1984). Fenbendazole is not significantly toxic in pigs at doses of up to 125 mg/kg body weight per day over 5 days (Booze and Oehme, 1983; Hayes *et al*., 1983) nor is flubendazole when given at multiples of the therapeutic dose (Campbell *et al*., 1983).

Oxibendazole was well tolerated by cattle and sheep (Theodorides *et al*., 1977), while albendazole had no major adverse effects on developing bovine fetuses (Theodorides *et al*., 1993). Pigeons

and doves treated with 50 mg/kg body weight per day fenbendazole over several days tolerated the drug well, although those given 100 mg/kg doses showed weight loss and higher mortality than those given the lower dose (Howard *et al*., 2002). Thus, the drugs are well tolerated in mammalian and avian species, although they may be more toxic to some types of bird when given at higher doses.

The results of some studies with benzimidazoles in pregnant livestock are given in *Table 32.1*.

The results suggest that benzimidazoles are safe for use in pregnant animals even at doses in excess of those normally recommended for anthelmintic use. Although adverse effects were seen in the offspring of pregnant sheep after treatment with netobimin, these occurred at doses over 3 times in excess of those recommended for normal use. However, care should be taken with off-label use of this class of drugs as this has resulted in abnormalities in neonates (Scholes *et al*., 2008).

Examination of the toxicology data in laboratory animals (*Table 32.2*) reveals a similar pattern of embryotoxicity and anomalies with a number of benzimidazoles, generally at the higher doses employed in the studies.

From the data presented in *Table 32.2* it is evident that the benzimidazoles can exert embryotoxic effects when given to laboratory animals during sensitive periods of gestation. The effects are dose-related and no-observed effect levels can be identified from the data in the literature and the JECFA monographs. The mechanisms by which they exert these effects are unknown, although it seems likely that their antimitotic effects arising from interaction with tubulin probably play a crucial role. The data suggest that several of the metabolites of each drug are inactive whereas others, notably the sulphoxides, are active (Delatour, 1983; Delatour *et al*., 1984; McKellar and Scott, 1990; Dayan, 2003). The data also suggest that these adverse effects are generally seen at high doses, often in excess of those used therapeutically. Nevertheless, the laboratory animal studies were predictive of the adverse reproductive effects seen occasionally in animals treated for parasitic diseases.

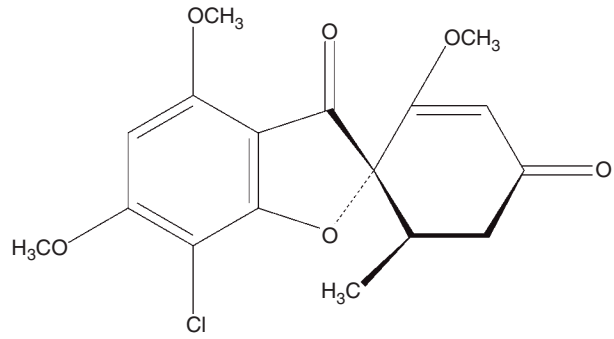

Fig. 32.2 Griseofulvin.

Griseofulvin

Griseofulvin (*Figure 32.2*) is a fungal metabolite produced by *Penicillium griseofulvum* and *Penicillium patulum* strains. It is used in human medicine for the treatment of dermatomycoses in skin, hair and nails and until recently was used widely in veterinary medicine for the treatment of fungal infections, mainly against ringworm infections and, increasingly, in the treatment of tinea capitis in children (Russel and Russel, 1992; Knasmuller *et al*., 1997; Ali *et al*., 2007).

Older toxicology studies showed it to have low acute and repeat dose toxicity in rodents and cats, and there were apparently no effects on reproduction in some limited studies in rats (Sharpe and Tomich, 1960). However, therapeutic treatment of pregnant cats for ringworm resulted in malformations in the offspring including cleft palate, exencephaly, caudal displacement and hydrocephaly, along with multiple skeletal abnormalities including cranium bifidium, spina bifida and abnormal vertebrae. Cyclops and anophthalmia also occurred (Scott *et al*., 1975). Similar cases have been reported by Gillick and Bulmer (1972), Gruffydd-Jones and Wright (1977) and Turner (1977). Cats appear to be more susceptible to the toxic effects of griseofulvin (Kunkle and Meyer, 1987), but it is not known if this species is more

Table 32.1 Results of studies of the effects of benzimidazoles in pregnant livestock animals.

Benzimidazole	*Species*	*Dose (typical therapeutic dose) (mg/kg)*	*Administration period (days after mating)*	*Positive (+) or negative (–) findings*	*Reference*
Oxfendazole	Pigs	4.5	12, 19, 26, 23	–	Morgan, 1982
			14, 21, 28, 35	–	
			16, 23, 30, 37	–	
		13.5 (5)	12, 19, 26, 23	–	
			14, 21, 28, 35	–	
			16, 23, 30, 37	–*	
Netobimin	Sheep	20 (7.5)	17	+**	Navarro *et al.*, 1998
Oxibendazole	Sheep	30	10, 17, 24, 32, 45, 52, 59	–	Theodorides *et al.*, 1977
Fenbendazole	Cattle	5 (7.5)	Days 12–21, then at 3-week intervals until month 5, then 2-month intervals until calving	–	Muser and Paul, 1984
Fenbendazole	Cattle	50	Days 12 and 21 of gestation	–	JECFA, 1991a
Fenbendazole	Sheep	15	Four doses at variable times	–	JECFA, 1991a
		50	96 hours after mating.	–	
		15 (7.5)	Every 4 weeks during gestation; total of seven doses	–	
Fenbendazole	Horses	10 or 25 (7.5)	Last trimester of pregnancy	–	JECFA, 1991a
Albendazole	Cattle	30 (5)	Four consecutive weeks, spanning weeks 5–33 of gestation; additional doses in weeks 35 and 39	–	Wetzel, 1985
Albendazole	Sheep	0–20 (5)	17	–†	JECFA, 1990
Albendazole	Sheep	Unknown	4 weeks after mating	+††	Scholes *et al.*, 2008
Mebendazole	Sheep	Unknown	50–60	Equivocal§	Scholes *et al.*, 2008
Triclabendazole	Sheep	Up to 10	At various stages of gestation – 12, 17, 21, 24 or 28	–	JECFA, 1993
		50 (10)		–	

* No effects on behaviour and no anatomical abnormalities.
** Abortions and skeletal, renal and vascular abnormalities.
† Increases in premature births at 20 mg/kg; all premature lambs stillborn. Increases in prognathia, scoliosis and spina bifida. Displaced or absent kidneys at 15 and 20 mg/kg.
†† Product used off-label; administration to pregnant ewes contraindicated, effects included scoliosis, hemimelia and arthrogryposis, hydrocephalus and renal agenesis.
§ Inbreeding could not be excluded; effects included amelia, hemimelia and arthrogryposis.

Table 32.2 Summary of data from developmental toxicity studies with benzimidazoles.

Species	*Doses (mg/kg bw)*	*Days of gestation dosed*	*Effects and reference*
Thiabendazole			
Mice	0–2,400	7–15	Resorptions increased at 1,300 mg/kg. Increased malformations at all doses – cleft palate and vertebral anomalies (JECFA, 1993, 2002)
Mice	30–2,400	9	Limb reduction deformities at doses above 480 mg/kg; fusion of vertebral arches at doses above 240 mg/kg. No treatment-related effects at 30 mg/kg (Ogata *et al.*, 1984)
Mice	0–200	6–15	Dose-related decreases in numbers of implantations and fetal weights; delayed ossification in all groups, but this was not dose related (JECFA, 2002)
Rats	0–80	8–15	No adverse effects (JECFA, 1993, 2002)
Rats	0–500	6–15	No adverse effects (Khera, 1979)
Rats	0–400	6–17	No effects on resorptions or fetal morphology, but reductions in fetal weights in treated rats (JECFA, 1993)
Rats	0–80	6–17	Reduction in fetal weights only (JECFA, 1993)
Rabbits	0–800	8–16	Reduction in fetal weights only (JECFA, 1993)
Rabbits	0–600	6–18	Domed head, hydrocephalus and enlarged fontanelles in one fetus from 120 mg/kg group and in two from 600 mg/kg group (JECFA, 1993, 2002)
Rabbits	0–600	6–18	Variations in lung lobation and decreases in metacarpal ossification at 600 mg/kg (JECFA, 1993, 2002)
Febantel			
Rats	0–100		Reduced pregnancy rates at 100 mg/kg. No effects on resorptions and fetuses at doses of up to 30 mg/kg. At 100 mg/kg, delayed ossification and anomalies – anophthalmia, dysplastic microphthalmia and multiple deformities of the ribs and spine (JECFA, 1991a)
Fenbendazole			
Rats	0–2,500	7–16	No adverse effects (JECFA, 1991a)
Rats	60 or 120	8–15	No adverse effects. No adverse effects with the 6-hydroxy or sulphone metabolites, but the sulphoxide (i.e. oxfendazole) caused 80% embryolethality and external malformations at 16 mg/kg and 100% embryolethality at 21 mg/kg (JECFA, 1991a)
Rabbits	0–63	7–19	Skeletal anomalies at the highest dose. No adverse effects at 0–25 mg/kg (JECFA, 1991a)

Table 32.2 *Continued*

Species	*Doses (mg/kg bw)*	*Days of gestation dosed*	*Effects and reference*
Triclabendazole			
Rats	0–100	6–15	Reduced fetal weights at 100 mg/kg but no other adverse effects (JECFA, 1993)
Rats	0–200	8–15	Reduced fetal weights at 100 and 200 mg/kg (Yoshimura, 1987)
Rabbits	0–20	6–18	Omphocoele in one fetus at 20 mg/kg; no effects at 3 or 10 mg/kg (JECFA, 1993)
Albendazole			
Mice	0–30	6–15	No adverse effects (JECFA, 1990)
Rats	0–40	16–20 or 16 of gestation to 20 of lactation	Small lungs and kidneys, and anasarca in some fetuses from dams given 40 mg/kg; no adverse effects at 20 mg/kg (JECFA, 1990)
Rats	0–30	6–15	No adverse effects at 10 mg/kg or below. At 30 mg/kg reduced fetal weights and sizes, with multiple gross visceral and skeletal anomalies including micromelia, ectromelia and curved femurs (JECFA, 1990)
Rats	0–10	6–15	Reduced fetal weights and sizes at 10 mg/kg, with delayed ossification, micromelia and microfetalis. No adverse effects at 5 mg/kg (JECFA, 1990)
Rats	0 or 27.5	6–15	Small increased incidence of shortened limb bones in fetuses from treated dams (JECFA, 1990)
Rats	0–13.25	8–15	Skeletal anomalies at 6.6 mg/kg and above. Craniofacial defects. Similar effects with sulphoxide metabolite (i.e. albendazole sulphoxide) but not with eight other metabolites (JECFA, 1990)
Rabbits	0–30	7–19	Reductions in numbers of implantations, increases in resorptions and ectodactyly, with reductions in fetal sizes and weights at the higher doses. No adverse effects at 5 mg/kg (JECFA, 1990)

susceptible than other animals to the teratogenic effects of the drug.

Griseofulvin has been tested in a number of assays for genotoxicity. In general, it has given negative results in tests for point mutations in bacterial systems, including the Ames test with strains of *Salmonella typhimurium*, and in a number of mammalian cell lines (Kuczuk *et al*., 1978; Leonard *et al*., 1979; De Zimmerman *et al*., 1984; Carli and Larizza, 1988; Zeiger *et al*, 1992). There is some limited evidence that griseofulvin is mutagenic in the mouse lymphoma TK^+/TK^- L5178Y assay (Sofuni *et al*., 1996). Results in the micronucleus test have generally been negative (Heddle *et al*., 1983; Kersten *et al*., 1999; Labay *et al*., 2001), although positive results were obtained in V79 cells, in a gut micronucleus test system and in L5178Y cells (Kalweit *et al*., 1999; Vanhauwaert

et al., 2001; Oliver *et al*., 2006). Griseofulvin gave negative results in a test for DNA repair using rat and mouse hepatocytes (Mori *et al*., 1984) and in bacterial systems (Leifer *et al*., 1981).

However, in a number of studies for aneuploidy and other tests for chromosomal damage arising during mitosis and meiosis, clear positive results were seen (De Carli *et al*., 1973; Larizza *et al*., 1974; Grant, 1982; Curry *et al*., 1984; Waters *et al*., 1986; Marchetti *et al*., 1992, 1996; Tiveron *et al*., 1992; Mailhes *et al*., 1993; Kolachana and Smith, 1994; Inoue *et al*., 1995; Fahmy and Hassan, 1996; LeBoeuf *et al*., 1996; Parry *et al*., 1996; Bourner *et al*., 1998; Migliore *et al*., 1999; Qinghua *et al*., 1999). The evidence demonstrates that griseofulvin is a potent aneugen in somatic cells and in germ cells. This effect may lead to loss of chromosomes and altered gene expression (Knasmuller *et al*., 1997). The results demonstrate that griseofulvin is an antimitotic agent and this may account for its teratogenic activity. The mechanism is unclear since it does not disrupt microtubules like some other spindle poisons, but it does appear to bind to tubulin or at least to microtubule-associated proteins (Grisham *et al*., 1973; Wehland *et al*., 1977; Ueno, 1985; De Carli and Larizza, 1988).

Moreover, studies in mice demonstrated that griseofulvin was hepatocarcinogenic in mice after oral dosing and it resulted in thyroid tumours in rats (Rustia and Shubik, 1978). Dietary administration to mice resulted in hepatotoxicity, disruption of hepatic architecture and lesions which had the appearance of liver tumours (DeMatteis *et al*., 1966). Parenteral administration of griseofulvin to infant mice resulted in a high incidence of liver tumours (Epstein *et al*., 1967). The mechanism of carcinogenicity is unclear (Williams, 1997). The International Agency for Research on Cancer (IARC) concluded that griseofulvin was hepatocarcinogenic in mice and that there were inadequate data to assess the evidence for carcinogenicity in humans but that the substance was possibly carcinogenic to humans (IARC, 1974, 2002).

Aneuploidy is regarded as an important change in the process of carcinogenesis (Oshimura and Barrett, 1986) and this, taken with the results in animal studies, confirms griseofulvin's status as a carcinogen. For such indirect carcinogens, it should be possible to determine a threshold dose or concentration (Parry *et al*., 1994; Kirsch-Volders *et al*., 2003), but the question arises as to which study to employ to determine this, as the drug gives different responses depending on the test system chosen (Kirkland, 1998).

Were griseofulvin being developed today for use as a drug, it is almost certain that its teratogenic activity would be detected during preclinical testing in laboratory species, and so it is possible to conclude that the toxicology tests would be predictive for its activity in cats. Significantly, as it produced generally negative results in genotoxicity studies that form the basis of the standard battery of tests used to predict carcinogenicity, and which if positive may lead to long-term studies in rodents, its carcinogenic potential may have been missed. However, any spurious results in a modern battery of genotoxicity tests may have led to further studies of other end-points, and its ability to produce aneuploidy may subsequently have been detected.

Avermectins

The avermectins are a class of drugs based on naturally occurring avermectins, which are widely used in veterinary medicine as antiparasitic agents (Fisher and Mrozik, 1989). These are macrocyclic lactones with a spiroketal structure. The first of these to be introduced, and perhaps the most successful veterinary drug ever produced, was ivermectin, which was first registered in France in 1981 (Di Netta, 1989) and which has now been authorised on virtually a global basis. Ivermectin is a mixture of two components, ivermectin B_1a and ivermectin B_1b (*Figure 32.3*).

Since ivermectin was first introduced a series of related compounds has become available for use in veterinary medicine, including abamectin, doramectin, emamectin, selamectin and eprinomectin. A related compound, moxidectin, a milbemycin (an avermectin macrocycle lacking a bisoleandrosyloxy substituent at the C-13

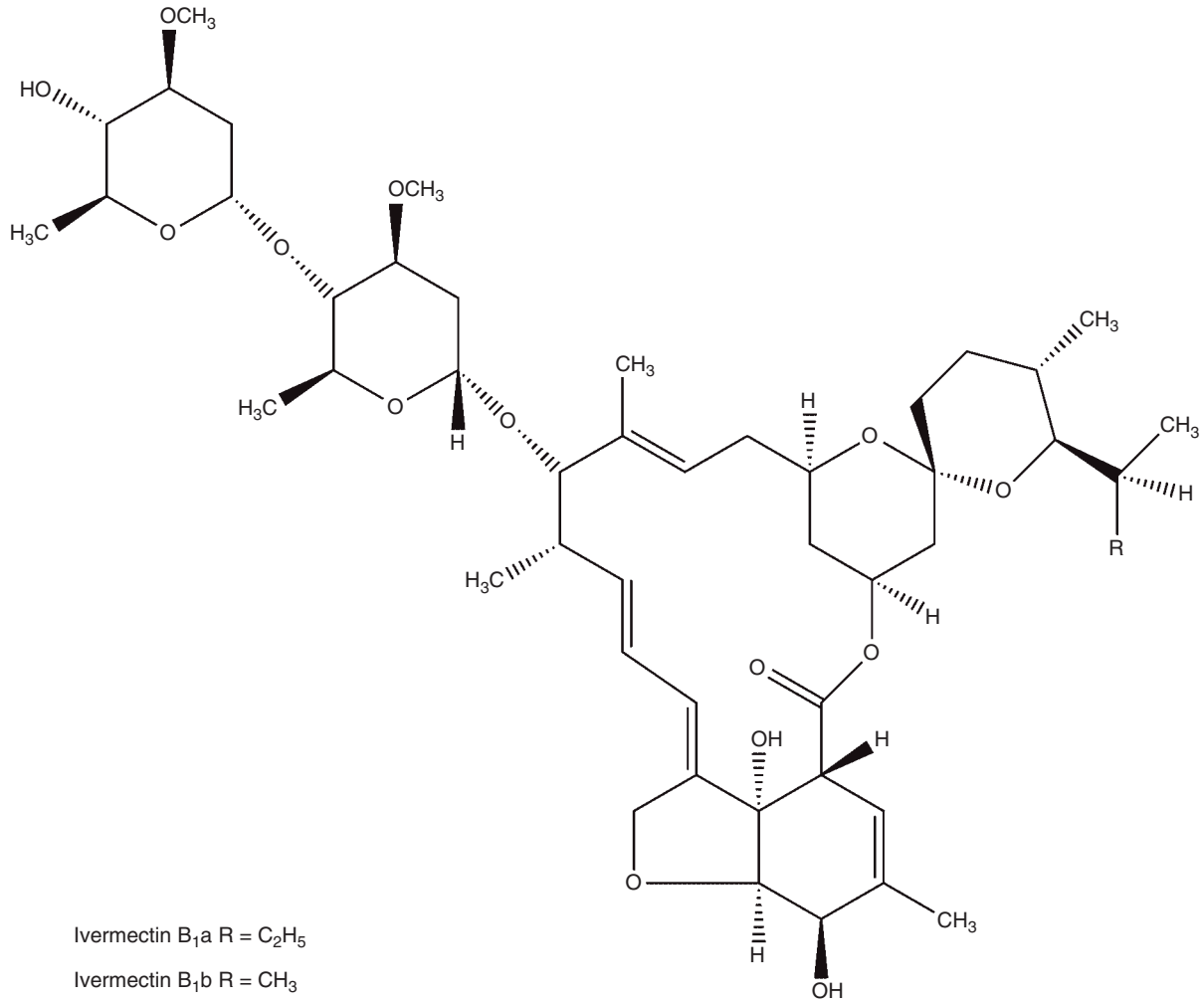

Fig. 32.3 Ivermectin.

position), has also been authorised. Avermectins are widely used as endectocides in sheep and cattle and other farmed species, except for emamectin which is used as a parasiticide in farmed salmon. Ivermectin, for example, is authorised for use in cattle, sheep, horses, goats, pigs, camels, reindeer and bison (Di Netta, 1989).

Many also have applications in companion animal medicine (Sutherland and Campbell, 1990; Shoop *et al*., 1995; McKellar and Benchaoui, 1996; Williams, 1997; Omura, 2008). Ivermectin is also used as a parasiticide in human medicine, largely for the treatment of onchocerciasis caused by *Onchocerca volvulus* and filariasis resulting from *Wuchereria bancrofti*, loiasis and strongyloidiasis (Greene *et al*., 1981; Aziz *et al*., 1982; Diallo *et al*., 1984; Lariviere *et al*., 1985; Kumaraswami *et al*., 1988; Richard-Lenoble, *et al*., 1988; Naquira *et al*., 1989; Cartwell *et al*., 1992; Oyibo and Fagbenro-Beyioku, 2002).

Avermectins, including ivermectin, have extremely good safety profiles and toxicity following exposure to them is rare during therapeutic use, although adverse effects have been reported in horses, notably following overdose with moxidectin (Karns and Luther, 1984; Khan *et al*., 2002), in cats, again following overdoses (Muhammad *et al*., 2004) and in a rhesus macaque monkey (Iliff-Sizemore *et al*., 1990), but tolerance in most species, including primates, is extremely good (Lankas and Gordon, 1989; Kužner *et al*., 2005).

However, some species, or more specifically, some breeds, appear to be more sensitive to the toxic effects of avermectins. Thus, signs of neurotoxicity appeared in a herd of Murray Grey cattle after therapeutic administration of avermectin B_1 (Seaman *et al*., 1987). These included signs of severe CNS toxicity, including death of some affected animals. The other major species with seemingly unique sensitivity to avermectins is the dog, and specifically the collie dog and related breeds such as the Australian shepherd. White Swiss Shepherd dogs are sensitive to the toxic effects of doramectin. Following therapeutic treatments, affected animals show evidence of CNS toxicity; in severe cases they may require mechanical ventilation and intensive nursing, the recovery period is often prolonged and death may occur (Pulliam *et al*., 1985; Houston *et al*., 1987; Paul *et al*., 1987; Tranquilli *et al*., 1987, 1991; Hadrick *et al*., 1995; Gray, 1997; Hopper *et al*., 2002; Yas-Natan *et al*., 2003; Geyer *et al*., 2007). Collies sensitive to ivermectin tolerated an imidacloprid-moxidectin formulation (Paul *et al*., 2004).

Ivermectin is neurotoxic and its mode of action appears to be through binding of the drug to glutamate-gated chloride channels, leading to increased chloride ion permeability, and eventually to hyperpolarisation of nerve and muscle cells. It may also interfere with γ-aminobutyric acid (GABA)-mediated transmission of nerve impulses. The overall consequence is paralysis and death of the parasite, the therapeutic aim of the drug (Schaeffer and Haines, 1989; Sutherland and Campbell, 1990; Martin, 1996; Roder and Stair, 1998; Dawson *et al*., 2000; Wolstenholme and Rogers, 2005). It is not generally neurotoxic in mammals at therapeutic doses because the blood–brain barrier protects the central nervous system.

However, ivermectin had been studied in toxicity tests using the CF_1 mouse. This strain is deficient in P-glycoprotein, a protein that is a constituent of cell membranes which determines their permeability (Didier and Loor, 1995; Schinkel *et al*., 1996; Sharom, 1997; Laffont *et al*., 2002; Ejsing *et al*., 2007) and this has been linked with a polymorphism in the *Mdr 1* gene (Macdonald and Gledhill, 2007). Hence, the CF_1 mouse, and neonatal animals, which are also deficient in P-glycoprotein, are more sensitive to the toxic effects of ivermectin, including its neurotoxic effects (Lankas and Gordon, 1989; Schinkel *et al*., 1994; Skopets *et al*., 1996; Lankas *et al*., 1989, 1997; Umbenhauer *et al*., 1997; Kwei *et al*., 1999; Marques-Santos, 1999; Skipor and Thiery, 2005). This extra sensitivity is displayed in acute toxicity, subchronic toxicity, reproductive toxicity and teratology studies with CF_1 mice, and, for the teratology results, in no-observed effect levels (NOELs) that are 5–10 times lower than those noted with normal rats or rabbits (JECFA, 1993).

As mentioned above, collie dogs and Murray Grey cattle are more sensitive to the toxic effects of ivermectin (Seaman *et al*., 1987; Fassler *et al*., 1991; Hopper *et al*., 2002). This may be due to decreased P-glycoprotein or to increased permeability due to other factors including concomitant drugs (Hopper *et al*., 2002). In fact, some collie dogs sensitive to the neurotoxic effects of ivermectin have been shown to possess a mutation of the *Mdr 1* gene (Mealey *et al*., 2001; Nelson *et al*., 2003) suggesting that the effects in this breed, and those noted in the CF_1 mouse, have a similar underlying genetic basis.

With hindsight, the toxic effects of avermectins seen in susceptible species would be difficult to predict under any circumstances. The CF_1 mouse is not normally used in routine toxicology studies and so the toxic effects would not normally have been observed, although avermectins developed since ivermectin have been tested in this mouse strain. Furthermore, the effects seen in collie dogs and Murray Grey cattle would have been almost impossible to predict in advance. It is simple to associate the P-glycoprotein associated effects retrospectively but not prospectively, although the knowledge now gained can be applied to future development of new avermectins. Indeed, the newer avermectin selamectin and the milbemycin moxidectin have a wider safety profile in the collie. In fact, selamectin has a good safety profile in general in both cats and dogs

(Krautmann *et al*., 2000; Novotny *et al*., 2000; Paul *et al*., 2000; Hovda and Hooser, 2002).

Non-steroidal anti-inflammatory drugs

Non-steroidal anti-inflammatory drugs (NSAIDs) are used in companion animals, particularly in dogs, for the treatment of musculoskeletal disorders including osteoarthritis. The major adverse effect of NSAIDs in dogs treated therapeutically, and in many other species, including humans, is gastric ulceration, and this has been noted in dogs with a range of these agents including diclofenac, ibuprofen, indomethacin, aspirin, mofezolac, piroxicam, proquazone and naproxen (Stewart *et al*., 1980; Van Ryzin and Trapold, 1980; Roudebush and Morse, 1981; Boulay *et al*., 1986; Stephenson, 1988; Yeats, 1988; Ohkubo *et al*., 1990; Jackson *et al*., 1991; Spellman, 1992; Vollmar, 1993; Poortinga and Hungerford, 1998; Ramesh *et al*., 2002; Coruzzi *et al*., 2007; Chiba *et al*., 2008).

These effects are well known (McCormack and Brune, 1987), and are due to loss of cytoprotection due to inhibition of prostaglandins, a side-effect of their mode of action (Kore, 1990; Vollmar, 1993; Waller *et al*., 2001). Gastroprotectants such as cimetidine seem to have limited potential in protecting against NSAID-induced gastric damage, but newer classes of the drugs, including the dual-acting lipooxygenase/cyclooxygenase (LOX/COX) and specific COX-2 inhibitors, may have more advantages and be less damaging to the gastric mucosa (Boulay *et al*., 1986; Hawkey, 1999; Neiger, 2003).

These adverse gastrointestinal effects of NSAIDs have been seen in laboratory animals including rats (Bolte *et al*., 1980; Van Ryzin and Trapold, 1980; Elliott *et al*., 1988) and so preclinical studies can be entirely predictive for this type of effect with these drugs.

NSAIDs, including COX-2 inhibitors, may also induce nephrotoxicity in a number of species, including dogs and humans (Kore, 1990; Catella-Lawson *et al*., 1999; Rossat *et al*., 1999; Perazella and Eras, 2000; Swan *et al*., 2000; Boothe, 2001), although the incidence in animals is thought to be lower than in humans, probably because they are usually given for relatively shorter periods of time (Boothe, 2001). The mechanism involves inhibition of vasodilatory prostaglandins, with loss of vasodilation and reduced urinary output (Shelley, 1978; Dunn *et al*., 1984; Black, 1986).

Like the gastrointestinal effects of NSAIDs, the renal effects have been noted in laboratory species such as rats (Bolte *et al*., 1980; Van Ryzin and Trapold, 1980) and so again these have predictive value for adverse effects that might come to light during therapeutic use in animals.

Sulphonamides

Sulphonamide drugs have been reported to cause disruption of thyroid function in dogs when given at high therapeutic doses (Daminet and Ferguson, 2003; Trepanier *et al*., 2003).

It was shown in 1943 that sulphonamides could induce thyroid hyperplasia in rats (Astwood *et al*., 1943; MacKenzie and MacKenzie, 1943). Sulphadimidine (sulfamethazine, *Figure 32.4*) induced thyroid hyperplasia in rats but not in mice after 90 days of administration (Heath and Littlefield, 1984a, b). Administration to mice for up to 24 months resulted in thyroid tumours (Littlefield *et al*., 1989, 1990), while in a two-generation study, administration to rats for up to 24 months resulted in adenocarcinomas of the thyroid (Littlefield *et al*., 1990).

Sulphadimidine is goitrogenic in rodents, resulting in stimulation of the thyroid by thyroid stimulating hormone, and leading to decreases in thyroid hormones (Nishikawa, 1983a, b;

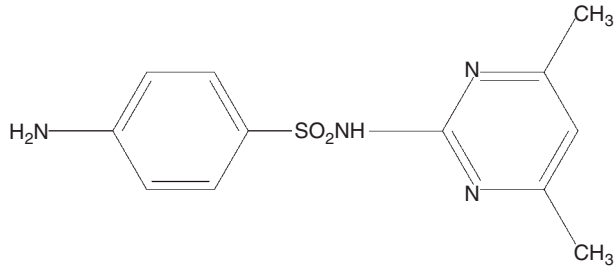

Fig 32.4 Sulphadimidine (sulfamethazine).

Fullerton *et al*., 1987; Gupta *et al*., 1992; JECFA, 1994; Shaw and Jones, 1994; McClain, 1995; Hill *et al*., 1996; Poirier *et al*., 1999; Altholtz *et al*., 2006).

Similar effects have been noted in pigs but not in primates (JECFA, 1994). Sulfanilamidoindazole, a sulphonamide that induces an acute arthritis in rats, and sulfamethoxazole also produce thyroid follicular hyperplasia (Ohmachi *et al*., 1998; Torii *et al*., 2001) as does sulfadimethoxine, which is also a thyroid carcinogen (Swarm *et al*., 1973; Shimo *et al*., 1995, 1997; Imai *et al*., 2004, 2005).

Hence, it seems that some mammalian species may be susceptible to the thyroid effects of sulphonamides, and those noted in the dog, while not perhaps predictable, might be seen in retrospect to be not entirely unexpected. Primates, including humans, appear to be refractory to the thyroid effects of sulphonamides, at least at therapeutic doses. Moreover, knowledge of the non-genotoxic mechanism of carcinogenicity, and the ability to identify NOELs for the underlying mechanisms, permit the calculation of acceptable daily intake values and allow for the safe use in food animals of sulphonamide drugs (Woodward, 1991b, 1992b; McClain, 1992, 1995; Hill *et al*., 1998).

Carbadox and olaquindox

Carbadox and olaquindox (*Figure 32.5*) are synthetic antimicrobial compounds which have been used therapeutically in the prevention and treatment of swine dysentery in pigs, and for growth promotion, again in pigs (Thrasher *et al*., 1969; Rainier *et al*., 1973; Downing, 1974; Schneider *et al*., 1977; Jager *et al*., 1986; Nabuurs and van der Molen, 1989). Chemically, they are quinoxaline-di-N-oxide derivatives.

Toxicity due to carbadox and olaquindox has been reported in pigs during treatment, and particularly after overdosage. Adverse effects have included adrenal lesions, abnormal renal function, hypoaldosteronism, hyponatraemia, hyperkalaemia and haemoconcentration (van Schie,

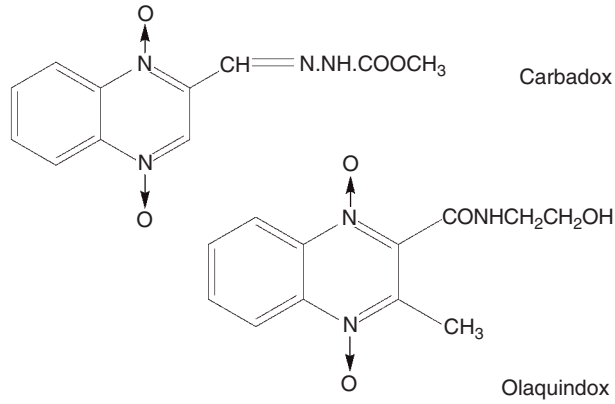

Fig 32.5 Carbadox and olaquindox.

1982; Power *et al*., 1989; Waldmann *et al*., 1989; Jager and Vroomen, 1990). Experimental studies in pigs have revealed adrenal toxicity with carbadox and olaquindox, and with another related drug, cyadox. The most obvious lesions were found in the adrenal cortex. The glomerular cells were swollen with hydropic changes, and some cells had pyknotic nuclei. Degenerative changes and necrosis were seen in the medulla and pelvis of the kidney.

The severity of the lesions increased with dose and with duration of drug administration. These changes were accompanied by decreases in aldosterone and sodium concentrations in blood, with increases in potassium levels. In animals given high doses of carbadox, the adrenal lesions were persistent and the hypoaldosteronism was slow to resolve (van Miert *et al*., 1984; van de Kerk, 1985; van der Molen *et al*., 1985, 1986a, b, 1989a–c; Baars *et al*., 1988; van der Molen, 1988; Nabuurs and van der Molen, 1989; Nabuurs *et al*., 1990). Carbadox has produced inhibition of aldosterone biosynthesis by pig adrenal preparations in in vitro studies (Spierenburg *et al*., 1988).

No major toxicological effects were seen in rats given carbadox at doses of up to 100 mg/kg body weight per day for up to 30 days or in dogs given up to 50 mg/kg body weight per day, but when given to rats for up to 26 months at doses of 5, 10, 25, 50 or 100 mg/kg body weight per day, adrenal changes were noted in animals given 25 mg/kg body weight per day and above. These included adrenocortical haemorrhages, necrosis and degeneration, but there was no major evi-

dence of nephrotoxicity (JECFA, 1991b). Adrenal atrophy was noted in rats given olaquindox at doses of 60 or 180 mg/kg body weight per day for 5 days per week for 13 weeks and fatty degeneration in the cells of the kidney tubules of dogs given 60 or 180 mg/kg body weight per day for 90 days (JECFA, 1991b).

No adrenal toxicity was noted in monkeys given carbadox at doses of up to 20 mg/kg body weight per day, 5 days per week for up to 24 months, although it was not clear to what extent organs were examined grossly or histopathologically. However, rhesus monkeys given olaquindox at doses of 20 or 40 mg/kg body weight per day, 5 days per week for 19 weeks showed evidence of minor kidney and adrenal toxicity (JECFA, 1991b). Fatty degeneration of the cells of the kidney tubules was seen in dogs given 60 or 80 but not 20 mg/kg body weight per day olaquindox for 90 days (JECFA, 1991b).

The adrenal toxicity seen with this group of compounds in pigs was also evident in the toxicity studies in rodents and, to a lesser extent, in dogs. However, the toxic effects seen in porcine kidneys were not seen in rats, but were evident, though to a lesser degree, in dogs and possibly primates. The laboratory studies involving carbadox and olaquindox were well conducted but perhaps less rigorous in terms of the organs examined by both gross pathology and microscopically than would now be considered acceptable and it is possible that more modern studies may have provided more convincing evidence of nephrotoxicity. Nevertheless, it is evident that the major toxic effect seen in pigs treated therapeutically with the drugs, usually in overdosage, would clearly be predicted, albeit by longer-term studies, in laboratory species.

Nitrofurans

Nitrofurans are synthetic antimicrobial drugs used for the treatment of a number of bacterial diseases in poultry, cattle and pigs. The most widely used nitrofuran drug in veterinary medicine is furazolidone, whose bacteriostatic activity

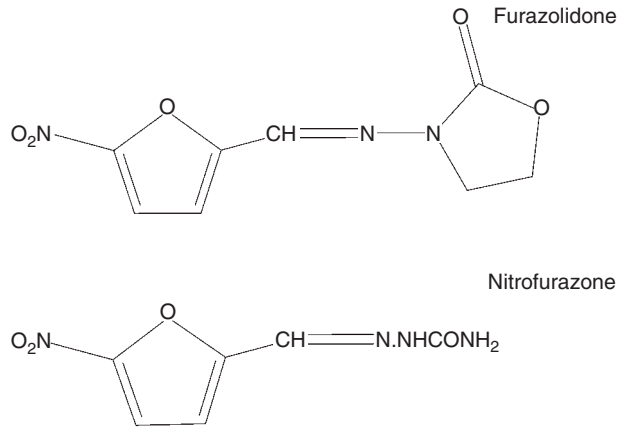

Fig 32.6 Furazolidone and nitrofurazone.

was first described in 1944, while nitrofurazone (nitrofural) is another important member of the class (*Figure 32.6*) (Dodd and Stillman, 1944; van Miert *et al*., 1984; Ali, 1999).

Furazolidone has been shown to be toxic, usually when used in overdose, in a variety of species including pigs, goats, poultry, ducks and cattle (Jankus *et al*., 1972; St Omer, 1978; Borland, 1979; Van Vleet and Ferrans, 1983a, b; Ali and Khogali, 1984; Czarnecki, 1984; Jager *et al*., 1984; van Miert *et al*., 1984; van de Kerk, 1985; Wilson *et al*., 1988; Ali, 1989, 1999; Jager and Vroomen, 1990; Taylor *et al*., 1991), while nitrofurazone toxicity has been reported in dairy calves (Lister and Fisher, 1970; Fankhauser *et al*., 1981).

The major toxic effect in avian species following therapeutic treatment or in experimental studies is a cardiomyopathy characterised by enlargement of the ventricles, which was more pronounced in the right ventricle. Congestion of blood vessels, abnormalities of electrocardiograms and myocardial necrosis with inflammation and fibrosis, pulmonary hypertrophy, neurotoxicity and congestion, and hepatic vacuolation and capsular fibrosis have also been noted (Jankus *et al*., 1972; St Omer, 1978; Simpson *et al*., 1979; Good and Czarnecki, 1980; Van Vleet and Ferrans 1983a, b, 1986; Ali and Khogali, 1984;, Wilson *et al*., 1988; Czarnecki, 1989; O'Brien *et al*., 1993; Zaman *et al*., 1995; Ali, 1999).

In dairy calves, the major signs of furazolidone and nitrofurazone toxicity are neurological

including lethargy, epistaxis and mild convulsions and haemorrhagic diathesis (Lister and Fisher, 1970; Fankhauser *et al*., 1981; Taylor *et al*., 1991; Finnie, 1992). Signs of neurotoxicity have been reported in Nubian goats and pigs following furazolidone treatments (Borland, 1979; Ali *et al*., 1984; Jager *et al*., 1984; van Miert *et al*., 1984; van der Kerk, 1985). Furazolidone has resulted in testicular degeneration in ducklings and chickens, with concomitant effects on spermatogenesis (Webb *et al*., 1990; Ali, 1999).

Furazolidone has been investigated in short-term studies in rats and dogs, and in carcinogenicity studies in rats and mice. Although a number of toxic responses were noted, including clear evidence of carcinogenicity, there was no indication of cardiotoxicity (Aiso *et al*., 1962; JECFA, 1993). Similarly, there was no cardiotoxicity noted in a number of studies in mice and rats with nitrofurazone, but some evidence of carcinogenicity was observed in longer-term studies (Morris *et al*., 1969; National Toxicology Program (NTP), 1988; Kari *et al*., 1989; Nomura *et al*., 1992), as noted with other nitrofurans and related compounds (Cohen *et al*., 1973), although in this case there may be an underlying endocrine rather than genotoxic mechanism (Takahashi *et al*., 2000; Takegawa *et al*., 2000).

Neurotoxicity was seen in toxicity studies in dogs dosed with furazolidone (JECFA, 1993), while hyperexcitability and convulsive seizures were noted in mice, and convulsions were observed in rats given high doses of nitrofurazone (National Toxicology Program, 1988; JECFA, 1993). Furazolidone resulted in testicular atrophy with decreased sperm and testicular tubular degeneration in dogs in a short-term toxicity study and in rats in a number of reproduction studies, possibly by disruption of the hypothalamic pituitary gonadal axis and a direct effect on the hypothalamus (JECFA, 1993; Zimmerman *et al*., 1993). Evidence of reduced spermatogenesis was seen in male Nubian goats given furazolidone (Mustafa *et al*., 1987). Testicular atrophy and other adverse reproductive effects occurred in mice and rats given nitrofurazone (Miyaji *et al*., 1964; National Toxicology Program, 1988; Nishimura *et al*., 1995; George *et al*., 1996; Ito *et al*., 2000). Nitrofurans are also able to act as sperm immobilising agents (Albert *et al*., 1974, 1975).

Thus, standard toxicity studies in conventional laboratory animals were not predictive of the cardiotoxic effects of furazolidone seen in avian species, and it is possible that these effects are restricted to birds and even to ducks, chickens and turkeys. However, the studies were predictive of the neurotoxic effects reported in goats, pigs and cattle and the adverse testicular effects seen in ducks and chickens, and thus these might be expected in other livestock species. Other studies have suggested that, as a group, the nitrofurans are carcinogenic, at least in rodents.

Fluoroquinolones

A number of fluoroquinolone antimicrobials are used in veterinary medicine, but of these, the most widely used is enrofloxacin, a drug closely related to ciprofloxacin, a drug extensively used in human medicine; in fact some ciprofloxacin is formed as a metabolite of enrofloxacin in a number of species (*Figure 32.7*) (Greene and Budsberg, 1993).

Fluoroquinolones are well tolerated in animals and humans. In humans they tend to be associ-

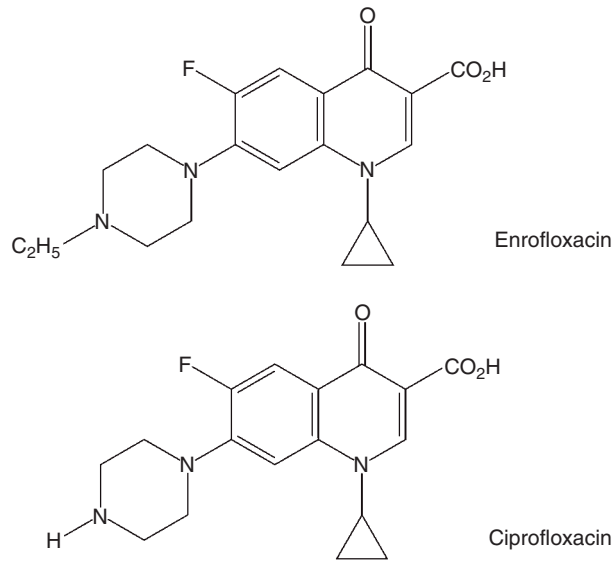

Fig. 32.7 Enrofloxacin and ciprofloxacin.

ated with fewer adverse reactions than some other antimicrobial drugs, although in rare cases they have produced central nervous system toxicity (Christ, 1990; Stahlmann, 1990; Vancutsem *et al*., 1990; Patterson, 1991; Greene and Budsberg, 1993; Hooper and Wolfson, 1993; Hori and Shimada, 1993; Giguere *et al*., 1999; Stahlmann and Lode, 1999; Papich and Riviere, 2001; Leone *et al*., 2003; Meropol *et al*., 2008). They are not without some adverse effects in animals.

In cats, the therapeutic use of enrofloxacin has been associated with irreversible retinal degeneration and blindness (Davidson, 2001; Abrams-Ogg *et al*., 2002; Crispin *et al*., 2002; Watson, 2002). In a retrospective study of 17 cats that had received systemic enrofloxacin, and which had developed retinal degeneration, presenting signs were mydriasis and blindness. All the animals had diffuse retinal degeneration, with increased tapetal reflectivity and retinal vascular attenuation. Vision returned in some of the animals, but the retinal damage persisted or progressed. Histopathology revealed outer retinal degeneration, with diffuse loss of the outer nuclear and photoreceptor layers and hypertrophy and proliferation of the retinal pigment epithelium (Gelatt *et al*., 2001).

This ocular toxicity has been cited as a class effect caused by fluoroquinolones. However, other fluoroquinolones, including marbofloxacin and orbifloxacin, have not resulted in these dramatic changes (Wiebe and Hamilton, 2002). For example, doses of 45 or 75 mg/kg body weight per day of orbifloxacin for 30 consecutive days resulted in only tapetal hyper-reflectivity and minimal degenerative changes (swollen photoreceptor cells). Thus, there remains some doubt as to whether the effects noted with enrofloxacin are a class effect. Fluoroquinolones can cause adverse ocular (and other) effects in humans after local exposures, including irritation, chemosis, hyperaemia and corneal damage, but there is no evidence to suggest any adverse retinal effects (Rubinstein, 2001; Melhorn and Brown, 2007; Thompson, 2007; Li *et al*., 2008).

Although retinal damage has been reported in Europe with enrofloxacin (Crispin *et al*., 2002; Holm and Winther, 2003), the majority of the cases emerged following therapeutic use in the US. In the US, flexible dosing had been permitted in the product literature, allowing doses of 5–20 mg/kg body weight per day, whereas in Europe, the dose is generally 5 mg/kg per day (Wiebe and Hamilton, 2002; Wilson, 2002). It may well be therefore that the effects seen in cats treated with enrofloxacin are dose-related toxicity rather than idiosyncratic in nature, and they may be specific to enrofloxacin. These effects are minimal at the recommended dose of 5 mg/kg body weight per day but increase in frequency and severity at higher doses (Wiebe and Hamilton, 2002). Evidence from the UK's adverse reporting scheme supports the view that retinopathy with enrofloxacin occurs with overdosing (Dyer *et al*., 2007).

While fluoroquinolones such as enrofloxacin, danofloxacin and sarafloxacin have been extensively tested in laboratory animal studies, there have been no indications of retinal damage (Nomura *et al*., 1992; Rootman *et al*., 1992; JECFA, 1995, 1998). This is not surprising if the retinal effects, as seems likely, are restricted to cats, as this species is not routinely used in toxicity testing.

Fluoroquinolones and quinolones have also been shown to cause chondrotoxicity in juvenile animals. Ciprofloxacin, difloxacin, fleroxacin, irloxacin, perfloxacin, ofloxacin and grepafloxacin result in fluid-filled vesicles that project above the articular surfaces, chondrocytes with shrunken cytoplasm, mitochondrial swelling and enlargement of cytoplasmic vacuoles in immature animals including rats, dogs, horses and poultry (Gough *et al*., 1979, 1985, 1996; Corrado *et al*., 1987; Schlüter, 1987; Christ *et al*., 1988; Burkhardt *et al*., 1990, 1997; Patterson, 1991; Greene and Budsberg, 1993; JECFA, 1995, 1998; Kashida and Kato, 1997; Beluche *et al*., 1999; Guzman *et al*., 1999, 2000; Takizawa *et al*., 1999; Stahlmann *et al*., 2000; Kappel *et al*., 2002; Nagai *et al*., 2002; Peters *et al*., 2002; Sridevi *et al*., 2002; Khaliq and Zhanel, 2003).

Concerns have been expressed regarding the use of fluoroquinolones in children (Camp *et al*.,

1994; Jick, 1997; Gendrel and Moulin, 2001), but their use in paediatric human populations appears to be relatively safe, and there have been no reports of adverse effects on joints (Jick, 1997; Gendrel and Moulin, 2001), although tendonitis has been reported in adults, generally over the age of 60 years (Hooper and Wolfson, 1993; Hayem and Carbon, 1995; van der Linden *et al*., 2001; Leone *et al*., 2003). The absence of significant numbers of adverse reactions relating to effects on joints in animals treated therapeutically with fluoroquinolones is likely to be a reflection of the fact that they are usually contraindicated for use in immature animals.

Glucocorticoids

The two major glucocorticoids used in veterinary medicine are betamethasone and dexamethasone, although others including prednisolone, flumethasone, mometasone, cortisol and cortisone are also used. In fact, dexamethasone and betamethasone are isomers, differing only in the orientation of a methyl group (*Figure 32.8*).

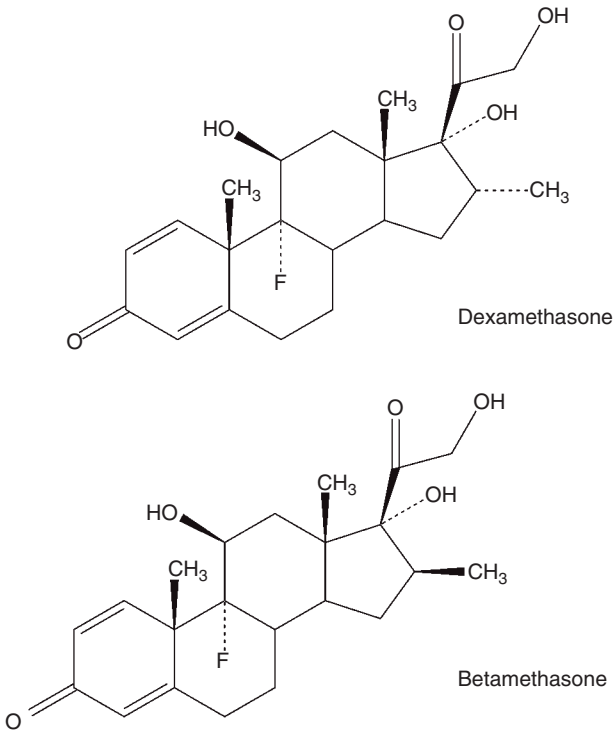

Fig. 32.8 Dexamethasone and betamethasone.

Cushing's syndrome with adrenal suppression, depression and irritability on withdrawal, cirrhosis, hepatomegaly, colonic perforation, excessive catabolism and muscle atrophy, dermatologic effects, immunosuppression and adverse reproductive effects including abortion has been reported in animals treated therapeutically with dexamethasone, particularly in the dog, the species most widely administered the drug (Ferguson and Hoenig, 2001).

Dexamethasone has been shown to induce adrenal atrophy in rats and increase their susceptibility to infections. Atrophy of the adrenal glands was noted in dogs treated with dexamethasone. It resulted in higher resorption rates when pregnant rats or rabbits were given the drug, with increased incidences of fetal abnormalities (Preziosi *et al*., 1967; Walker, 1967, 1971; Shimo *et al*., 1982; Hansen and Grafton, 1994; JECFA, 1994; Stewart *et al*., 1997; Hansen *et al*., 1999; Nevagi and Kaliwal, 2001; Yu *et al*., 2002). Similar effects have been seen following administration of betamethasone, usually in rodents or rabbits (Walker, 1971; Barrada *et al*., 1980; Pratt *et al*., 1999; Christensen *et al*., 2001).

Prenatal exposure to dexamethasone or betamethasone may lead to renal effects including reductions in the numbers of nephrons and glomeruli, with subsequent hypertension, and to adverse effects on cardiac and pulmonary development (Wu *et al*., 1993; Torres *et al*., 1997; Benešová *et al*., 1999; Okajima *et al*., 2001; Ortiz *et al*., 2001, 2003; De Vries *et al*., 2002). However, dexamethasone had no apparent adverse effects on sheep fetuses, at least when given in early gestation (Moritz *et al*., 2002). In primates, dexamethasone resulted in depletion of hippocampal neurons, multiple gastric ulcers and adrenal cortical hypoplasia; hippocampal damage was noted in the fetuses of rhesus monkeys when the maternal animals were treated with dexamethasone (Uno *et al*., 1989, 1990, 1994).

Thus, many of the adverse effects of glucocorticoids are predictable from animal toxicity studies, even though there is a lack of temporal sequencing in that the use of these drugs and the discovery of adverse effects, predate some of the

studies. Other effects are also predictable from knowledge of their pharmacological activities. The effects on skin are arguably less predictable or at least less obvious. However, they have been used for many years in human medicine where they are known to cause sensitisation and dermatitis (Sneddon, 1969; Verbov and Abell, 1969; Pasricha and Gupta, 1983; Boyle and Peachey, 1984; Dunkel *et al*., 1991; Hisa *et al*., 1993; Whitmore, 1994, 1995; Alexiou *et al*., 1998; Nucera *et al*., 2002) and to induce dermal atrophy and telangiectasia (MacDonald, 1971; Lubach *et al*., 1989). Their ability to cause adverse skin effects in animals is therefore unsurprising.

Calcipotriol

Calcipotriol is a structural analogue of calcitriol (*Figure 32.9*), the biologically active form of vitamin D. It is used in human medicine for the treatment of psoriasis and other skin disorders as a topical ointment.

Calcipotriol has caused toxicity in dogs which have accidentally ingested the product prescribed for human disease (Campbell, 1997; Fan *et al*., 1998; Durtnell, 1999; Torley *et al*., 2002; Welch, 2002). Signs of toxicity include those typical of vitamin D toxicosis including hyperglycaemic renal tubular necrosis, hypercalcaemia, soft tissue mineralisation, smooth muscle proliferation and death (Ramos *et al*., 1996; Fan *et al*., 1998; Misulis *et al*., 1999; Torley *et al*., 2002; Saedi *et al*., 2007). Similar toxicity has been reported in cats and dogs after ingestion of calcipotriene and in animals given vitamin D preparations therapeutically (Chew and Capen, 1980; Berger and Feldman, 1987; Hare *et al*., 2000; Pesillo *et al*., 2002).

The toxicity of calcipotriol has been investigated in single dose studies in rats and dogs, and in 4-week and 26-week studies in dogs (Imaizumi *et al*., 1996a–c). The substance was acutely toxic to rats, resulting in a number of signs of toxicity including nasal discharge, ptosis, diarrhoea, nasal and vaginal bleeding, subnormal temperatures and death. Although less acutely toxic to dogs, animals vomited, had decreases in activity and defecation, eye discharges and desquamation of skin. Histopathology showed calcium deposits in the renal tubules and thyroids. After topical administration for 4 weeks, urinary calcium levels were elevated and there was squamous cell hyperplasia and hyperkerato-

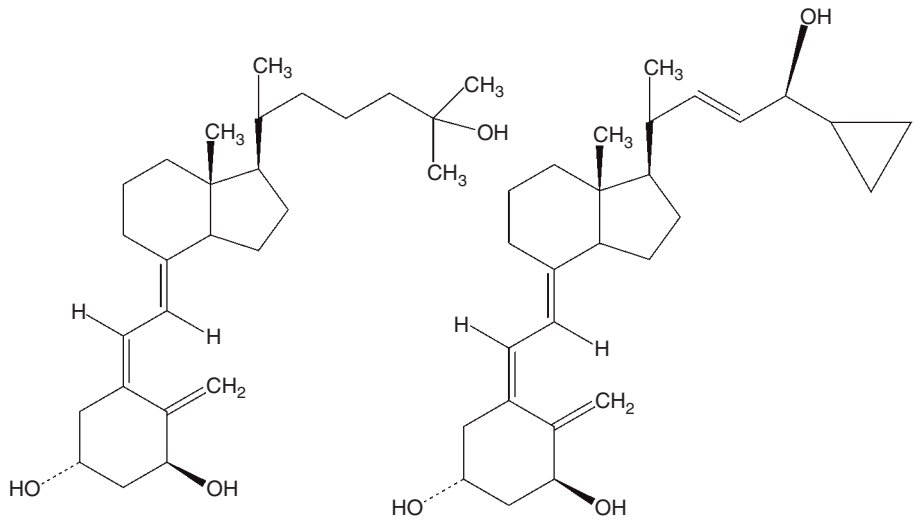

Fig. 32.9 Calcitriol and calcipotriol.

sis of the skin. Similar effects were noted after 26 weeks of application. Such effects have occurred after ingestion of calciferol (vitamin D2) when used as a rodenticide (Lorgue *et al*., 1996). Thus the effects of calcipotriol are predictable from animal toxicity tests, and, indeed, from poisoning episodes with other compounds related to vitamin D.

Discussion

Evidently, the examples described above are partially self-selecting in that they involve drugs for which both adverse reaction and preclinical toxicity data are available, and in some of the examples described above, the adverse reactions predate the availability of toxicity data generated to modern standards. This is particularly true of the older drugs. Nevertheless, the data underline the fact that for type A, toxicity or pharmacologically based adverse reactions, many of the adverse effects noted in target animal species are predictable from the toxicity data. This is similar to the situation with human medicines where there is often good predictability between animal toxicity studies and effects in humans, but where, nevertheless, there are limitations on the use of such data (Zbinden, 1990; Olson *et al*., 2000; Descotes, 2003), despite the level of advancement and sophistication seen in recent years in experimental toxicology.

Limitations may arise because of adverse effects seen post marketing that occurred in populations not represented by the experimental studies (or even clinical trials), such as patients with renal failure, liver disease or other conditions which might affect the behaviour of the drug, or in other susceptible populations including the very young or the very old. For example, benoxaprofen was shown to have extended half-lives in elderly humans and those with renal impairment, and these kinetic variations may account for some of its adverse effects in this group – the group most likely to be treated with the drug (Chatfield and Green, 1978; Brogard *et al*., 1981; Aronoff *et al*., 1982; Hamdy *et al*., 1982; Kamal and Koch, 1982; James, 1985); these limitations are not restricted to human populations and they may also be seen in their animal counterparts.

Species differences undoubtedly also play a part. For example, the rat and dog are more susceptible to the gastrointestinal effects of non-steroidal anti-inflammatory drugs than guinea-pigs and some primates (Rainsford *et al*., 1984; Heywood, 1990). Standard toxicological studies are less able to predict idiosyncratic or type B reactions, so that while conventional toxicity studies were able to predict the gastrointestinal and haematological effects of NSAIDs, the neurotoxicity due to clioquinol, the liver damage due to halothane and possibly the dyskinesias resulting from phenothiazine in humans – all type A reactions (with the possible exception of halothane) – they were not predictive of the anaphylaxis seen with alphaxalone, the photosensitivity and deaths due to benoxaprofen in the elderly, the retroperitoneal fibrosis arising from methysergide and the aplastic anaemia with chloramphenicol or phenylbutazone – all type B, idiosyncratic reactions (Heywood, 1990; Dayan, 2000).

In testing veterinary medicines, investigators have one major advantage over their counterparts in human medicine – the products being investigated for their safety for animal patients are usually tested, depending on local regulatory requirements, at multiples of up to 5 times the intended therapeutic dose, so that any toxicity specific to that animal, or specific to higher doses of the drug in that animal, will often be seen. Taken together with the results of laboratory animal toxicity studies and clinical trials in animals, a high degree of predictability for type A reactions can be realised. However, it must still be recognised that the population sizes used in animal clinical trials and target animal safety tests are usually too small to detect rare idiosyncratic type B reactions.

Overall, the results of laboratory animal studies, together with those from target animal safety studies, clinical trials and field safety evaluations, should provide a significant degree of predict-

ability for adverse effects in treated animal patients (Carakostas and Colaianne, 1996), which can be enhanced to a degree by a knowledge of any adverse effects seen in humans treated with the same or related drugs, although it must be recognised that it is sometimes difficult to identify adverse effects from non-adverse effects in animal studies (Lewis *et al*., 2002). This knowledge can then be enhanced by data from spontaneous adverse reaction reporting schemes and, where appropriate, from post-marketing surveillance studies and pharmacoepidemiological techniques. The latter techniques will also be useful for investigating adverse reactions in susceptible subgroups not normally examined in laboratory species or target animal safety studies, and perhaps only investigated to a limited degree in clinical trials, including elderly animals, neonates and those with reduced hepatic or renal function, especially when the drug in question is aimed at one of those groups. For example, NSAIDs are frequently used in elderly dogs with muscular skeletal disorders, and these animals may also have hepatic and renal impairment.

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