Toxicological Effects of Veterinary Medicinal Products in Humans

Volume 2

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Toxicological Effects of Veterinary Medicinal Products in Humans

Volume 2

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Preface

The term ''veterinary medicinal product'' describes those medicines developed specifically for use in animals. The development of these products involves an enormous amount of intellectual effort and physical labour as well as a considerable amount of financial investment in order to ensure that animals have available products that are of the appropriate quality and with the correct degree of effectiveness. These products also need to be safe for the animal patient as well as for the user, for the consumer of edible animal products and for the environment. On the other hand, the term ''veterinary drug'' is misleading, as the majority of drugs used in veterinary medicine, with very few exceptions, either are used in human medicine or have been used in the past in human medicine. As a consequence, we tend to know a considerable amount about the toxicity of veterinary drugs from their use in human medicine. We only know a little regarding the safety of veterinary medicinal products in humans from their use in animals.

This books attempts to bring together some of this knowledge and experience to assess the safety of veterinary medicinal products. As described in the pages that follow, this involves user safety and safety of those who consume products derived from animals treated with veterinary medicines, and for the most part this means examining their toxicological and pharmacological properties. However, some veterinary drugs are also microbiologically active, and this presents certain hazards that also need to be taken into account. Finally, like human drugs, these products also eventually find their way into the environment. As a result, to examine the potential hazards arising from veterinary medicine, we need to evaluate their toxicological and pharmacological properties, *and* we need to consider their microbiological properties and their eventual fate in the natural environment. This latter aspect is of concern not only because organisms might encounter the remnants of veterinary medicines as a result of environmental contamination, but also because of the potential effects for human health from the contamination of land and drinking

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water. I have tried to reach a balance, and review the main issues that might impact on human safety arising from the use of veterinary medicinal products. It is not possible to cover every product or drug in a work of this nature, and I have made no attempt to do so. Some products are used infrequently, and some are only used in certain countries. Many others are human drugs that are used off-label in animals. I have attempted to cover the major drug classes as well as some individual drugs of interest. Some of these are now of historical interest as many have fallen out of use or have been replaced with more effective and safer alternatives. Nevertheless, it would be remiss to avoid discussion of these where they may have impacted human safety in the past, so I have included them here.

I would like to thank the authors who have invested significant efforts by providing chapters for this book – Dr Tim Marrs, Derek Renshaw and Professor Peter Silley. I would also like to thank my family – and dogs – for their forbearance and patience while I have been working on this project.

Kevin Woodward
Surrey

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CHAPTER 9

Human Safety of Coccidiostats: A European Perspective

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9.1 Introduction

In the European Union (EU) substances and preparations used to treat or prevent diseases in animals are normally regarded as veterinary medicinal products and are regulated under the veterinary medicines and related legislation (Chapters 2 and 3). The only exception to this is the coccidiostats group of substances and their related formulations. These may be regulated as veterinary medicinal products or they may be controlled under the EU's feed additives legislation. The main legal instrument for the regulation of feed additives was Directive 70/524/EEC but this has now been replaced by Regulation (EC) No 1831/2003 (on additives for use in animal feed) and its implementing rules under Regulation (EC) No 429/2008. Substances being evaluated for use in animal feed are assessed in much the same way as veterinary medicinal products, particularly for safety and efficacy. Once approved, they are entered into the European Union Register of Feed Additives. The majority of substances in this Register are micronutrients, flavouring agents, defined botanicals, digestibility enhances, preservatives, colouring agents, enzymes and microorganisms. However, the Register contains a small number of coccidiostats.

9.1.1 Coccidiosis, Coccidiostats and Anticoccidial Medicines

Coccidiosis is a disease caused by protozoal intracellular parasites known as coccidia, which commonly occur in animals, including poultry, cattle, pigs,

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rabbits, guinea-pigs, dogs and cats. Coccidiostats are substances that are administered to inhibit or delay the development of coccidiosis. In some instances the same substances are used as veterinary medicines to treat coccidiosis or other diseases.

9.1.2 Human Exposure

Humans can be directly exposed to coccidiostats as a result of handling them or feeds containing them. Such exposure is likely to be mainly by dermal and inhalation routes. Consumers of animal-derived foods can be orally exposed to residues of coccidiostats in food. These residues may be the result of intentional treatment of the animals with coccidiostats or the result of unintentional contamination of their feed.

9.1.3 Committees that Evaluate the Safety of Coccidiostats

The European Union (EU) agencies responsible for the scientific evaluation of the safety of feed additives and veterinary medicinal products are, respectively, the European Food Safety Authority (EFSA) and the European Medicines Agency (EMA). The EFSA is advised on scientific aspects of feed additives, including the safety of target species, consumers, users and the environment, by the Panel on Additives and Products or Substances Used in Animal Feed (FEEDAP). Some of the older evaluations of feed additive uses of coccidiostats under Directive 70/524/EEC were performed by the Scientific Committee on Animal Nutrition (SCAN), which reported to the European Commission prior to the establishment of EFSA. The Committee for Medicinal Products for Veterinary Use (CVMP) advises the EMA on scientific aspects of veterinary medicines. The distinction between medicinal and feed additive uses is not always clear, as some uses to prevent coccidiosis are considered to be medicinal (*e.g.* use of lasalocid in poultry).

Internationally, the safety of both veterinary medicines and pharmacologically active feed additives is evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA).

9.2 Approaches Taken to Ensure the Human Safety of Coccidiostats

9.2.1 Consumer Safety

The scientific committees mentioned earlier assess the safety for consumers of foods derived from animals treated with coccidiostats by assessing the available data from studies of the pharmacokinetics (including metabolism and residue depletion) in the target species and laboratory animals, toxicological studies and any available observations in exposed humans. Consumer safety is expressed numerically as an acceptable daily intake (ADI) and legal limits applied to concentrations of residues permitted in foods are established as a

series of MRL values. Withdrawal periods are set for each product and each target species to ensure that the residues in foods are maintained below the MRLs. The assessment of consumer safety is conducted in a similar manner to the processes employed with veterinary medicines (Chapter 3).

9.2.2 User Safety

User safety is addressed by considering whether exposure to the coccidiostat-containing product is likely to occur in the workplace and assessing whether there is a risk of toxicity following inhalation, irritation to skin or eyes or of skin sensitisation. Physical data (dusting potential and particle size distribution), laboratory animal studies and any observations in exposed humans are used in this assessment. The results of the evaluation of user safety data are used by risk managers to decide on the need for protective measures for workers and on the warning labels needed on product packages. Again, the principles applied are similar to those used for the assessment of user safety for veterinary medicinal products (Chapter 4).

9.2.3 Inconsistencies in the Values of ADIs

Some of the coccidiostats have been evaluated by more than one expert committee and some of these committees have calculated ADI values at different values to others. One reason for this is that some committees may have had access to data that were not available to other committees at the time that they performed their reviews. In addition, committees sometimes alter their ADI to take account of new information. Different experts may on occasion interpret the available data differently to each other or may choose to use different uncertainty factors when calculating the ADI (see Chapter 3). Furthermore, some committees have adopted different approaches to establishing an ADI to others. For instance, JECFA and the CVMP have made use of microbiological data based on possible effects on the human gut flora as part of the calculation of the ADIs for antimicrobial substances, whereas the FEEDAP has not pursued this for feed additives.

The FEEDAP practices a product-based assessment in which applicants each provide data in support of their own product, with the result that, occasionally, different sets of data can be provided for the same coccidiostat. Therefore, there is potential for different ADIs and MRLs being set for the same substance. The CVMP and JECFA examine the data on the active ingredient from all sources when calculating ADI values and elaborating MRLs.

There are several guidelines available for the conduct of studies for safety, quality and efficacy of veterinary medicinal products. As described in Chapter 2, the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) has developed a series of guidelines which have been adopted by a number of

regulatory agencies, and notably by those in the EU, USA and Japan. Clearly, this includes the EMA and the EU national regulatory authorities. VICH Guideline GL36[1] gives general advice on how to estimate microbiological ADIs. One approach to calculating a microbiological ADI that has been used extensively by the CVMP and JECFA is to use a mathematical formula based on *in vitro* MIC_{50} (minimum inhibitory concentrations) data for a range of bacterial species representing the human gut flora. Other factors taken into account in the calculation include the typical size of a human faecal bolus, faecal binding potential and ease of transference of genes for resistance. The methods used by JECFA and CVMP to calculate microbiological ADIs from *in vitro* MIC_{50} data have been modified and refined from time to time, but the two committees have not always been in agreement over the most appropriate way of calculating microbiological ADIs. The microbiological ADI is used as the overall ADI for a substance only if it is lower than the ADI calculated by applying an appropriate uncertainty factor to a no observed effect level (NOEL) or benchmark dose level (BMDL) derived from the toxicological and pharmacological data. As described in Chapter 3, where there are several ADI values for a particular substance, *e.g.* derived from toxicological, pharmacological and microbiological data, the lowest value is normally chosen in the elaboration of MRLs.

The FEEDAP has taken a different approach to the use of microbiological data, considering them in a qualitative way and not using them in the calculation of the ADI.

9.2.4 Inconsistencies in the Approaches Used to Establish MRLs

The MRL value for a coccidiostat or veterinary drug is the highest concentration of a marker residue that is legally allowed to be present in a food derived from treated animals. The marker residue may be the parent drug or a metabolite and it is representative of the total of potentially harmful residues which remain in food. A series of MRLs are set for representative foods (muscle, liver, kidney, fat, milk, eggs) derived from each target species. The MRLs are elaborated in the EU by the European Commission on the basis of the advice and opinions of CVMP (for veterinary medicines) and FEEDAP (for feed additives). The EU is also committed to taking account of international MRLs set by the Codex Alimentarius Commission (Codex MRLs) on the scientific advice of JECFA.

The FEEDAP,[2] CVMP[3] and JECFA[4] use different methods in elaborating MRL values. Therefore, there is potential for them to have access to identical data but to calculate three different MRLs for the same substance in the same tissue or food commodities, from the same target species. In practice it is uncommon for CVMP and FEEDAP to propose different MRLs for the same tissue in the same target species, as the target species considered by FEEDAP for feed additive use of coccidiostats are usually different from those considered by CVMP for veterinary medicinal use. When JECFA proposes different

MRLs to FEEDAP or CVMP, efforts are made to harmonise the values that should be used, either by trying to persuade the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF) to use the value proposed by the EU or by adopting the Codex MRL established by the CCRVDF. If agreement cannot be reached, the EU MRL is used.

9.2.5 Cross-contamination of Feed with Coccidiostats

Surveys of residues in foods have sometimes detected coccidiostats in foods derived from species in which their use is not authorised.[5,6] This can be the result of misuse or it can be due to unintentional cross-contamination of feed from a batch containing a coccidiostat with one that is intended to be coccidiostat-free. Normal practice at feed mills can result in some carry-over of a small amount of an earlier batch of feed into the next batch to be mixed. The level of cross-contamination resulting from this would be expected to be less than 10%. A risk assessment showed that the level of residues of each coccidiostat in edible animal tissues was sufficiently low that the exposure of consumers would not exceed the ADI of each coccidiostat.[5,6] It was concluded that such cross-contamination of animal feeds would not be expected to adversely affect the health of consumers. However there were a few instances in which a high level of cross-contamination by feed additives containing high levels of coccidiostats could cause some non-target animals to be exposed to levels of coccidiostats that were potentially detrimental to their health. Therefore the EFSA Panel on Contaminants in the Food Chain recommended limits for levels of cross-contamination of lasalocid and maduramycin in some foods and these limits have been introduced into legislation.[7] Cross-contamination limits were not set for those coccidiostats that were unlikely to leave harmful levels of residue.

9.3 The Safety of Authorised Coccidiostats

9.3.1 Ionophoric Polyether Coccidiostats

Polyether coccidiostats, such as monensin and lasolocid, act against coccidia by interrupting the flow of ions across membranes of these single-celled organisms. The ionophoric properties of the polyether coccidiostats also have consequences for the health of animals and humans as they can also disrupt ion flows across membranes in mammalian cells. The nervous system, muscles and the cardiovascular system are particularly vulnerable to effects on ion flows.

Ionophores modify the permeability of biological membranes by forming lipid-soluble reversible cation complexes that can transport cations across biological membranes. Polyether ionophores may differ in their polarity and each has its own distinctive pattern of ion selectivity. Thus, the ionophoric coccidiostats are not identical in their ways of interacting with different membrane systems and in their toxicological effects.

It is likely that the lethal cardiotoxicity caused by high doses of ionophores in acute toxicity tests, the positive inotropy (increased force of heart contraction, causing an increase in blood flow from the heart) produced at lower doses, and the myopathy in skeletal muscles and neurotoxicity are probably all related to ion flow disrupting properties. It is plausible that there is a common mechanism for these effects of polyether ionophore coccidiostats. When such a common mechanism of toxicity exists for a group of substances, the default assumption is that their toxicity will be additive.[8] As such, the ionophoric effects of polyether coccidiostats in a mixed exposure may be regarded as additive, whilst the toxicological properties of coccidiostats that are not due to disruption of ion flows are less likely to be caused by a common mechanism and are not considered to be additive.

Some polyether ionophore coccidiostats have been shown to cause positive inotropy. The potential for residues to produce this effect in consumers of food of animal origin is of particular concern for people with coronary vascular and other cardiac diseases. It has been suggested that, although drug-induced inotropy would not be expected to present a serious threat to people with healthy blood vessels that can readily dilate, it could pose special problems for those with coronary diseases.[9] If a particular coronary vessel is partially occluded through disease, the resultant flow impairment could produce some degree of hypoxia, which would trigger the autoregulatory process, causing the vessel to dilate. If that vessel was dilated close to its limit, its ability to dilate further when exposed to an ionophore would be less than that of healthy vessels. Thus ionophore-induced dilation of healthy coronary vessels in parallel with less responsive partially-occluded vessels would divert blood flow away from the diseased vessels and towards the healthy vessels, thereby exacerbating the hypoxia in the myocardium supplied by the partially-occluded vessels. The affected individual could subsequently suffer adverse effects such as angina. This phenomenon is called ''coronary steal''.[10] Irrespective of whether or not polyether ionophore-induced inotropy is regarded as a potentially serious adverse effect on health due to consumption of residues, it is clearly an undesirable effect that consumers should not expect as a result of eating food of animal origin.

There has been inconsistency of approach to assessing the potential of ionophoric coccidiostats to cause inotropy. The JECFA did not regard inotropy as an adverse effect to be considered when setting an ADI, whereas the CVMP and the FEEDAP have both set ADIs for some ionophores on the basis of their inotropic effects. For some polyether coccidiostats, there has been a requirement to perform special oral-dosing studies in dogs to identify an NOEL for this effect (*e.g.* the evaluation of monensin by the CVMP), but for some other polyether coccidiostats an ADI has been proposed without an NOEL for inotropy being identified (*e.g.* CVMP assessment of lasalocid). Although general toxicological studies incorporating clinical chemistry, gross pathology, histopathology and electrocardiogram measurements can be relied upon to detect most toxicological effects on the cardiovascular system, these investigations cannot be relied on to detect inotropy.

9.3.1.1 Lasalocid

Two different EU committees have assessed the consumer safety of lasalocid (Figure 9.1). The FEEDAP considered the use of the lasalocid-containing product Avatec 150G as a coccidiostatic additive to the feed of chickens[11] and the CVMP considered the use of lasalocid as a veterinary medicine in feed for poultry for the prevention of coccidiosis.[12] The two committees had access to similar packages of safety data, including information on pharmacology, microbiology, short-term toxicity, long-term toxicity, mutagenicity, carcinogenicity, reproductive toxicity and developmental toxicity, but came to different conclusions.

Lasalocid has similar metabolic profiles in chickens, turkeys and rats, with excretion of unchanged lasalocid in the bile being the major route of clearance. A small proportion of the lasalocid undergoes hydroxylation prior to excretion *via* the kidneys. Oral doses are rapidly absorbed and are also rapidly cleared within one day of dosing. Most of the remaining tissue residues occur in the form of unchanged lasalocid.[11,12,56]

The most sensitive toxicological effects of lasalocid were increased adrenal weight and minor changes to some blood biochemistry parameters that were seen in a two year rat feeding study, and reductions in pregnancy and fetal bodyweight in a rabbit developmental study. An NOEL of 0.5 mg/kg bw/day was identified for all these effects. The CVMP noted that published studies showed that high doses could cause peripheral neuropathy in birds, but also noted the absence of special studies of neurotoxicity in mammals.

Inotropy was seen in dogs given a single intravenous dose of 1 mg/kg bw. An NOEL for inotropy was not identified. No adverse effects on the cardiovascular system were found in a two year dog oral toxicity study, although the routine tests performed in the study would not be expected to detect inotropy.

Microbiological data included a series of *in vitro* MIC_{50} values for bacterial species representative of the human gut flora. The CVMP used these to calculate a microbiological ADI, but noted that toxicological effects occurred at lower doses than those that affected the gut flora.[12] The FEEDAP chose not to identify a microbiological ADI, but noted that the inhibitory concentrations for susceptible strains of human gut flora bacteria are far greater than the residual concentrations of lasalocid found in edible tissues from animals given the recommended dose.[11]

The CVMP[12] had concerns over the paucity of data on metabolism in rats and chickens, so it applied a 200-fold safety factor to the NOEL of

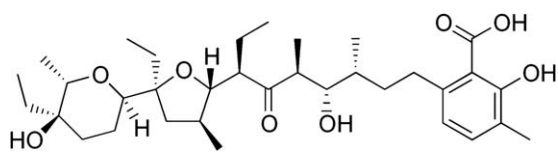

Figure 9.1 Chemical formula of lasalocid.

0.5 mg/kg bw/day to calculate an ADI of 0.0025 mg/kg bw for lasalocid. The FEEDAP[11] did not share the concerns of CVMP about the importance of these gaps in the available data and had access to additional studies on metabolism,[56] so it used a more conventional safety factor of 100 to derive an ADI of 0.005 mg/kg bw.

A series of MRLs were established for poultry tissues under Regulation (EC) No 470/2009 on the advice of the CVMP.[12–14] The FEEDAP[56] adopted this same set of MRLs for lasalocid in poultry tissues.

User safety was assessed by FEEDAP.[11] The results of acute inhalation studies in mice, rats and guinea pigs indicated that lasalocid sodium dust has the potential to cause local toxicity to the respiratory tract and lungs and systemic toxicity to other organs. Acute dermal toxicity in rabbits was low, reflecting poor skin absorption. Lasalocid sodium did not cause skin irritation in rabbits or skin sensitisation in guinea pigs, but did cause eye irritation in rabbits.

9.3.1.2 Maduramycin

Maduramycin (Figure 9.2) is the most toxic of the authorised coccidiostats. The consumer safety for the use Cygro 1%, a coccidiostat feed additive based on maduramycin ammonium alpha, was initially assessed within the EU by SCAN. Cygro 1% was authorised for use in feed for chickens for fattening[15] and for turkeys[16] on the advice of SCAN. It has been since been reformulated as a less dusty additive, Cygro 10G, which was evaluated by FEEDAP for use in feed for chickens for fattening[17] and turkeys.[18]

The metabolism of maduramycin ammonium alpha following oral dosing of chickens, turkeys and rats[16,17] showed that maduramycin-α was the main compound excreted (26%) and the other major metabolites were maduramycin-alpha, maduramycin glucuronide, di-*O*-desmethyl-maduramycin and mono-*O*-desmethyl-maduramycin. In the rat, the metabolites identified in the liver were maduramycin-alpha and an *O*-demethylated metabolite. After cessation of treatment, levels of residues rapidly decreased in all tissues investigated.

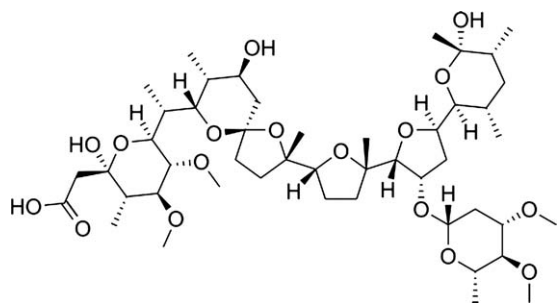

Figure 9.2 Chemical formula of maduramycin.

Unpublished reports of toxicological studies of maduramycin have been assessed by SCAN[16] and FEEDAP;[17] their assessments gave limited details of them. Oral doses of maduramycin were tested in animal studies (mice, rats, rabbits and dogs) of acute toxicity, 28 day, 90 day and 12 month repeat dose toxicity, carcinogenicity, chronic toxicity and reproductive toxicity, including developmental toxicity. No teratogenicity was seen. Neurotoxicity was seen at high doses. The FEEDAP[17] concluded from chronic toxicity/carcinogenicity studies in mice and rats that maduramycin ammonium alpha is not carcinogenic.

Maduramycin caused chromosome breaks in CHO cells, but gave negative results in all other *in vitro* tests performed, namely a *Salmonella*/microsome reverse mutation test, HGPRT mutation test and an unscheduled DNA synthesis (UDS) test. Two tests for *in vivo* mutagenicity in two different somatic tissues (a comet assay in liver cells from orally dosed rats and a cytogenetics assay in bone marrow of intraperitoneally dosed rats) gave negative results. Overall, the results showed that maduramycin was not genotoxic.[16]

The FEEDAP[17] calculated an ADI of 0.001 mg/kg bw by applying a safety factor of 100 to the NOEL of 0.1 mg/kg bw/day for litter weight depression in a two-generation rat reproduction study and to the NOEL of 0.12 mg/kg bw/day for reduced thyroid/parathyroid weight in a two-year chronic toxicity study in rats. None of the studies examined endpoints that would be suitable for identifying whether or not exposure to maduramycin could cause inotropy (the critical endpoint in the evaluation of another ionophore, monensin). Possible effects on the gut microflora were not taken into account when the ADI was considered, but SCAN[16] noted that maduramycin has moderate activity against Gram-negative bacteria but no activity against Gram-positive bacteria. The SCAN further noted that oral treatment of chickens had no effect on their gut flora. A set of MRLs was recommended for chickens.[17]

There has been a report of seven people poisoned with maduramycin.[19] The group had eaten a total of approximately 450 g of maduramycin (an average of about 65 g/person) mixed with vegetable oil. There were early manifestations of quadipareisis. Within two days, two of the victims died of respiratory failure and hyperkalaemia. At 8 days after exposure, the survivors were admitted to hospital. The clinical presentation was polyneuropathy with rhabdomyolysis. Electrocardiograph and echocardiograph results were normal and cardiomyopathy was not detected in any of the surviving patients. Nerve conduction studies showed polyradiculopathy. Acute renal failure developed at 8–10 days after exposure in 4 of the 5 survivors. Some patients needed mechanical ventilation for several days. Muscle pain subsided three weeks after exposure.

With regard to user safety, dermal exposure to maduramycin-α caused high systemic toxicity in rats and rabbits.[17] Maduramycin was irritant to skin and corrosive to eyes of rabbits,[16] but the formulated feed additive, Cygro 10G, did not show skin and eye irritating or skin sensitising potential when tested in animal studies.[17] Cygro 10G had high acute toxicity when inhaled by rats and was considered by FEEDAP[17] as being potentially harmful if inhaled by humans. Although Cygro 10G is formulated as granules that produce little dust

when handled, the FEEDAP[17] recommended that action should be taken whenever possible to minimise operator exposure to dust.

9.3.1.3 *Monensin*

The consumer safety of monensin (Figure 9.3) has been assessed within the EU by the CVMP[20] and by FEEDAP[21–23,25,26] and internationally by JECFA.[24] The FEEDAP evaluated the use of the monensin-containing products Elancoban and Coxidin as coccidiostat additives for incorporation into the feed of chickens, turkeys, calves and cattle for fattening, whereas the CVMP considered the use of monensin as an active ingredient in veterinary medicines administered orally to dairy cattle to treat ketosis. JECFA considered the safety of food residues of monensin resulting from any uses in food producing animals.

Metabolism of monensin in laboratory animals was mainly by *O*-demethylation and hydroxylation forming substances with reduced ionophoric activity. Acute oral toxicity was high in mice, rats and rabbits with LD_{50} values of 22–96 mg/kg bw, but it was lower in monkeys (160 mg/kg bw caused no mortality). Repeated dosing caused degeneration of skeletal and cardiac muscle in mice, rats and dogs with effects at doses as low as 15 mg/kg bw/day. Monensin was not carcinogenic and was not genotoxic when tested for bacterial gene mutation, *in vitro* cytogenetics and in an *in vivo* bone marrow micronucleus test in mice. It did not cause reproductive or teratogenic effects at doses below those that were maternally toxic. In humans,[24] ingestion of 500 mg caused rhabdomyolysis, kidney failure and heart failure and death within 11 days.

The CVMP[20] and JECFA[24] took account of microbiological data when calculating ADI values, but noted that the value of the ADI was governed by the toxicological data rather than the effects on microorganisms. The FEEDAP did not use the MIC data in the calculation of its ADI for monensin.

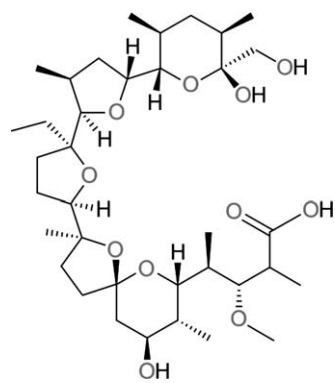

Figure 9.3 Chemical formula of monensin.

The CVMP[20] and the FEEDAP[21–23] calculated an ADI of 0.003 mg/kg bw for monensin, based on the NOEL of 0.345 mg/kg bw/day for inotropy in dogs and the NOEL of 0.3 mg/kg bw/day for maternal toxicity in a rabbit developmental toxicity study. JECFA noted the cardiovascular effects in dogs, but did not consider them to be adverse.[24] Instead, JECFA calculated an ADI of 0.01 mg/kg bw by applying a 100-fold safety factor to the NOEL of 1.14 mg/kg bw/day for decreased bodyweight gain in a two-year rat dietary study.

The FEEDAP[25] recommended a set of MRLs relating to use of Elancoban and Coxidin, in chickens and turkeys for fattening. It proposed[26] that there is no need to set MRLs or withdrawal periods for feed additive use of Elancoban in calves for rearing and cattle for fattening, as tissue residues are sufficiently low that consumers will not receive doses of monensin in excess of the ADI if the product is used as recommended. Nevertheless, MRLs have been set for bovine species[27] because the CVMP identified a need for limits on residues resulting from medicinal use of monensin in cattle.

When the FEEDAP addressed the user safety of Coxidin,[23,28] it noted that the product showed a high dusting potential with a high proportion of particles being of respirable size. Monensin is highly toxic by inhalation (LC_{50} 69 mg m^{-3} in male rat and 51.9 mg m^{-3} in females). Monensin sodium was non-irritant to skin and slightly irritant to eyes of rabbits and it was not a skin sensitiser in a maximisation test in guinea pigs.[22] FEEDAP concluded that there was a high risk to health from occupational exposure by inhalation and it recommended that appropriate personal protective measures should be taken when handling Coxidin. It was further recommended that undiluted Coxidin should not be used on-farm and use should be restricted to the premises of feed compounders.[28]

9.3.1.4 Narasin

Narasin (Figure 9.4) is used in coccidiostat products both as the sole anticoccidial agent (Monteban) and in combination with nicarbazin (Maxiban). The consumer safety of narasin has been assessed by the FEEDAP[29,30] and JECFA.[24] The FEEDAP assessed it as part of the evaluation of the narasin-based feed additive Monteban G100[29] and the effects of narasin in combination with nicarbazin have been addressed in the evaluation of Maxiban G160.[30] Combined dosing of nicarbazin and narasin did not appear to increase the toxicity of either substance, although co-administration enhances their efficacy as coccidiostats.

The main metabolic route for narasin in chickens and rats involved oxidation to yield hydroxy- and keto-narasins. Oral LD_{50} values were 16 mg/kg bw in mice and 20 mg/kg bw in rats. The major toxic effect of repeat dosing was degeneration of muscles and nerves. The lowest NOEL these findings and for neurological findings (leg weakness and increased salivation) was 0.5 mg/kg bw/day in a one-year dog study. A NOEL of 1.53 mg/kg bw/day was identified for inotropy in dogs. Narasin was not genotoxic. It was not carcinogenic in

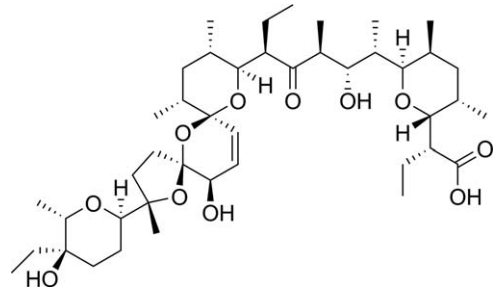

Figure 9.4 Chemical formula of narasin.

mice and rats. It caused no reproductive or developmental toxicity at doses below those that caused maternal toxicity.

Acute *in vitro* MIC (minimum inhibitory concentration) data were submitted for effects of narasin on a range of bacteria, but FEEDAP[29] did not use these data in their calculation of the ADI. The JECFA[24] noted that there was no need to calculate a microbiological ADI as a faecal-binding study showed that most of the narasin in the gut is bound and inactive. In addition there is no risk of selection of resistance to antibiotics that are important for human medicine. Both FEEDAP[29] and JECFA[24] calculated an ADI of 0.5 mg/kg bw/day by applying a 100-fold safety factor to the NOEL of 0.5 mg/kg bw/day from the one-year dog study.

The FEEDAP investigated the user safety of Monteban G100[29] and Maxiban G160.[30] Tests showed narasin and Monteban G100 to be irritant to skin and eyes of rabbits. A guinea-pig maximisation test gave a negative result for skin sensitisation by narasin, but a local lymph node assay in mice showed Monteban G100 to be a potential skin sensitiser. Two workers developed IgE-mediated allergy to narasin that has been confirmed by skin testing. In inhalation toxicity studies of dogs, 1.1 mg m^{-3} of dust of Monteban G100 (containing 740 g kg^{-1} of narasin) caused ataxia, limb paresis, tremors, eye irritation and degeneration of skeletal muscles and peripheral nerves, with a NOEL of 0.11 mg m^{-3}. These results indicate that narasin should be regarded as an irritant, a potential skin sensitiser and as potentially harmful by inhalation.

9.3.1.5 Salinomycin

The consumer safety of salinomycin (Figure 9.5) has been assessed within the EU by the FEEDAP. Three salinomycin-containing feed additive products have been evaluated by FEEDAP: Kokcisan 120G, Sacox 120 microGranulate and Bio-Cox 120G (now re-named Salinomax 120G). Different sets of data were supplied in support of each product, which were assessed in isolation from one-another. No ADI could be derived for salinomycin on the basis of the data supplied for Kokcisan,[31] but an ADI of 0.005 mg/kg bw was

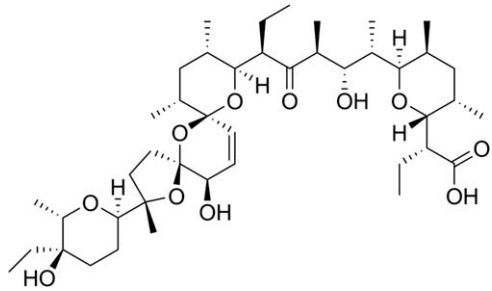

Figure 9.5 Chemical formula of salinomycin.

calculated for salinomycin on the basis of the data supplied for each of the other two products.[32–35] This was calculated by applying a 100-fold safety factor to the NOEL of 0.5 mg/kg bw/day for serious neurotoxic effects (including peripheral neuropathy) seen in a 1-year dog study. The results of electrocardiogram measurements in this 1-year dog study did not reveal any adverse effects, but a special study of effects on the cardiovascular system of dogs showed inotropic effects at doses down to the lowest one tested: 0.32 mg/kg bw/day. A NOEL could not be identified for the inotropy. It is not clear from the EFSA Opinion why the FEEDAP chose to calculate an ADI for salinomycin when an NOEL had not been identified for its inotropic effects.

The other studies that were considered included repeat dose oral toxicity studies, carcinogenicity studies, reproduction toxicity studies, developmental toxicity studies and mutagenicity tests. One of the *in vitro* mutagenicity gave a positive result for the production of chromosomal aberrations. However, salinomycin was regarded as non-genotoxic as other *in vitro* tests for bacterial mutations, mammalian cell gene mutations and UDS gave negative results, and a range of *in vivo* studies, including a bone marrow micronucleus test and a liver UDS assay, gave uniformly negative results.

Although microbiological data were available, the FEEDAP chose not to use these in its consideration of an ADI. Studies of *in vitro* MIC values of 109 strains of bacteria showed that the strains were mostly insensitive to salinomycin. This suggests that dietary salinomycin, at doses below the ADI, would be unlikely to cause changes to bacterial populations of the gut flora that could adversely affect the health of consumers.

The FEEDAP[34] proposed MRLs for salinomycin in tissues from chickens for fattening.

Assessments of user safety indicated that salinomycin sodium biomass was irritant to skin and eyes and Bio-Cox 120 G caused skin sensitisation in mice.[33] Sacox 120 microGranulate was slightly irritant to skin and eyes of rabbits and was a skin sensitiser in guinea-pigs.[34] Neither Sacox microGranulate[32] nor Bio-Cox 120G[33] produce much dust when handled and none of the particles in Bio-Cox are of respirable size. However, about 10% of the Sacox product consisted of respirable particles ($<10\,\mu m$). No adverse effects were seen in an acute inhalation toxicity study of Sacox in rats, but the air concentration

achieved was low and this result did not give full assurance of the inhalation safety of salinomycin.[34] Nevertheless, FEEDAP concluded that there was minimal risk of inhalation toxicity in users handling these products but precautions should be taken to minimise skin exposure.

9.3.1.6 *Semduramycin*

The consumer safety of semduramycin (Figure 9.6) was assessed by SCAN,[36] which considered its use as an additive to the feed of chickens. SCAN calculated an ADI of 0.00125 mg/kg bw for semduramycin by applying a safety factor of 100 to the toxicological NOEL of 0.125 mg/kg bw/day for decreased serum protein and sodium levels at 0.25 mg/kg bw/day in a two-year rat chronic toxicity/carcinogenicity study. In dogs (1, 6 and 12 month studies) there was a NOEL of 0.3 mg/kg bw/day for retinal changes (degeneration of rods and cones) seen at 1 mg/kg bw/day or more. At higher doses, there were histopathological changes to skeletal muscle and an oral dose of 3 mg/kg bw/day caused ataxia as a result of myopathy. No evidence of effects on the heart were revealed by electrocardiogram readings at doses of up to 4 mg/kg bw/day, although some dogs from the top-dose group of the 12-month study (given 1 mg/kg bw/day) had increased systolic blood pressure at the end of the study. An NOEL for inotropy was not identified for oral doses. A three-generation reproduction study in rats showed an NOEL of 0.5 mg/kg bw/day based on decreased maternal bodyweight gain. No developmental toxicity was seen at doses below those that were maternally toxic. A suitable battery of

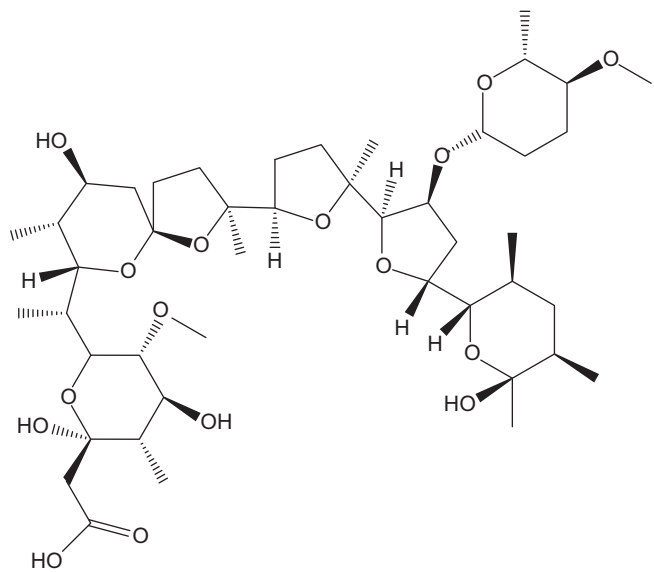

Figure 9.6 Chemical formula of semduramycin.

mutagenicity tests gave negative results and the results of carcinogenicity studies in mice and rats provided no evidence of carcinogenicity. Microbiological data were submitted and were discussed by SCAN, which chose not to use these data in calculating the ADI for semduramycin.

SCAN concluded on user safety that the main risk from semduramycin was from inhalatory exposure.[36] Acute inhalation toxicity was high (rat LC_{50} 67 mg m^{-3}), but dusting was minimised by an antidust formulation for the additive. It was nevertheless recommended that workers should use a dust mask.

9.3.2 Non-ionophoric Coccidiostats

9.3.2.1 Amprolium

Amprolium (Figure 9.7) is a thiamine antagonist. Its oral use as a veterinary drug is permitted for control of coccidiosis in poultry.[36,37] However, its use as a feed additive is no longer permitted. Authorisation as a coccidiostatic feed additive was withdrawn in 1992 when data requested by SCAN for evaluation of amprolium-containing products (Pancoxin and Pancoxin Plus)[74] were not provided.

Amprolium is metabolised to numerous metabolites, mostly unidentified. It is of low acute oral toxicity. High doses of amprolium were given to rats in repeat dose oral studies of up to 56 weeks duration without any clear toxicological effects. In a two-year rat feeding study, bodyweight gain was suppressed at 200 mg/kg bw/day or more, but there was no evidence of other adverse effects at up to 1000 mg/kg bw/day. There was increased mortality in dogs given 300 mg/kg bw/day or more (NOEL 100 mg/kg bw/day) in oral studies of 12 weeks or two years duration.

Mutagenicity data showed *in vitro* genotoxicity in a micronucleus test and a cytogenetics assay, inconsistent results in the *Salmonella*/microsome reverse mutation assay, but negative results in a bacterial *rec* assay. However, *in vivo* tests (mouse micronucleus assay and rat liver UDS assay) gave negative results. As there were no signs of genotoxicity in tests performed *in vivo* in two different somatic tissues, it was concluded that amprolium was not a genotoxic hazard to humans.[37] Amprolium has been administered to AIDS patients at doses of up to 45 mg/kg bw/day for up to 8 days without ill effect, but doses greater that 90 mg/kg bw/day had adverse effects on the nervous and cardiovascular systems.[38]

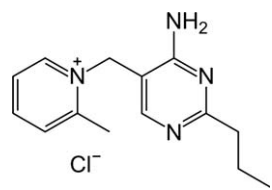

Figure 9.7 Chemical formula of amprolium.

The CVMP considered amprolium to have no antibacterial properties, so it did not request data on possible effects on the human gut flora. It[37] set an ADI of 0.1 mg/kg bw for amprolium by applying a safety factor of 200 to the NOEL of 20 mg/kg bw/day for suppressed bodyweight gain in the two-year rat feeding study. The large safety factor was used to compensate for the poor quality of the critical study.

No MRLs were established for residues in poultry tissues and eggs, as the CVMP[38] considered that the authorised use of amprolium as a veterinary medicine in poultry would not result in harmful concentrations of residues in foods.

9.3.2.2 Decoquinate

Decoquinate (Figure 9.8) is a 4-hydroxyquinolone antimicrobial. The FEEDAP considered the use of the decoquinate-containing product Deccox as a coccidiostat feed additive for chickens for fattening, and the CVMP considered its use as an active ingredient in coccidiostat veterinary medicines administered to calves and lambs. The committees evaluated consumer safety based on similar packages of data and came to similar conclusions.[39,40,41]

Information on the metabolism of decoquinate was limited. In chickens, unchanged decoquinate made up 23% of the radiolabel found in the bile, 85% of that in the liver and 59% of that in the kidneys, but the metabolites making up the remainder of the radioactivity were not identified. Metabolites noted in studies in rats, sheep and calves were not identified.

The FEEDAP[39] and CVMP[40,41] noted that the levels of use of decoquinate would not result in concentrations which would kill bacteria and thus residues would not affect the human gut flora. They would not be expected to cause bacterial resistance. Consequently it was not necessary to identify a microbiological ADI.

Acute oral toxicity was low in rats ($LD_{50} \leq 5000$ mg/kg bw). Repeat dose oral toxicity studies were performed in rats (several studies of 16 day to 2 year duration) and dogs (12 week and 2 year studies), with the lowest NOEL seen in these studies being 15 mg/kg bw/day for subdued behaviour in dogs given 62.5 mg/kg bw/day in the 12 week study. No adverse effects were seen in the rat studies at doses of up to 37.7 mg/kg bw/day for up to two years. No carcinogenicity studies were available, but the two-year toxicity study in rats showed no treatment related effects on tumour incidences. A three-generation study in rats produced no adverse effects on reproduction, even at the highest dose level

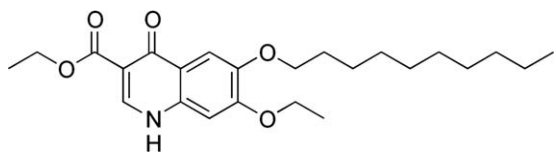

Figure 9.8 Chemical formula of decoquinate.

of 60.6 mg/kg bw/day. A rat developmental study resulted in some fetotoxicity (retarded skeletal development) at a dose of 300 mg/kg bw/day (NOEL 100 mg/kg bw/day) but no effects on embryotoxicity or teratogenicity. A developmental toxicity study in rabbits produced embryotoxicity at 100 mg/kg bw/day (NOEL = 60 mg/kg bw/day) but no effects on fetotoxicity or teratogenicity.

Mutagenicity studies were not performed to modern standards, but their results suggested that decoquinate was not genotoxic. A mouse lymphoma assay indicated that decoquinate caused gene mutation only at the highest concentration tested in the presence of metabolic activation, but it was questionable whether this should be regarded as a positive result for mutagenicity as most of the cells were killed at this concentration. Other *in vitro* mutagenicity tests (*Salmonella*/microsome reverse mutation assays, a bacterial *rec*-assay and a cytogenetics test) gave negative results. No *in vivo* mutagenicity tests were available.

Both committees calculated an ADI value of 0.075 mg/kg bw for decoquinate by applying a safety factor of 200 to a toxicological NOEL of 15 mg/kg bw/day for subdued behaviour, reduced activity and emesis in dogs in a 12-week oral toxicity study. The large safety factor was used to account for uncertainty about the NOEL due to the fact that the critical dog study and some of the other toxicity studies (including the rabbit developmental toxicity study) were not conducted to modern standards.

There are no EU MRLs for decoquinate. The FEEDAP[39] could not establish any MRLs because the risk for consumers exposed to decoquinate residues in chicken tissues could not be adequately assessed as none of the metabolites had been identified or quantified in either chickens or laboratory rats. The CVMP[41] advised that the concentrations of residues left in muscle, liver, kidney and fat as a consequence of the authorised use of decoquinate in cattle and sheep were unlikely to be sufficiently high to be hazardous to consumers.

The safety of workers handling Deccox was considered by the FEEDAP.[39] It was shown to have a low dusting potential and only a small proportion of its weight consisted of particles of respirable size. Furthermore, decoquinate was of low acute inhalation toxicity when tested in rats in two studies. Decoquinate was non-irritant to skin and eyes of rabbits and showed no potential to cause skin sensitisation when tested in two studies using guinea pigs. FEEDAP recommended that the normal handling of Deccox/decoquinate on-farm or in feed mills would present little risk of ill health in workers.

9.3.2.3 Diclazuril

Diclazuril is a benzeneacetonitrile derivative (Figure 9.9). The consumer safety of diclazuril has been assessed by the FEEDAP,[42,43] the CVMP[44,45] and the JECFA.[46–49] The FEEDAP reviewed the safety of the use of Clinacox 0.5% (a diclazuril-based coccidiostat) in chickens,[50,51] turkeys,[52] guinea fowl[53] and rabbits.[54] The CVMP considered the use of diclazuril as the active ingredient in anticoccidial veterinary medicines administered orally to all ruminant and

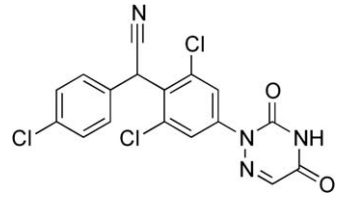

Figure 9.9 Chemical formula of diclazuril.

porcine species.[44,45] JECFA considered the safety of residues resulting from any use.

Diclazuril was of low acute toxicity in mice, rats, rabbits and dogs when tested by oral, dermal, subcutaneous and intraperitoneal routes. Repeated oral doses caused vacuolation of hepatocytes in mice, rats and dogs, with the lowest NOEL being 2.9 mg/kg bw/day in male mice in a chronic toxicity/carcinogenicity study. Uniformly negative results were obtained in a wide range of *in vitro* and *in vivo* mutagenicity tests, including bacterial tests, a mouse lymphoma test, an *in vitro* micronucleus test, an *in vivo* mouse bone marrow micronucleus test and a mouse dominant lethal test. There was no evidence of carcinogenicity in studies in mice and rats. In a two-generation rat reproduction study, there were decreases in the weight and survival of pups with a NOEL of 5 mg/kg bw/day. In a rat developmental toxicity study there was maternal toxicity and decreased litter weight, with a NOEL of 5 mg/kg bw/day, with slight fetotoxicity at maternally toxic doses but no teratogenicity at any dose tested. In rabbits, there was teratogenicity at high doses (1280 mg/kg bw/day), but the NOEL for developmental toxicity of 80 mg/kg bw/day was identified on the basis of fetotoxicity.

Diclazuril was devoid of antimicrobial activity against a range of fungi and bacteria, including several pathogens,[44,46,47] but the range of micro-organisms tested was not typical of a healthy human gut flora.

FEEDAP, CVMP and JECFA all identified an ADI for diclazuril of 0.03 mg/kg bw (when expressed to one significant figure), by applying a 100-fold safety factor to a NOEL of 16 mg/kg feed (2.9 mg/kg bw/day) for hepatic changes (hypertrophy of centrilobular hepatocytes and sinusoidal cells, histiocytosis and pigmentation of macrophages in mesenteric lymph nodes) in male mice in the two-year chronic toxicity/carcinogenicity study.

The CVMP[45] advised that there was no need to establish MRLs for use of diclazuril by dietary administration to pigs and ruminants, as the recommended dosing procedures for each species would not cause harmful levels of residues to occur in edible tissues. The FEEDAP recommended MRLs for foods derived from chicken,[55] turkeys,[55] guinea fowl[53] and rabbits.[54] On the advice of JECFA, the Codex Alimentarius Commission set MRLs for tissues from sheep, rabbits and poultry, but within the EU these MRLs are only applicable for sheep tissues, as the EU MRLs established on the advice of FEEDAP and CVMP take precedence for other species.

When assessing the safety of Clinacox 0.5% for workers, the FEEDAP[53,54] noted that rabbit irritancy studies of diclazuril and of Clinacox showed no irritation to skin and only minimal eye irritation. The results of a skin sensitisation study in guinea pigs gave no indication of any sensitisation potential. In an acute inhalation toxicity study in rats, there were no mortalities, but an air concentration of 2.24 g m^{-3} for 4 hours caused changes to the lungs in 60% of the animals. It was not clear from the reports[53,54] whether diclazuril or Clinacox had been the test article in the studies of sensitisation and inhalatory toxicity.

9.3.2.4 Halofuginone

Halofuginone is a quinazolone antimicrobial agent (Figure 9.10; see also chapter 12). The *trans*-isomer of halofuginone hydrobromide (HBR) is used in feed additives for turkeys and chickens for fattening and in veterinary medicines for bovine animals, other than those from which milk is produced for human consumption.

The consumer safety of halofuginone has been assessed by FEEDAP[57] and CVMP[58,59] on the basis of similar packages of data. The FEEDAP considered an application for a generic approval of use of HBR as an additive to the feed of turkeys and chickens, whereas the CVMP considered elaboration of MRL values for the use of HBR as an active ingredient in veterinary medicines administered to calves to treat diarrhoea caused by *Cryptosporidium parvum*.

The consumer safety of halofuginone has been investigated in studies that were performed on HBR or on halofuginone lactone (HLC). Some of these studies were not performed to modern protocols.

The CVMP[58] considered that it was not necessary to identify a microbiological ADI as no significant antimicrobial effect had been noted during *in vitro* MIC tests of HBR using a wide range of micro-organisms from the gut flora of calves and of humans. The FEEDAP[57] noted that there were no data on the potential for halofuginone to cause bacterial resistance to antibiotics used in human medicine.

HLC and HBR were of similar acute oral toxicity in rats (LD_{50} 30 mg/kg bw) and mice (LD_{50} 5 mg/kg bw) and the *cis*-isomer of halofuginone was of low oral toxicity (LD_{50} 430 mg/kg bw) in mice.[58] Repeat dose studies of HBR and HLC in mice, rats and dogs showed decreases in erythrocyte counts and related haematological parameters, with a compensatory increase in erythropoiesis. Mice were the most sensitive species with an NOEL for this effect of 0.07 mg/kg bw/day. In rats there was also fatty vacuolation of hepatocytes at 0.47 mg/kg bw/day or more of HBR. Both HBR and HLC were clearly

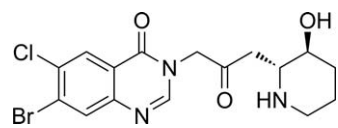

Figure 9.10 Chemical formula of halofuginone.

mutagenic in *Salmonella*/microsome reverse mutation assays, but were negative in other tests (HBR in a host-mediated *Salmonella* gene mutation assay, a mouse lymphoma assay, an *in vitro* cytogenetics assay, an *in vitro* UDS assay, an *in vivo* metaphase analysis of rat bone marrow, and HBR and HLC in *in vivo* micronucleus assays in mouse bone marrow). There was no evidence of carcinogenicity in studies of HBR in mice and rats. Treatment with HBR did not adversely affect the fertility of mice or dogs, but it caused low litter weight and reduced bodyweight gain of pups during lactation at 1 mg/kg feed in a three-generation reproduction study in mice. The CVMP and FEEDAP had different interpretations of the data on maternal bodyweight gain from this study with only CVMP considering a reduction to be treatment-related with a NOEL of 0.25 mg HBR kg^{-1} feed (equal to 0.034 mg/kg bw/day). There was no evidence of HBR causing any developmental toxicity in studies in rats and rabbits but maternal toxicity was seen with NOELs of 0.8 mg/kg bw/day in rats and 0.03 mg/kg bw/day in rabbits.

The CVMP[58] calculated an ADI of 0.3 mg/kg bw for halofuginone on the basis of NOELs for maternal toxicity (reduced bodyweight gain) seen in a rabbit developmental toxicity study and a mouse three-generation reproduction study. However, the FEEDAP did not derive an ADI as it still had concerns over the uncertainty about the possible genotoxicity of halofuginone. The FEEDAP required two negative results for genotoxicity in relevant *in vivo* studies in somatic tissues to provide it with the necessary degree of assurance that the clear positive result in an *in vitro* test was not indicative of a genotoxic hazard for exposed humans.[57]

CVMP recommended MRLs for halofuginone in tissues from bovine species,[59] but no MRLs have been elaborated for poultry.

Occupational safety was investigated by CVMP[58] and FEEDAP.[57] Halofuginone, HBR and HLC were irritant to skin and eyes of rabbits and caused "delayed systemic toxic effects".[58] Skin sensitisation potential of HBR was studied in guinea pigs with a Buehler test showing no sensitisation.[57,58] However, a maximisation test indicated that halofuginone caused slight skin sensitisation.[58] The dermal LD_{50} in rabbits was 16 mg/kg bw.[58] On inhalation of HBR dust by rats, there was eye and respiratory irritation, adverse systemic effects on the gastrointestinal and urogenital tracts, muscle incoordination and death with an LC_{50} of 0.053 mg L^{-1}.[57,58] Workers were recommended to use personal protective equipment to minimise dermal and inhalation exposure when handling halofuginone.

9.3.2.5 *Nicarbazin*

Nicarbazin (Figure 9.11) is a complex of two substances: 4,4′-dinitrocarbanilide (DNC) and 2-hydroxy-4,6-dimethylpyrimidine (HDP). Nicarbazin is the sole active ingredient in the coccidiostat products Koffogran, Monteban and Clinacox, but both nicarbazin and narasin are present in another product, Maxiban. Co-administration of nicarbazin with an ionophore such as narasin gives greater anticoccidial activity than using the same amount of either substance alone.

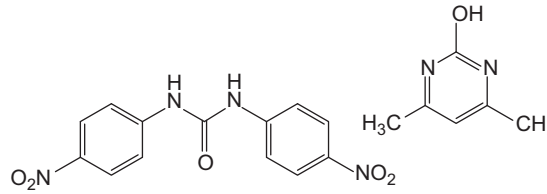

Figure 9.11 Chemical formula of nicarbazin. (HDP can exist in keto or enol forms – only the latter is shown)

Nicarbazin is stable in feed but it rapidly breaks down to form DNC and HDP in the lumen of the gut following ingestion by the target animal. Consumers of foods derived from treated animals are therefore potentially exposed to residues of DNC, HDP and their metabolites, but they are not exposed to nicarbazin. On the other hand, workers who handle nicarbazin and feeds containing nicarbazin may be exposed to nicarbazin dermally and by inhalation.

Toxicological studies have been performed on nicarbazin and on mixtures of DNC and HDP and of nicarbazin and narasin. Some assessments[48,60] of the consumer safety of the use of nicarbazin in food-producing animals have set limits based on the toxicity of nicarbazin, but a more recent assessment has focused on the safety of residues of DNC.[61,62] As DNC is absorbed less readily than nicarbazin and is of lower oral toxicity, it has been possible to recommend lower MRLs that more realistically reflect the risk to consumers from food residues of DNC. In the past, there have been a number of instances in which residues of DNC (the marker residue) have exceeded the low values for MRLs that were at the time based on the safety of nicarbazin.[5,6] Higher and more realistic MRL values based on the safety of DNC make MRL violations less likely without compromising consumer safety.

The consumer safety of the use of nicarbazin in food-producing animals has been assessed by FEEDAP.[61,63–66]

Nicarbazin had no activity against a range of pathogenic and non-pathogenic bacteria and no effect on the gut colonisation or excretion of *Salmonella enteritidis*, so it was considered to be microbiologically safe.

The metabolic fate of nicarbazin was similar in chickens and rats with DNC and HDP being metabolised independently of one another. HDP underwent little metabolism and was rapidly cleared from tissues. DNC was metabolised by reduction and acetylation and was cleared less rapidly. Residue depletion studies in chickens showed that normal use of nicarbazin would not result in toxicologically significant levels of HDP in tissues and that consumer safety would be dictated by the concentrations of DNC. Co-administration of nicarbazin and narasin to chickens increased concentrations of residues of DNC by 20% in liver to 50% in muscle.[62]

Nicarbazin was of low acute oral toxicity when administered alone or in combination with narasin. The sub-chronic oral toxicity of nicarbazin in rats was higher (NOEL 1.45 mg/kg bw/day, based on damage to the kidneys and testes) than that of DNC (NOEL 709 mg/kg bw/day, the highest dose tested).

Two-year oral toxicity studies of a mixture of DNC and HDP in dogs showed an NOEL of 154 mg/kg bw/day of DNC and 51 mg/kg bw/day of HDP based on elevated serum alanine aminotransferase (ALT). No adverse effects were seen in a two-year rat study at up to the highest dose tested: 300 mg DNC + 100 mg HDP/kg bw/day. Multigeneration reproduction studies in rats showed no adverse effects at up to 300 mg DNC + 100 mg HDP/kg bw/day. A developmental study of nicarbazin in rats showed fetotoxicity (delayed ossification) at 200 mg/kg bw/day with an NOEL of 70 mg/kg bw/day (JECFA[48] regarded the dose of 200 mg/kg bw/day as the NOEL). No developmental toxicity was seen in rabbits at up to a maximum dose of nicarbazin of 120 mg/kg bw/day, with an NOEL for maternal toxicity of 60 mg/kg bw/day.

Nicarbazin gave positive results for mutagenicity in two *Salmonella*/microsome reverse mutation tests, but it gave negative results in a series of tests in mammalian systems (mouse lymphoma assay, *in vivo* mouse micronucleus test and *in vivo* rat liver UDS test), including *in vivo* tests in two somatic tissues. In addition, negative results were obtained in a series of *in vitro* and *in vivo* mutagenicity tests on a mixture of nicarbazin and narasin. No carcinogenicity studies were available but it was considered that the overall findings of toxicity and mutagenicity studies suggested that nicarbazin was unlikely to be carcinogenic.

The 50th meeting of JECFA[48] calculated an ADI of 0.4 mg/kg bw for nicarbazin by applying a safety factor of 500 to the NOEL of 200 mg/kg bw/day for maternal toxicity (increased mortality, reduced bodyweight gain and feed intake) and fetotoxicity (delayed ossification and reduced fetal weights) in a rat developmental toxicity study. The large safety factor was used ''to account for limitations in the available data''.

As DNC is the major food residue resulting from use of nicarbazin, the FEEDAP addressed the consumer safety for the feed additive use of nicarbazin by calculating an ADI for DNC. It was not possible to tell from the studies of mixtures of DNC and HDP which substance was responsible for any adverse effects. Therefore, the FEEDAP cautiously assumed that all of the toxicity was due to DNC. An ADI for DNC of 0.77 mg/kg bw was calculated on the basis of toxicological studies with DNC/HDP taking into account the genotoxicity and reproduction/developmental toxicity studies of nicarbazin. A 200-fold safety factor was applied to the NOEL of 154 mg DNC/kg bw/day for elevated serum ALT in the two-year dog study. The high safety factor was used to account for shortcomings in the design and protocol of this study.

The FEEDAP[61,62] has recommended a series of MRLs for tissues from chickens for fattening. These are based on the toxicity of DNC and are lower than the MRLs previously established on the basis of the toxicity of nicarbazin.

Nicarbazin was not irritating to the skin of rats or rabbits and Koffogran was not irritating to rabbit skin.[63] Koffogran caused only a slight transient irritation to the eyes of rabbits.[63] A Buehler test in guinea-pigs showed that Koffogran did not have skin sensitising potential.[63]

The acute inhalation toxicity of Koffogran dust (47% of respirable size) was tested in rats, which were exposed to an air concentration of 0.147 mg L^{-1} for 4 hours.[63] There were no deaths or lasting adverse effects apart from a reduced rate of bodyweight gain in females in the days following exposure. As Koffogran is not a dusty product (dustiness was 0.3 to 0.8 g m^{-3} as measured by the Stauber–Heubach method),[61] workers would not inhale much of the product during handling.

9.3.2.6 Robenidine

Robenidine is a chlorophenylbenzylidine derivative (Figure 9.12). Its consumer safety has been assessed by the FEEDAP,[67–70] which considered the use of the robenidine hydrochloride-containing product Cycostat 66G as an additive to the feed of chickens for fattening, rabbits for breeding and fattening and turkeys.

Microbiological MIC data showed that robenidine was active against Gram-positive bacteria.[70] The FEEDAP did not take account of the MIC data in calculating the ADI because of its policy of not deriving ADIs from such data.

The metabolism of robenidine is broadly similar in chickens, turkeys, rabbits and rats,[67,69] involving hydrolysis[67] of the semicarbazide bonds followed by oxidation to form *p*-chlorobenzaldehyde and *p*-chlorobenzoic acid. Some absorption occurs and excretion is rapid (biliary in rabbits).

Robenidine hydrochloride was of low to moderate acute oral toxicity in mice, rats and rabbits, with LD_{50} values of around 150, 3000 and 2000 mg/kg bw, respectively.[67] Renal toxicity (nephritis and degeneration of tubules) was seen in 90-day studies of mice and rats with NOELs of 14 and 37 mg/kg bw/day, respectively. However, it did not occur in a 90-day dog study. No evidence of carcinogenicity was seen in poor quality studies of inadequate duration in rats (84 weeks) and dogs (2 years), but the numbers of animals examined were small and NOELs could not be identified. Hepatocellular vacuolation was seen in the dog studies with an NOEL value of 7.5 mg/kg bw/day.[67] Mutagenicity tests (*Salmonella*/microsome reverse mutation, *in vitro* cytogenetics, and an *in vivo* mouse micronucleus test) gave negative results.[67] No adverse effects on reproduction or fetal development were seen in a two-generation rat study at up to the highest dose tested (500 mg/kg feed, equal to 33 mg/kg bw/day). A limited reproduction study in rabbits showed no adverse effects when Cycostat 66G was fed at a level giving a robenidine concentration of 66 mg/kg feed (equivalent to a dose of 4 mg/kg bw/day).[67] Adverse effects on maternal fertility, stillbirth rate and growth of neonatal animals were seen

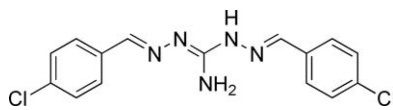

Figure 9.12 Chemical formula of robenidine.

at 330 mg/kg feed in a tolerance study in female breeding rabbits, with a NOEL of 198 mg/kg feed (11 mg/kg bw/day).[70] Maternal toxicity, low foetal body-weights and adverse effects on skeletal development were seen at 50 mg/kg bw/day or greater (NOEL 20 mg/kg bw/day) in a rabbit developmental toxicity study.[67]

An ADI of 0.11 mg/kg bw was calculated by FEEDAP[70] by applying a safety factor of 100 to the NOEL of 11 mg/kg bw/day from the tolerance study in breeding rabbits.

An MRL of 0.2 mg/kg (twice the limit of quantitation of the analytical assay) was recommended for rabbit liver and kidneys, with robenidine as the marker residue.[70] No withdrawal period would be needed to ensure that residues remained below this MRL, but a withdrawal period might be needed to ensure that food was not tainted by the bitter taste of robenidine. As MRLs were established[69] for chickens and turkeys for fattening on the basis of an earlier and higher ADI based on hepatic effects in dogs,[71] it was recommended[70] that the European Commission should consider amending the MRLs for chickens and turkeys in line with the new lower ADI for robenidine.[70]

The FEEDAP[67,70] evaluated the worker safety of Cycostat 66G. Robenidine hydrochloride was shown to be non-irritant to the skin or eyes of rabbits and it produced no skin sensitisation in guinea pigs in a maximisation test. Its acute inhalation toxicity was low ($LC_{50} > 5.2\,mg\,L^{-1}$). Cycostat 66G had a low dusting potential and contained no particles of respirable size. Therefore, it was concluded by FEEDAP that handling of Cycostat 66G presented little risk to workers.

9.3.2.7 Toltrazuril

Toltrazuril is a triazine derivative (Figure 9.13) authorised as a veterinary medicinal product for treatment and prevention of coccidiosis. The CVMP has assessed the safety data on toltrazuril.[72] Metabolism was similar in all species tested, involving step-wise sulfoxidation to sulfoxide and sulfone, and a small amount of hydroxylation.

Acute oral toxicity was low and the LD_{50} was about 2000 mg/kg bw in rats. Short-term studies showed no consistent adverse effects. There was no treatment-related carcinogenicity in mice, but in rats there was an increased incidence of tumours of the uterus with a NOEL for pre-neoplastic lesions of 1 mg/kg bw/day. There was no evidence of a genotoxic mechanism as all

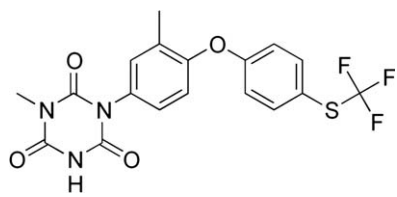

Figure 9.13 Chemical formula of toltrazuril.

mutagenicity tests (five *in vitro* studies covering gene mutation, clastogenicity and UDS, plus an *in vivo* micronucleus test) gave negative results. A rat reproduction study found increased numbers of stillborn foetuses at all doses (lowest observed effect level was 0.3 mg/kg bw/day). Four developmental toxicity studies in rats and rabbits showed a variety of adverse effects on the placenta, embryo and foetus (including teratogenic effects such as microphthalmia and cleft palate at maternally toxic doses) with an NOEL of 0.5 mg/kg bw/day for the most sensitive effect, increased placental weight in rabbits. Studies of toltrazuril sulfone, a major metabolite, showed that it was less toxic than toltrazuril. Toltrazuril had no antibacterial activity at concentrations of up to 0.13 mg mL^{-1}. The CVMP calculated an ADI of 0.002 mg/kg bw by applying a safety factor of 500 to the NOEL for the production endometrial tumours. This gave a safety margin of 250 for increased placental weight in rabbits (NOEL 0.5 mg/kg bw/day).

A series of MRLs have been established for non-laying chickens, turkeys and all mammalian food-producing species.

9.3.3 Other Anticoccidial Substances

Other substances that are authorised in some non-EU countries for use against coccidiosis include clopidol, dinitolmide, ethopabate (used in combination with amprolium) and methylbenzoquate.[73] There was insufficient information available on any of these substances to allow ADI values to be calculated. SCAN requested companies to supply further information on the safety of clopidol, ethopabate and methylbenzoquate, but data were not provided.

9.4 Conclusions

A variety of substances are used for the treatment and prevention of coccidiosis. In the EU, these may be regulated as feed-additives and/or as veterinary medicinal products under different legislation and with different expert committees providing opinions on consumer and user safety.

The majority of these substances are of low toxicity. NOEL values have been identified and MRLs elaborated. As a result of differences in approach by expert committees, and particularly in the use (or otherwise) of microbiological data derived from studies intended to investigate potentially harmful effects on the human gut flora, there may be differences in the ADI values calculated, which may be reflected in differences in MRL values. However, considering that two EU approaches (CVMP and FEEDAP) and one international approach (JECFA) are operating, it is perhaps remarkable that there is a degree of agreement resulting from parallel assessments.

Coccidiostats are valuable tools in the prevention and treatment of disease in domestic animals. Some of these agents are more toxic than others and the ionophores as a group exemplify this. Maduramycin is the most acutely toxic substance within this group. However, if the necessary precautions are taken to protect consumer and user health, then they can be used safely.

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74. European Commission, Report of the Scientific Committee for Animal Nutrition on the use of Pancosin and Pancosin Plus in feedstuffs for poultry, Opinions of SCAN on Antibiotics and Coccidiostats, 1984, pp. 13–27, http://ec.europa.food/fs/sc/scan/outcome_eu.html.

CHAPTER 10

Organophosphorus Veterinary Medicines

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10.1 Introduction

A number of organophosphorus (OP) compounds have been used as veterinary medicines, mostly as ectoparasiticides, but also as anthelmintics.[1–3] In the European Union (EU), the main uses of OPs in veterinary medicine are on sheep for ectoparasites (diazinon[†] and formerly propetamophos and chlorfenvinphos) and also against sea lice in farmed Atlantic salmon (*Salmo salar*) (azamethiphos). OPs were used against warble flies (a parasite of cattle and deer) (phosmet, famphur and fenthion) but warble fly has largely been eradicated. Diazinon is used in the USA on non-lactating cattle in an insecticidal ear tag and was formerly used in pet collars designed to control fleas and ticks.[4] These compounds have roles in arable agriculture and horticulture as well as veterinary medicines, while a few are used as human pharmaceuticals and similar compounds have been manufactured as chemical warfare agents.[5]

OPs are phosphorus derivatives, many of which are esterase inhibitors, most notably inhibitors of cholinesterases. Their structures vary (see Table 10.1).[6] The anticholinesterase OPs are esters of phosphoric, phosphonic or phosphorothioic or related acids. The term OP is often used as a shorthand for

[†]Dimpylate is the international non-proprietary name (INN) as a drug and diazinon the International Organization for Standardization (ISO) name.

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Table 10.1 Main groups of organophosphates (adapted from Marrs).[6]

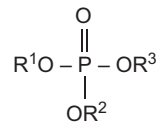
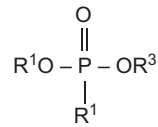
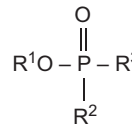
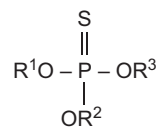
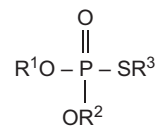
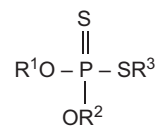
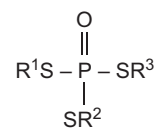
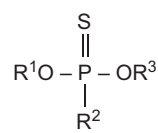
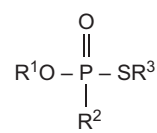
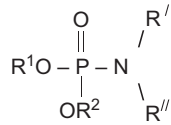
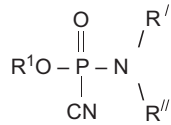
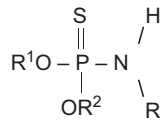
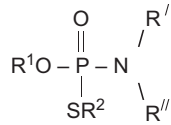
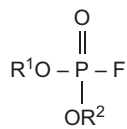
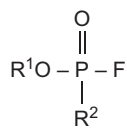
Type	*Structure*	*Examples*
Phosphate		Dichlorvos[a] Chlorfenvinphos[a, b]
Phosphonate		Trichlorfon/metrifonate[a, c]
Phosphinate		
Phosphorothioate = S type (phosphorothionate)		Diazinon/dimpylate[a, b] Parathion[a] Bromophos[a] Pyrazophos[d] Fenitrothion[a] Chlorpyrifos[a] Triazophos[a]
Phosphorothioate S-substituted (phosphorothiolate)		Demeton-*S*-methyl[a] VG/Amiton/tetram[e, f, g] Ecothiopate[h] Azamethiphos[a, b]
Phosphorodithioate (Phosphorothionothiolate)		Malathion[a] Dimethoate[a] Disulfoton[a] Terbufos[a]
Phosphorotrithioate		*S*,*S*,*S*-Tributyl phosphorotrithioate (DEF)[i]
Phosphonothioate = S type (Phosphonothionate)		Leptophos[a] EPN[a]
Phosphonothioate S-substituted (Phosphonothiolate)		VX [*S*-2-(diisopropylamino)ethyl *O*-ethyl methylphosphonothioate][e] VR, Russian VX, [*N*,*N*-diethyl-2-(methyl-(2-methylpropoxy)phosphoryl) sulfanylethanamine][e]

Table 10.1 (*Continued*)

Type	*Structure*	*Examples*
Phosphoramidate		Fenamiphos[a]
Phosphoramidocyanidate		Tabun[e,j]
Phosphorothioamidate = S		Propetamphos[a,b]
Phosphorothioamidate S-substituted		Methamidophos[a] Acephate[a]
Phosphorofluoridate		Diisopropylphosphorofluoridate (DFP)[k]
Phosphonofluoridate		Sarin[e] Soman[e] Cyclosarin[e] GE [isopropyl ethylphosphonofluoridate][e]

[a]Insecticide (ISO name).
[b]Veterinary ectoparasiticide, international non-proprietary name (INN).
[c]Trichlorfon (ISO) is the same substance as metrifonate, the latter being the INN when used as a human pharmaceutical.
[d]Fungicide (ISO name).
[e]Chemical warfare agent.
[f]Trade name when it was marketed as a pesticide.
[g]Russian common name (transliterated).
[h]Pharmaceutical (INN).
[i]Cotton defoliant.
[j]Tabun is unique amongst G agents and has the IUPAC name ethyl-*N*,*N*-dimethyl phosphoramidocyanidate.
[k]Laboratory chemical.

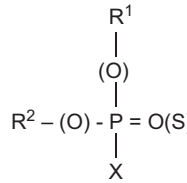


Figure 10.1 General formula of anticholinesterase OPs.

OP anticholinesterase, but strictly speaking herbicides such as glyphosate and glufosinate-ammonium are OPs. The general formula of anticholinesterase OPs is shown in Figure 10.1:

The R groups in pesticides and veterinary ectoparasiticides are generally either both methoxy groups or ethoxy groups, however propetamphos is an exception. In the phosphonates, one R group is attached directly to the phosphorus atom, not through oxygen. Many pesticidal OPs are phosphorothioates: those that contain P=S groups such as diazinon and propetamphos (thionates) tend to be of lower acute mammalian toxicity than their corresponding phosphates and phosphonates, because thionates require metabolism to their corresponding oxons, by oxidative desulfuration carried out by cytochromes P450 (CYPs).[7,8] The thionates are not powerful anticholinesterases, whereas the corresponding oxons are.[9] Thus, the oxon corresponding to diazinon (diazoxon) would be expected to be much more toxic than diazinon. The X or leaving group of anticholinesterase OPs can be any one of a large variety of moieties, the key property being that the link between the leaving group and the phosphorus atom is more labile than that between the alkoxy (R) groups and the phosphorus atom.[10] OPs have been classified by the nature of the leaving group,[11] but most of the compounds discussed in this chapter fall into group IV under this classification. The acute cholinergic syndrome is the most obvious effect of the anticholinesterase OPs, but certain other effects are also seen with some OPs. The clinical effects of OPs are discussed in section 10.3.

All of the anticholinesterase OPs have the same qualitative anticholinesterase action, and this property is responsible for their acute lethal effects. However, quantitative differences in toxicity occur and these are partly due to differences in absorption, distribution, metabolism and excretion. Also, the rates of formation of the OP-acetylcholinesterase complex, of hydrolysis of this complex, and of the aging reaction (see below) must be considered.

The amount of information available on OPs is enormous (see Table 10.2), not only in the scientific peer-reviewed literature but also in documents produced by national and international regulatory bodies and departments of health, agriculture and food in various countries. Also, much information on OPs has been produced by departments of defence in relation to OP nerve agents and by associated research facilities. As a generalization, where substances are regulated as both pesticides and veterinary medicines, more information is obtainable from pesticide regulatory authorities than from veterinary medicine regulators.

Table 10.2 Sources of information on health effects of OP anticholinesterases.

Use of OP	*Cause of exposure*	*Exposed subject*	*Example Refs.*
Insecticides/acaricides (used as pesticides or veterinary ectoparasiticides)	Accidental	User, treated animal General public	Numerous case reports in scientific literature; some summarized by JMPR,[a] JECFA,[b] EFSA,[c] EMA[d] also government publications such as the Annual reports of the Veterinary Products Committee from 2005 and the Veterinary Appraisal Panel before 2005[e]
	Deliberate self harm	Suicide	
Contaminants	Accidental	Consumers of contaminated foods/ drinks	Scientific literature
Human pharmaceuticals	Deliberate medicinal use	Patients	EMA[d]
Regulatory data	Deliberate experimental	Mostly in rodents, rabbits, guinea pigs and dogs, sometimes in farm animals, occasionally in human volunteers.	Metabolism and toxicology summarized by JMPR,[a] JECFA,[b] EFSA,[c] EMA[d] also government publications: USEPA OPP fact sheets,[f] Annual reports of the UK Veterinary Products Committee,[e] Advisory Committee on Pesticides Evaluation Documents[g]
Nerve agents	Deliberate aggressive use	Targets of aggression (armed forces in wartime and civilians in terrorist use)	Marrs, Maynard and Sidell[h]
	Experimental	Animals, volunteers	Marrs, Maynard and Sidell[h]

[a]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). Geneva, Switzerland: World health organization, http://www.inchem.org/pages/jmpr.html.

[b]Toxicological monographs: Joint Food and Agricultural Organization/World Health Organization Expert Committee on Food Additives (JECFA) monographs. Geneva, Switzerland: World health organization, Food Additive Series, http://www.who.int/ipcs/publications/jecfa/monographs/en/index.html.

[c]European Food Safety Authority peer review of active substances European Food Safety Authority (EFSA), Parma http://www.efsa.europa.eu/en/efsajournal and Summary dossiers and rapporteur Member State assessment reports. European Food Safety Authority (EFSA), Parma, http://www.efsa.europa.eu/en/pesticide-speerreview/assessmentreports.htm.

[d]EMA, European Agency for the Evaluation of Medicinal Products, Committee for Medicinal Products for Veterinary Use Summary Reports (human and animal exposures to veterinary medicines), Committee for Medicinal Products for Human Use opinions and assessment reports.

[e]Annual reports of the UK Veterinary Products Committee http://www.vpc.gov.uk/Public/reportsAR.html.

[f]United Sates Environmental Protection Agency, Office of Pesticide Programs Fact Sheets http://www.epa.gov/pesticides/factsheets/index.htm.

[g]Advisory Committee on Pesticides Evaluation Documents. CRD, York, http://www.pesticides.gov.uk/guidance/industries/pesticides/advisory-groups/acp/acp-evaluation-documents.

[h]Chemical Warfare Agents: Toxicology and Treatment, Second Edition, Editor(s): T. C. Marrs, R. L. Maynard, F. R. Sidell, John Wiley and Sons, Chichester, UK, 2007.

10.2 Anticholinesterase Activity

Acetylcholine is a neurotransmitter at cholinergic sites and acetylcholinesterase is the esterase, which brings about the hydrolysis of acetylcholine after it has performed its purpose of neurotransmission at synapses and cholinergic effector sites. Acetylcholine binds to acetylcholinesterase at two sites, namely the anionic site and the esteratic site. The quaternary nitrogen of choline forms an electrostatic link at the anionic site, while the carbonyl group binds to a serine residue at the esteratic site. The reaction for acetylcholine can be visualized as shown in eqn (10.1) below, E being the enzyme, AX acetylcholine, EAX is a reversible Michaelis–Menten complex and A is acetate:

$$\mathrm{E} + \mathrm{AX} \underset{k_{-1}}{\overset{k_{+1}}{\longleftrightarrow}} \mathrm{EAX} \xrightarrow{k_2} \underset{+\mathrm{X}}{\mathrm{EA}} \xrightarrow{k_3} \mathrm{E} + \mathrm{A} \qquad (10.1)$$

where k_{+1}, k_{-1}, k_2, k_3 are rate constants.

Reactivation by hydrolysis of the acetylated enzyme ($\mathrm{EA} \rightarrow \mathrm{E} + \mathrm{A}$) occurs very quickly.[12] In a reaction similar to that of acetylcholine with the enzyme, inactivation of cholinesterases by OPs involves a reaction in which the leaving group (X) is lost; in most cases this results in a dialkylphosphoryl enzyme. The inhibitory potency of OPs (unlike the reactivation and aging rate of the inhibited complex with cholinesterases) depends on the structure of the whole molecule and structure–activity relationships for this have been reviewed.[13,14] Since most OPs used on animals contain two methoxy or two ethoxy R groups, inactivation of cholinesterase produces either a dimethoxyphosphorylated enzyme or a diethoxyphosphorylated enzyme. Reactivation of the phosphorylated enzyme occurs by hydrolysis, but reactivation is much slower with the phosphorylated enzyme than with the acetylated enzyme, and while the enzyme remains phosphorylated it cannot catalyze the hydrolysis of acetylcholine. It should be noted that the kinetics of reactivation are the same for each derivative regardless of the structure of the leaving group of the OP. Reactivation of the dimethoxy phosphorylated enzyme that is produced by, for example, azamethiphos will occur within a few hours and is considerably quicker than the diethoxy equivalent, such as is produced by chlorfenvinphos and diazinon, while phosphorylated complexes containing one alkylthio group and one alkoxy group reactivate faster than those containing two alkoxy groups. By contrast, spontaneous reactivation of complexes containing larger R groups such as isopropoxy and di-*sec*-butoxy is slow or non-existent,[15,16] and this might also be the case with propetamphos (no spontaneous reactivation was detected in human plasma cholinesterase inhibited by propetamphos, which produces a methoxy-ethylamino phosphoryl cholinesterase)[17] (see Wilson *et al.*[15] for a list of reactivation half-lives of various inhibited cholinesterases). Aging (monodealkylation) of the inhibited enzyme prevents spontaneous and oxime-induced reactivation; aging rates with OPs discussed in this chapter are generally thought to be slow (aging rates were also tabulated by Wilson *et al.*),[15] but might be important if treatment of poisoning were delayed.

The binding affinity for inhibitors such as OPs to acetylcholinesterase is described by the dissociation constant for the complex EAX, K_D. This is equal to k_{-1}/k_{+1}, in equation 10.1. For inhibitors whose complexes with acetylcholinesterase reactivate slowly, and these include OPs, k_3 can be ignored and the reaction with acetylcholinesterase can be described by a bimolecular rate constant, k_i as follows in eqn (10.2):

$$E + AX \xrightarrow[k_i]{} X + EA \qquad (10.2)$$

It follows that $k_i = k_2/K_D$.[18] Also $k_i = \ln 2/I_{50}$ (see Aldridge,[19] Wilson[20] and Main[21]), which allows for easy estimation of these constants.

10.3 Clinical Effects

10.3.1 Syndromes Associated with OP Exposure

A number of syndromes have been associated with OP exposure, and these are listed in Table 10.3. Some of these syndromes, notably the acute cholinergic syndrome, are largely caused by acetylcholine accumulation, whereas another, organophosphate-induced delayed polyneuropathy (OPIDP), clearly is not.

10.3.1.1 Acute Cholinergic Syndrome

The clinical effects of OP anticholinesterases are largely a consequence of acetylcholine accumulation although there is some evidence for other mechanisms and these are discussed in section 10.3.2. Although qualitatively the clinical symptomatology produced by OP anticholinesterases is similar, they vary considerably in potency. Moreover, as was discussed in section 10.2, there are differences in rates of inactivation and reactivation of acetylcholinesterases, together with dissimilar rates of aging of the inhibited enzyme. Because anticholinesterase OPs are heterogeneous, particularly with respect to the leaving group, differences in absorption, distribution, metabolism and excretion are frequently observed.

Table 10.3 Syndromes associated with OPs.

Syndrome	*Time in association with exposure*
Acute cholinergic syndrome	Immediate; lasting a few days
Intermediate syndrome	Starting a few days after poisoning.
Organophosphate-induced delayed polyneuropathy (OPIDP)	Starting a week **or** two after poisoning: to some extent persistent
Chronic OP-induced neurological damage (COPIND)	Persistent

The symptoms and clinical signs of acute OP anticholinesterase poisoning have been reviewed.[6,22,23] The effects of OPs may be divided into 3 groups: muscarinic, mediated by muscarinic receptors in parasympathetic effector organs; nicotinic, mediated by nicotinic receptors in autonomic ganglia and the neuromuscular junction; and effects in the central nervous system, which are mediated by receptors of both types (see Table 10.4). The muscarinic symptoms and signs result from increased activity at parasympathetic effector sites and include bronchorrhoea, salivation, constriction of the pupil of the eye (miosis), which results in dimming of vision, abdominal colic and bradycardia. Nicotinic effects at autonomic ganglia can produce tachycardia and hypertension together with pallor of the skin. From the above, it can be seen that two opposing effects may be seen on the heart and the result depends on whether muscarinic or nicotinic effects predominate: additionally, in some cases, cardiac arrhythmias may occur, including *torsade de pointes*. At the neuromuscular junction, nicotinic signs include muscle fasciculation, weakness and later paralysis, which may involve the muscles of respiration. If the patient survives the acute cholinergic syndrome, the effects are for the most part reversible, although in

Table 10.4 Main effects of nerve agents at various sites in the body.

Receptor	*Target Organ*	*Symptoms and Signs*
Muscarinic		
	Glands	
	(a) Bronchial mucosa	Bronchorrhea
	(b) Nasal mucosa	Rhinorrhoea
	(c) Lachrymal	Lachrymation
	(d) Salivary	Salivation
	(e) Sweat	Sweating
	Smooth Muscle	
	(a) Iris	Miosis
	(b) Biliary muscle	Failure of accommodation, eye pain
	(c) Gut	Abdominal cramp, diarrhoea, involuntary defecation,
	(d) Bladder	Frequency, involuntary micturition,
	(e) Heart	Bradycardia
Nicotinic	(a) Autonomic ganglia	Sympathetic effects including pallor, tachycardia, hypertension
	(b) Skeletal muscle	Weakness, fasciculation, respiratory depression, paralysis
Central	Central nervous system	Failure to concentrate, anxiety, restlessness, headache, confusion, convulsions, respiratory depression, respiratory arrest

certain circumstances there may be long-term changes in the central nervous system (CNS) (see section 10.3.1.4). Where death occurs, it is said to be caused by respiratory paralysis, which may be central or due to the anticholinesterase action at the neuromuscular junction, although the ventricular arrhythmias frequently seen in severe intoxications are potentially lethal,[24] (see also discussion by Lotti and review by Bar-Meir and colleagues[25,26]).

10.3.1.2 Intermediate Syndrome (IMS)

In 1987, Senanayake and Karalliedde[27] reported a syndrome that occurred after apparently successful therapy for the acute cholinergic effects of OPs. Because the syndrome was seen after the acute cholinergic syndrome and before any late effects of OPs, especially organophosphate-induced delayed polyneuropathy (OPIDP), Senanayake and Karalliedde called the condition the intermediate syndrome (IMS). In fact IMS is probably the same as the type II syndrome described by Wadia and colleagues.[28] IMS is a proximal limb paralysis starting a few days after poisoning and the respiratory muscles are commonly involved. IMS is probably caused by down-regulation of nicotinic acetylcholine receptors at the neuromuscular junction as a consequence of acetylcholine accumulation.[29] Antidotal therapy for OP poisoning does not appear to be successful in the treatment of IMS and respiratory support is necessary, because of the involvement of the muscles of respiration.

10.3.1.3 Organophosphate-induced Delayed Polyneuropathy (OPIDP)

OPIDP has been the subject of numerous reviews, *e.g.* by Lotti and Moretto[30] and Jokanović and colleagues.[31] OPIDP is a neurodegenerative condition affecting peripheral nerves, both sensory and motor, which tends to be most severe in the long axons. There is a central component involving ascending and descending tracts of the spinal cord and the condition occurs 2–3 weeks after exposure. In the peripheral nervous system, there is degeneration of axons accompanied by Schwann-cell proliferation[32–34] together with changes in the spinal cord and medulla oblongata.[35] Clinically, the most disabling feature is the paralysis of the legs which may result. Less severe cases exhibit a characteristic high stepping gait, associated with bilateral foot drop, and some recovery may occur, but there is no specific treatment. The pathogenesis of OPIDP appears to involve the inhibition of neuropathy target esterase (NTE), a serine hydrolase present at several sites including neurones, where it is an integral membrane protein.[36] Inhibition of NTE is followed by an aging reaction similar to that described in section 10.2 for anticholinesterase OPs with acetylcholinesterase.[37] On the basis of studies with NTE-null mice and *Drosophila*, Glynn[38] hypothesized that organophosphates producing OPIDP caused a transient loss of NTE activity, disrupting membrane phospholipid homeostasis and endoplasmic reticulum functions, such as glial-axonal interaction and axonal transport: the distal parts of long axons would

be particularly vulnerable to perturbations caused by loss of these functions. But the precise sequence of events whereby inhibition of NTE causes OPIDP are unclear and it is noteworthy that mutations in the NTE gene can cause autosomal recessive motor neuron disease.[39]

The structure–activity requirements for inhibition of acetylcholinesterase and NTE are different: this is demonstrated by the fact that many OPs with powerful anticholinesterase properties do not have the capability to produce OPIDP. Thus, the OP chemical warfare nerve agents that are very potent anticholinesterases, have little propensity to cause OPIDP,[40] although, at supralethal doses, sarin can cause delayed neuropathy in antidote-protected chickens[41] while tri-*o*-cresyl phosphate, which has little anticholinesterase activity, is powerfully neuropathic.[42,43]

Regulatory authorities typically demand that OPs be tested for their capacity to bring about the development of OPIDP. As a result most OPs that are capable of producing OPIDP are no longer marketed. The usual test that is carried out is one using domestic hens (*Gallus gallus domesticus*) (there is an OECD guideline),[44] the reason for the use of hens being that these creatures are very susceptible to the syndrome. Several OPs not in current use as pesticides produce OPIDP, including mipafox and diisopropyl phosphorofluoridate (di(propan-2-yl) phosphorofluoridate (IUPAC), and one or other of these is commonly used as a positive control in the hen test, which consists of dosing the hens with high doses of the test substance, with concurrent antidotal protection. The endpoints sought are clinical (staggering), biochemical (NTE estimation) and histopathological examination of the CNS and peripheral nervous system (PNS) *post mortem*. It has been opined that the hen test may give misleading results with chiral OPs, which are often used as insecticides as racemic mixtures;[45] the vast majority of insecticidal OPs are not optically active, but a few are, for instance propetamphos and methamidophos.

10.3.1.4 Other Delayed Effects of OPs on the Nervous System

The behavioural and neuropsychiatric toxicity of OPs, has been reviewed.[46–49] Some of the material in these reviews, which are also discussed below, concerns the chemical warfare nerve agent OPs but, since the mechanism of acute intoxication is the same for insecticidal and chemical warfare agent OPs, this material is relevant to the present discussion. Because acute OP poisoning can bring about effects such as convulsions, respiratory failure and cardiac arrhythmias, all of which can cause cerebral anoxia, it is not unexpected that substantial poisoning has been associated with adverse long term central nervous system (CNS) consequences.[50–60] Much useful information has become available in follow-up studies undertaken on survivors of the use of sarin, an organophosphate nerve agent, in Tokyo (see review by Okumura *et al.*).[61]

The biological plausibility of long-term nervous system effects of acute poisoning is clear. It is less biologically credible, but not implausible, that long-term low dose exposure produces delayed or chronic effects; regrettably the two problems have been the subject of conflation. Moreover there are many

intermediate patterns of exposure (see below). Studies on subjects exposed to OPs, but not experiencing frank acute poisoning, have been reviewed (*e.g.* by Eyer,[62] the Institute for Environment and Health,[63] and the Committee on Toxicity of Products in Food Consumer Products and the Environment).[64] Steenland[65] concluded that studies on subjects previously poisoned by OPs, had shown chronic effects in the CNS and peripheral nervous system (PNS) but that outcomes after long-term low-level exposure was less consistent. An interesting study of sequelae of exposure to diazinon in common marmosets was undertaken by Muggleton and colleagues.[66] Diazinon was administered intramuscularly as a single dose of 10, 90 or 130 mg/kg and there were vehicle controls. Cognitive performance was measured using parts of the Cambridge Neuropsychological Test Automated Battery (CANTAB), and the electrocorticogram and sleep patterns were also monitored. Acetylcholinesterase inhibition of up to 82% was observed together with short-term changes in sleep patterns, but data collected for 12 months showed no evidence of biologically significant long-term changes in any measurements. In relation to the controversy over OP sheep dips in the UK (see also chapter 15), the existence of a definite syndrome has been proposed, with an acronym (chronic organophosphate induced neuropsychiatric disorder, COPIND).[67–69] Also in relation to sheep dip, it has been suggested that depression might be a consequence of OP exposure and a report was commissioned the English Department of Health into this.[70] There was no evidence for any greater amount of depression in sheep farmers than other farming occupations.

Transient parkinsonism as a sequela of acute organophosphate poisoning has been reported[71] and it has been suggested that OPs might play a role in the development of Parkinson's disease.[72]

A connection has been made between exposure to phosmet, a treatment for warble fly in cattle, and bovine spongiform encephalopathy (BSE, mad cow disease).[73,74] The scientific steering committee of the European Commission considered that use of and exposure to organophosphates and the occurrence of BSE were not connected.[75]

10.3.1.5 Developmental Neurotoxicity

Because of the effects of OPs on cholinergic neurotransmission, there is a solid theoretical basis for concern that adverse effects which in adults would be reversible, would in the embryo, fetus and neonate be irreversible. The effects of low-level exposure to organophosphate pesticides on fetal and paediatric health have been reviewed.[76,77] Poisoning of pregnant women with organophosphates has been reported, *e.g.* by Kamha and colleagues,[78] Solomon and Moodley,[79] Sebe and colleagues[80] and Adhikari and colleagues.[81] Outcomes have included survival of mother and child, death of mother and child and survival of the mother with abortion or neonatal death. It is also worth noting that four pregnant women, who were exposed to sarin in the Tokyo exposure at 9–36 weeks' gestation, had normal offspring.[82] A very large number of epidemiological studies have been undertaken seeking to establish whether there is an

association between OP exposure and adverse outcomes in the offspring, many on the effects of chlorpyrifos (see reviews by Slotkin,[83] and Jurewicz and Hanke[84]). In the case of chlorpyrifos, and based on a hypothesis-based weight of evidence analysis, Pruiett and colleagues[85] concluded that a causal association between chlorpyrifos exposure and neurodevelopmental effects was not plausible in humans in the absence of acetylcholinesterase inhibition in the brain, and the few positive associations observed in epidemiology studies could probably be attributed to alternative explanations. There is evidence that different OPs may have differing effects on neurological development. Thus, Timofeeva and colleagues[86] found that neonatal parathion exposure evoked long-term changes in behaviour in rats, but the effects were less severe, and in some instances different from those seen with chlorpyrifos or diazinon. Because of concern that neuroactive pesticides might give rise to developmental neurotoxicity, the United States Environmental Protection Agency (USEPA) has developed guidelines for a developmental neurotoxicity study in the rat[87] as has the OECD.[88] A number of OP pesticides (and other compounds) have been tested in the USEPA's developmental toxicity study in the rat and the results of these studies are starting to become available.[89,90] It should be noted that there is no general requirement in the European Union to do developmental neurotoxicity studies, although in individual instances such studies have been requested from manufacturers, for example with deltamethrin (a synthetic pyrethroid insecticide),[91] but not so far with OPs.

10.3.2 Other Effects of OPs

As discussed above, OPs are enzyme inhibitors, most obviously antiesterases and the most important esterase OPs inhibit is acetylcholinesterase. However, there is increasing evidence that some toxic effects are not mediated by inhibition of acetylcholinesterase. In fact, OPs bind to a variety of enzymes including esterases other than acetylcholinesterase (*e.g.* carboxylesterase, long chain fatty acid hydrolase), serine peptidases, amidases and proteases and others, often modulating them (see review by Lockridge and Schopfer and section 10.3.2.1).[92]

10.3.2.1 Non-anticholinesterase Effects

One non-anticholinesterase effect of OPs has been discussed in section 10.3.1.3, namely OPIDP. The degree to which non-acetylcholinesterase inhibition effects contribute to acute OP toxicity continues to be a matter of interest and has been reviewed.[93] Thus, Chambers[94] noted a poor correlation between rat oral LD_{50} values of various phosphorothionate insecticides and inhibition potency of corresponding oxons for brain acetylcholinesterase. However, there are a number of possible explanations for this, other than differences in target molecule: notably, differences in site and magnitude of desulfuration of the thionate to the active oxon may be important. Studies by Duysen and colleagues[95] with acetylcholinesterase knockout mice suggested that non-acetylcholinesterase inhibitory effects must contribute to the acute toxicity of VX. Maxwell *et al.*[96]

mathematically modelled the lethal effects of OP compounds *in vivo* in order to determine the variation in OP toxicity that could be attributed to acetylcholinesterase inhibition. The compounds studied were mainly chemical warfare agents but also included paraoxon, the oxon metabolite of the pesticide parathion and diisopropylphosphorofluoridate. Regression analysis demonstrated that 93% of the variation in the median lethal doses (*i.e.*, LD_{50} values) of OP compounds in rats could be explained by the variation in their rate constants for inhibition of AChE *in vitro*. The authors opined that the residual unexplained variation in OP toxicity (< 10%) might be explained by non-anticholinesterase mechanisms. All the OPs used in this study are comparatively acutely toxic. With less acutely toxic OPs, the role of non-anticholinesterase effects may be more important than with the OPs studied by Maxwell *et al.*[96]

Possible mechanisms of action of OPs other than by inhibiting acetylcholinesterase are legion. There is evidence that some OP anticholinesterases act directly on muscarinic and nicotinic receptors and their subtypes[97–100] (see review by Eldefrawi *et al.*[101]). It has also been shown that the effects of OPs are not confined to cholinergic neurotransmission, but can affect other pathways of neurotransmission. Examples include γ-aminobutyric acid (GABAergic), glutamatergic, dopaminergic, somatostatinergic and noradrenergic systems.[102–111] Although much of the work has been done on chemical warfare nerve agents, some has been done on pesticides or pesticide metabolites such as paraoxon, and the data clearly show the potential of OPs to influence neurotransmission systems other than those where acetylcholine is the transmitter. It is likely that many but probably not all such effects are secondary to actions on cholinergic systems, but non-cholinergic systems may contribute to seizure activity[112] (see review by Tattersall[113]). The efficacy of some experimental treatments thought not to act on components of cholinergic systems indicate effects of organophosphates on other neurotransmission systems. Thus, Braitman and Sparenborg[114] and Sparenborg *et al.*[115] found that dizocilpine, which is an effective antagonist at the *N*-methyl-D-aspartate (NMDA)-type glutamate receptor, could ameliorate or prevent seizures induced in guinea pigs by soman. It has also been suggested that the protective action of caramiphen against the effects of OPs may be partly because of the ability of this drug, which is also an anticholinergic agent, to act at the NMDA glutamate receptor[116] (see review by Weissman and Raveh[117]). However, because successful treatments for OPs work on other systems this does not necessarily mean that OPs act directly on other neurotransmission systems: studies *in vitro* with paraoxon suggest that cholinergic overstimulation enhances glutamatergic transmission by enhancing neurotransmitter release from presynaptic terminals.[118]

There are some indications that oxidative stress might play a part in the toxic manifestations of OPs.[119–121]

10.3.2.2 Organ Specific Toxicity Outside the Nervous System

OPs may have properties which may be dependent or independent of their anticholinesterase effects, including mutagenicity and carcinogenicity,[122,123] in

fact, some OPs are alkylating agents, and so might be expected to be genotoxic and potentially carcinogenic. Other effects seen, such as developmental and reproductive toxicity,[124] may be secondary to anticholinesterase effects. These effects of OPs are normally investigated as part of the data package generated premarketing for veterinary medicines and pesticides, and are usually available from regulatory bodies and/or the World Health Organization in summary form.

Some aspects of cardiac toxicity of OPs have been discussed in section 10.3.1.1. The complex actions of OPs on the heart have also been reviewed elsewhere.[125–127]

A myopathy has been described *post mortem* in cases of human poisoning with organophosphates[128,129] *inter alia* with parathion[130] and also noted in experimental animals with soman,[131] paraoxon[132] and sarin[133,134] and it has been suggested that there might be a connection between this myopathy and IMS (see section 10.3.1.2). The myopathy appears to be initiated by calcium accumulation in the region of the motor endplate as a result of OP-induced acetylcholine accumulation. Karalliedde *et al.*[29] concluded that IMS was probably caused by down-regulation of cholinergic receptors and that there was no direct relationship of IMS and the myopathy.

Some features of the pulmonary toxicity of OPs have been discussed in section 10.3.1.1. Muscarinic effects cause bronchoconstriction and bronchorrhoea, whilst nicotinic actions at the neuromuscular junction may cause paralysis of the muscles of respiration. Further, there may be effects on the respiratory centre. IMS (see section 10.3.1.2) commonly results in paralysis of the respiratory muscles. These effects or a combination of them may be fatal. The pulmonary toxicity of OPs has been reviewed by Hilmas and colleagues.[135]

Ocular effects in acute OP poisoning, such as myosis and lachrymation, have been discussed in 10.3.1.1 and theses and other effects on parts of the eye such as the retina have been reviewed.[136–138] A strange story is that of Saku disease, a syndrome comprising reduced visual acuity, myopia and/or astigmatism and optic nerve damage associated with OPs and seen in Japan.[139] One possible explanation for this syndrome is enhanced susceptibility of Eastern races for the ocular effects of OPs, but the role if any of OPs in causing Saku disease has by no means been proven.

Immunotoxicity has been shown to be a property of a number of OPs and this subject has been reviewed,[140–143] OPs having immunotoxic properties include diazinon. Most data in this area come from animals, and data on human immunotoxicity are very limited. Whether immunotoxicity occurs in acute organophosphorus poisoning in humans is not known, but if it did occur such an effect would presumably be transient and unimportant in comparison with other aspects of the cholinergic crisis.

Pancreatitis has frequently been reported as a complication of acute OP poisoning (*e.g.* by Dagli and Shaikh).[144] There is evidence that pancreatic damage may be more common than formerly thought, if biochemical evidence is sought: Singh *et al.*[145] found that about 47% of patients in a series of anticholinesterase poisonings had elevated serum amylase activities. Necrotizing pancreatitis has been reported.[146] Parotitis has also been reported.[147]

10.3.3 Diagnostic Tests and Biomarkers

10.3.3.1 Diagnostic Tests and Biomarkers of Effect

The activities of two enzymes have been used as biomarkers of effects for OPs, namely acetylcholinesterase (EC 3.1.1.7) and butyrylcholinesterase, sometimes known as pseudocholinesterase (EC 3.1.1.8). The structure and function of these enzymes has been reviewed.[148] In humans the former is present in red blood cells and the latter in plasma, but such distribution is not true of all species. In dogs, both enzymes are present in plasma with a ratio of butyrylcholinesterase to acetylcholinesterase of 7 : 1,[149] while in the rat, plasma cholinesterase activity comprises more acetylcholinesterase with a butyrylcholinesterase to acetylcholinesterase activity of 1 : 3 in males and 2 : 1 in females;[150] in neither blood compartment are the functions of the enzymes fully understood.[151] Because of the possibility of confusion, the terms "plasma cholinesterase" and "erythrocyte cholinesterase" as synonyms for butyrylcholinesterase and acetylcholinesterase are to be deprecated, especially when used of enzymes in animals where serious confusion may result. It is often necessary to look in detail at animal studies to see what activity has been measured in each matrix. In particular, it is necessary to look at the substrate(s) used in the assay together with any inhibitors used. Methods for measuring acetylcholinesterase have been reviewed[152,153] and acetylcholinesterase and butyrylcholinesterase activities can be measured separately. In almost all cases it is the enzyme activity, rather than protein concentration, that is measured and many of the procedures used are variants of the Ellman method.[154] Correct storage of blood samples is important as reactivation of inhibited enzymes *ex vivo* can occur.

The action of acetylcholinesterase on its natural substrate has been discussed in section 10.2. It was also noted that the rate of reactivation of OP-inactivated acetylcholinesterase depended on the nature of the alkyl groups, being slower with diethoxy OPs than with dimethoxy OPs. Further, it was noted that in certain circumstances aging might be important. Acetylcholinesterase activity is normally measured in the red cell and is generally the preferred enzyme in human OP poisoning, as it is the same gene product as the enzyme whose inhibition in the nervous system is responsible for the acute cholinergic syndrome. Limiting its use as a biomonitor in poisoning may be the fact that there may be differences in accessibility between the red cell and nervous tissue, and Lotti[155] states that inhibition of erythrocyte acetylcholinesterase overestimates inhibition in the brain. Additionally, unlike nervous tissue, resynthesis of acetylcholinesterase cannot take place in the erythrocyte, as these cells cannot synthesize proteins. By contrast in nervous tissue, resynthesis of acetylcholinesterase can contribute substantially to restoration of enzymatic activity.[156] Moreover, after exposure to an OP, the kinetics of absorption, distribution, metabolism and excretion will affect the time course of acetylcholinesterase depression: some OPs are highly fat soluble, form a deep compartment and are slowly released into the blood and eliminated. During that time, if the OP is present in the blood at a sufficiently high concentration,

enzyme activity can remain diminished for extended periods and cases have been reported where this has resulted in prolonged poisoning, *e.g.* that reported by Merrill and Mihm[157] with fenthion. Acetylcholinesterase levels can be used as a biomarker of effect in occupational settings. Generally depression of acetylcholinesterase activity is less sensitive as a biomarker than urinary alkylphosphates and, in humans, there is a fair variability between individuals in activity levels. Acetylcholinesterase activity in red cells is measured during regulatory studies on OPs in experimental animals and often is the most sensitive index of exposure. Also in some studies, such as the neurotoxicity study in rats in accordance with the OECD test guideline 424,[158] brain acetylcholinesterase is measured. Occasionally human volunteer studies on OPs are carried out; in these acetylcholinesterase in red blood cells and often plasma butyrylcholinestease are measured.

Butyrylcholinesterase is an enzyme that resembles acetylcholinesterase, but with a preference for larger molecular weight choline esters as substrate. It is a different gene product from acetylcholinesterase with different inhibition, reactivation and aging characteristics (see tabulations by Wilson and colleagues),[15] although like acetylcholinesterase, butyrylcholinesterase is a serine esterase. Plasma butyrylcholinesterase is largely synthesized in the liver. There is considerable individual variation between humans in plasma activity of the enzyme and activity levels may be decreased in liver disease. A great deal is known about this enzyme because of the existence of genetic variants with decreased ability to hydrolyse, and therefore deactivate, the muscle relaxing drug suxamethonium chloride.[159] There are even some individuals with no butyrylcholinesterase activity who seem to be healthy,[160,161] which raises the question of what the role of this enzyme is: there is evidence that the enzyme plays a role in the degradation of poisonous esters and ester-containing drugs.[162] As a biomonitor of effect for OPs, butyrylcholinesterase has the disadvantage, that as already discussed, it is a different gene product from acetylcholinesterase with different inhibition characteristics, that there is considerable inter-individual variation in activity levels and that activity is affected by a large number of acquired and genetic factors (see review by Soliday and colleagues).[163] Many of these problems can be obviated if there are pre-exposure measurements of butyrylcholinesterase activity in plasma, but these are rarely available in acute poisonings. By contrast in regulatory studies in animals, it is possible to design studies so that pre-exposure measurements of activity are available.

Whole blood cholinesterase was used as a monitor in early studies of OP exposure.[164] The use of neurophysiological variables as biomonitors of effect has been proposed.[165,166]

10.3.3.2 Biomarkers of Exposure

Measurement of alkyl phosphates in the urine is a widely used biomarker of exposure to OPs but it is not suitable for diagnostic purposes. Alkyl phosphates can generally be metabolites of more than one OP, an exception being propetamphos, whose metabolite methylethylphosphoramidothioate, is more or less

specific for that insecticide.[167] With other OPs, provided the exposure is to a single OP, measurement of alkyl phosphates in the urine is a sensitive biomarker of exposure. As discussed in section 10.2, most OP insecticides are dimethyl or diethyl derivatives of phosphoric, phosphorothioic or phosphorodithioic acids. The dimethyl compounds are metabolized to dimethyl phosphate (DMP), dimethyl phosphorothioate (DMTP) and/or dimethyl phosphorodithioate, while the diethyl insecticides are metabolized to the diethyl homologues, diethyl phosphate (DEP), diethyl phosphorothioate (DETP) and/or diethyl phosphorodithioate. Alkyl phosphates found in urine after exposure to common OPs have been tabulated:[168] diazinon produces DEP and DETP, and azamethiphos would be expected to produce DMP and DMTP. These biomarkers are used in occupational settings and in epidemiological studies; alkyl phosphates have also been used to estimate cumulative exposure to OPs (see reviews by Cocker *et al.*,[169] Duggan *et al.*[170] and Sudakin and Stone).[171] In the last-named situation, where there will be likely exposure to a mixture of OPs, there is a serious problem in that the biological significance of the findings will be unclear. Furthermore, alkyl phosphates may be present in the environment (presumably from biotic or abiotic degradation of OPs) so it cannot be assumed that all alkyl phosphate seen in human urine is derived from metabolism of OPs in humans.[171] Substance-specific urinary metabolites can be measured as an alternative to, or in addition to alkyl phosphates,[172] and one such is discussed above in relationship to propetamphos. Unlike the metabolite of propetamphos, which is an unusual alkyl phosphate, these are generally metabolites of the leaving group and include 2-isopropyl-6-methyl-pyrimidin-4-ol, a metabolite of diazinon, and 3,5,6-trichloro-2-pyridinol, a metabolite of chlorpyrifos. For a list of substance-specific urinary analytes available for selected OPs, see Jain.[173]

OPs bind to a number of proteins, not all enzymes, often at serine residues, and less often at tyrosine ones. This binding can be measured, in the case of enzymes, by estimating catalytic activity, or with other proteins, by other means.[92]

10.3.4 Management of OP Poisoning

The management of OP poisoning has been reviewed.[174] Management of OP poisoning involves symptomatic treatment and the use of antidotes, especially atropine. Hypoxia, hypotension, cardiac arrhythmias and fluid balance and electrolytes may require attention.

Atropine is an anticholinergic compound at muscarinic receptors and its efficacy in OP poisoning has been recognized since the 1950s.[175] Other anticholinergic agents have been studied in OP poisoning but the use of atropine has become more or less universal (see review by Heath and Meredith).[176] The other antidotes that have been widely used are pyridinium oxime enzyme reactivators. Three of these have been used in human poisonings, salts of pralidoxime (chloride, iodide, mesylate or methylsulfate), obidoxime and, on a limited basis, asoxime (HI-6). However, a recent Cochrane systematic review has cast doubt on the usefulness of oximes in OP pesticide poisoning.[177] On the

face of it this is surprising as there is much evidence *in vitro* and *in vivo* in experimental animals for the efficacy of oximes in reactivating inhibited acetylcholinesterase (see review by Bismuth *et al.*[178]) but like most drugs, the pyridinium oximes have toxicological properties[179] and it is important to know whether or not they are beneficial. Possible reasons for inefficacy of oximes in clinical practice include aging, and continuing high blood concentrations of OP, causing reinhibition. Certainly experimental studies on the treatment of OP poisoning have to be interpreted with caution, because many animal studies have used unrealistic times between experimental poisoning and therapy or have even used prophylactic protocols.

Convulsions and muscle fasciculation respond to diazepam.[180] Diazepam, a benzodiazepine, acts allosterically at the $GABA_A$ receptor to increase the effect of GABA; as in adult mammals the effect of the GABA neurotransmission system is inhibitory, the effect is to ameliorate effects such as convulsions.[181] Midazolam has been suggested as an alternative to diazepam.[182]

10.4 Exposure and Regulatory Aspects

Humans may be exposed to OPs, used as veterinary medicines, as users in the domestic situation on pets or farmers when the products are used to treat farm animals. Others may be exposed, for example children playing with treated pets, and there is clear potential for exposure *via* the food chain when products are used on food-producing animals such as sheep or cows. For these reasons OPs used on animals are regulated by a pre-marketing approval system.

10.4.1 European Union

In the European Union, substances used on animals, such as OPs, are regulated as veterinary medicines, the initial legislative basis being Directive 65/65/EEC.[183] This was followed by implementing legislation in the form of Directive 81/851/EEC,[184] which established the regulatory framework, and by Directive 81/852/EEC,[185] which set out the requirements for testing, including testing for safety and efficacy (see Chapter 2). These two directives were frequently amended over the ensuing years, and supplemented by other legislative measures. Much of this was codified prior to the review of the EU legislation and the main legal text is now Directive 2001/82/EC (as amended). Veterinary medicines are regulated in the European Union by national competent authorities and by the European Medicines Agency (EMA), whose advisory committee on veterinary products is the Committee for Medicinal Products for Veterinary Use (CVMP), which meets at the EMA in London. Most veterinary medicines used in food-producing animals require maximum residue limits (MRLs; see Chapter 3). In the past, MRLs were established under Regulation (EEC) 2377/90[186] where they were assigned to one of four annexes: Annex I, which listed substances with full MRLs, Annex II, which listed substances which did not require MRLs, Annex III, which listed substances with

provisional MRLs and Annex IV, which contained substances where MRLs could not be established on human safety grounds. The effect of this was that Annex IV substances could not be used on food-producing animals. The arrangements were slightly changed under Regulation (EC) No 470/2009[187] and (EC) No 37/2010,[188] the former amending and repealing previous legislation, and the latter containing 2 tables. Table 1 lists "allowed substances" and their MRLs and Table 2 lists "prohibited substances," *i.e.* substances prohibited in the EU for use in food producing animals. MRLs are established on the basis of an acceptable daily intake (ADI) calculated on the basis of a suitable no-observed adverse effect level (NOAEL). This NOAEL is usually selected from a full set of toxicity studies in animals similar to that required for other food contaminants, although the NOAEL may also be derived from pharmacological or microbiological studies. Unless there is a clear reason to use another NOAEL, the lowest NOAEL in the most sensitive species (the critical NOAEL) is normally used to calculate the ADI, using a safety factor (usually 100–10 for interspecies variation and 10 for intraspecies [human] variation[189] but more if the database is defective or certain toxicological endpoints are seen). Then, with residues depletion data in the food animal of interest, and taking into account standard food intake data, the MRL can be elaborated. Unlike pesticide MRLs, veterinary drug MRLs are safety-based.

10.4.2 USA

In the USA, regulation of OP veterinary medicines is broadly similar to that in the EU but a major difference is that veterinary ectoparasiticides are regulated by the USEPA under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA)[190] as amended, as pesticides. The Food and Drug Administration's Center for Veterinary Medicine (CVM) regulates active ingredients that exert an effect by systemic absorption and distribution under the Federal Food, Drug, and Cosmetic Act (1938)[191] as amended.

10.4.3 Interpretation of Regulatory Studies

In regulatory studies, the critical endpoints for calculation of ADIs differ between compounds, but with OPs they are frequently based on acetylcholinesterase inhibition rather than organ-specific effects. For example, with diazinon, the Joint Food and Agricultural Organization/World Health Organization (FAO/WHO) Meeting on Pesticide Residues (JMPR) set an acceptable daily intake (ADI) on erythrocyte cholinesterase depression in 92-day repeat-dose study in rats, occurring at higher doses.[192] With azamethiphos, the then European Medicines Evaluation Agency (EMEA) (now the EMA) set the ADI on the basis of brain acetylcholinesterase inhibition in dogs.[193] This means that it is important to know whether important effects occur below those causing cholinesterase depression. Unfortunately, it is often unclear from published

studies in peer-reviewed journals and in regulatory studies whether blood for erythrocyte cholinesterase measurements has been taken at the optimum time for measuring cholinesterase activity; this is a particular problem in dog studies, where the use of fasting blood samples may result in a long period between the last exposure to inhibitor and blood letting, with the opportunity for enzyme reactivation. It is also a potential problem in rodent gavage studies, but much less of a problem in rodent feeding studies.

The interpretation of cholinesterase depression is an area of contention. There is general agreement that where acetylcholinesterase and butyrylcholinesterase data are both available in an experimental study, depression of the former should be viewed as an adverse effect, whereas depression of butyrylcholinesterase activity by itself should not. However where data on brain and erythrocyte acetylcholinesterase are both present, the issue is less clear: Carlock and colleagues[194] considered that acetylcholinesterase inhibition data in brain should take precedence over erythrocyte enzyme inhibition for determining NOAELs. However, the JMPR has been concerned that this might be insufficiently protective as acetylcholinesterase in the peripheral nervous system and the neuromuscular junction were likely to be more open to inhibition by OPs than that in the central nervous system.[195] Another issue is the degree of cholinesterase depression that is considered biologically significant: many regulatory bodies set a threshold of 20% reduction in activity compared with concurrent controls. The use of a degree of inhibition rather than statistical significance is a completely different approach to that used with other toxicological endpoints. The origin of the figure of 20% is unclear, but it is the same as the action limit to remove personnel from OP exposure adopted by establishments working on OP nerve agents in the 1950s onwards. In actual fact, the JMPR's position is more complicated and they recommend taking into account both the 20% threshold and statistical significance:[195] the attitude of Joint FAO/WHO Expert Meeting on Food Additives (JECFA) is somewhat similar for the few OPs it has examined.[196]

10.4.4 Pharmacovigilance

An essential part of regulation of veterinary products is pharmacovigilance, that is the gathering of information on adverse reactions to veterinary products post-marketing. This is done in most developed countries and data are collected both on human adverse reactions and adverse events in animals.[197]

10.5 Sheep Dips in the United Kingdom

10.5.1 Introduction

Sheep are affected by a number of external parasites (ectoparasites) (see Table 10.5).

Table 10.5 Some ectoparasites of sheep.

Ectoparasite		
English name	*Latin name*	*Effect*
Green bottle	*Lucile seriatim*	Fly strike
Blue bottle	*Phormia terrae-novae*	
Blue blow-fly	*Calliphora erythrocepala*	
Sheep mite	*Psoroptes ovis*	Scab, ovine psoroptic mange
Sheep keds,	*Melophagus ovinus*	
Biting louse	*Bovicola ovis*	

10.5.2 Treatments

Treatments and prophylaxes for these conditions involve the use of insecticides, which can be applied in several ways. Of these in the UK, plunge dipping (immersion) is the most widely used at present, but pour-ons (sprays) are also used and showering or jetting races are used in some countries; injectable preparations of endectocides (*e.g.* ivermectin and doramectin) are available for the control of some ecto- and endoparasites.[198] In recent years cyromazine pour-ons have been used but this compound is not effective against established fly-strike and has to be used early in the season.[199] Cypermethrin, a synthetic pyrethroid, was formerly used in the UK as a sheep dip, but in 2010, manufacturers of the cypermethrin sheep dips voluntarily withdrew their Marketing Authorisations following a number of environmental incidents.[200]

10.5.3 Organophosphate Plunge Dips

Largely confined to England, and during the 1990s, there was a considerable increase in reports of adverse effects in humans exposed to OP sheep dips (see also Chapter 15).[201] The symptoms observed in such reports could broadly be divided into 2 groups. (i) Acute – consisting of a transient influenza-like illness (dippers' flu). (ii) Long term. The symptoms and clinical signs were variable but included poor memory, depression, headache, pyrexia and sometimes signs referable to the peripheral nervous system.[202] These symptoms were characteristic of neither the acute cholinergic syndrome nor OPIDP (which is not known to be a problem with OPs used in sheep dips such as diazinon). The number of human adverse reactions to OP sheep dips reported to the VMD adverse reactions scheme peaked at 180 reports in 1993. Since then reports of adverse reactions to OP sheep dips have declined steadily probably as a result of changes to the products available, including the replacement, in 2001, of the old containers, with packaging which is intended to avoid contact of operators with the concentrated ship dip, before dilution. There were various initiatives to increase appreciation of the need for attention in handling the products. Also, a compulsory certification scheme for competence with diazinon was introduced in 1994. Because of concern that handlers of OP sheep dips might not appreciate the inherent toxicity of the OPs, a publicity campaign was mounted to increase awareness of hazard.[203–205]

Some of the data on long term effects of OPs on the nervous system were discussed above in section 10.3.1.4, including the review by the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT), initiated by the UK government. 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 consider the management of those asserting that they had been adversely affected by sheep dip. These Royal Colleges established a working group, which issued a report in 1998.[206]

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 relevant government departments and to draw together scientific evidence relevant to policy issues and to examine licensing of veterinary products.[207] A research programme to look at sheep dips was initiated, and an important study of sheep dippers was undertaken by the Institute of Occupational Medicine in Edinburgh, in collaboration with the University of Glasgow.[208–211] This study was a cross sectional study of 612 sheep dippers with two non-exposed groups (107 ceramics workers and 53 farmers who did not dip sheep). Neurological symptomatology and thermal and vibration sensory thresholds were studied. A weak positive association between exposure to OPs and neurological symptoms was seen but an association between exposure to OPs and either thermal or vibration sensory thresholds was not observed. Symptoms were more frequent in those handling sheep dip concentrate than those not. There was also some evidence for an increase in thermal and vibration sensory thresholds among those handling concentrate. There is thus no clear evidence of long-term effects of OP on sheep dips on farmers and shepherds. One possibility that could not be excluded was that OPs cause adverse neurologic or neuropsychiatric disease in a small subgroup of those exposed, a question that may remain permanently unanswered.[212]

10.6 Conclusion

Human exposure to veterinary medicines resembles that to pesticides in that exposure can occur through food, to operators and to bystanders. Furthermore owners and others, particularly children, may be affected by contact with treated pets and, less often, with farm animals.

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CHAPTER 11

Antifungal Drugs

11.1 Introduction

Animals, like humans, are susceptible to a number of infections caused by fungi. These may be localised infections, for example on the skin or in the ear, or they may be systemic diseases. The former include diseases caused by yeasts or yeast-like organisms such as *Candida* and *Malassezia*, while the latter include blastomycosis, coccidiomycosis, histoplasmosis and aspergillosis. There are also some subcutaneous conditions including sporotrichosis and pythiosis.

There are a number of antifungal drugs that may be given topically or systemically to treat these conditions. Of these, perhaps the most commonly encountered are those to treat otitis media and infections of the outer ear particularly in companion animals. Although these conditions may be primarily due to bacterial infections, they may be exacerbated by yeast such as *Malassezia pachydermatis*. Consequently, many otic products (ear drops) contain an antimicrobial drug and an antifungal agent, usually in combination with an anti-inflammatory drug.[1–6] The most commonly used antifungal agents in veterinary medicines are shown in Table 11.1. Most of these agents are also used in human medicine.[7–13]

As shown in Table 11.1, the majority of these drugs belong to one of three main classes, the polyenes that include amphotericin B, natamycin and nystatin, the imidazoles that include clotrimazole and ketoconazole, and the triazoles, which include itraconazole and posaconazole. Together, these latter two groups are often referred to as the azole antifungal drugs. Griseofulvin is the only member of its class. It is a natural product produced by *Penicillium griseofulvum*. Flucytosine is closely related to the cytostatic drug 5-fluorouracil and it is converted to the latter *in vivo* to provide the active agent. The polyenes are thought to exert their effects by binding to a sterol, ergosterol, present in

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Table 11.1 Some antifungal drugs used in veterinary medicine.

Drug	*Class*	*Typical Type of Infection*	*Type of Animal*	*EU MRL*
Griseofulvin	—	Skin, nails	Companion animals	No
Amphotericin B	Polyene	Systemic and local infections	Companion animals	No
Natomycin	Polyene	Skin, mucous membranes	Companion animal; food animal	Yes[a]
Nystatin	Polyene	Otic	Companion animal	No
Clotrimazole	Imidazole	Skin, otic	Companion animals	No
Ketoconazole	Imidazole	Skin, otic	Companion animals	No
Enilconazole	Imidazole	Skin	Companion animals; food animals	Yes[a,b]
Miconazole	Imidazole	Skin	Companion animals	No
Itraconazole	Triazole	Skin	Companion animals	No
Posaconazole	Triazole	Otic	Companion animals	No
Fluconazole	Bis-triazole	Skin	Companion animals	No
Flucytosine	Pyrimidine	Systemic	Companion animals	No

[a]No MRL required on public health grounds.
[b]Also known as imazalil (pesticide).

fungal membranes, and then creating greater permeability allowing leakage of small molecules especially potassium and magnesium ions. The imidazoles and triazoles do not bind to ergosterol. Instead, they inhibit its synthesis through inhibition of fungal cytochrome P450 which results in changes in permeability. They also affect the structure of the fungal cytoskeleton and produce reactive oxygen species which damage the cell.[1,2,7,14–21] Flucytosine is converted by fungal cytosine deaminase, an enzyme not present in mammalian cells, to 5-fluorouracil which, after further metabolism, inhibits thymidylate synthase and fungal DNA synthesis. Thus, it has similar antimetabolite activity on fungi as 5-fluorouracil has on tumour cells (Chapter 7).

11.2 Griseofulvin

Griseofulvin is a spirobenzofuran cyclohexenone derivative (Figure 11.1). It is widely used for the treatment of ringworm in humans and tinea capitis infections in children and for a number of fungal infections in animals.[1,2,7,22–24] In earlier toxicology studies, griseofulvin appears to have a very low order of toxicity.[25] However, therapeutic treatment of pregnant cats for ringworm resulted in range of malformations in their offspring which included cleft palate, exencephaly, caudal displacement, spina bifida, cyclops and anophthalmia.[26–29] Cats are seemingly more sensitive to the general toxic effects of griseofulvin,[30] but it is not known if this also applies to teratogenicity. In a population-based case control study of 38 151 women who delivered babies without any defects and 22 843 who delivered those with congenital abnormalities, seven case and 24 control women had been treated with oral

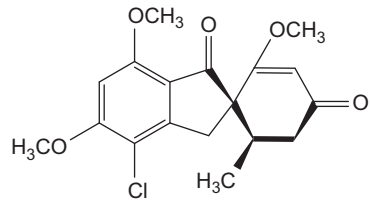

Figure 11.1 Structural formula of griseofulvin.

griseofulvin. There was no indication (crude odds ratio 0.49 with 95% confidence interval 0.21–1.13) that griseofulvin was associated with an excess teratogenic risk.[31]

Griseofulvin has been tested in a wide number of *in vitro* and *in vivo* tests for genotoxicity. Broadly, griseofulvin has produced negative results in tests for point mutations in bacterial systems, including in the Ames reversion assay using *Salmonella typhimurium* assay and in a number of mammalian cell lines.[32–36] However, there was limited evidence of a genotoxic effect in the mouse lymphoma TK^+/TK^- L5178Y assay.[37] Tests for the induction of micronuclei were largely negative,[38–40] but positive results were obtained in V79 cells, in a gut micronucleus test and in L5178Y cells.[41–43] It also gave negative results in tests for the induction of DNA repair in rat and mouse hepatocytes, and in bacterial systems.[44,45]

In contrast, several studies for aneuploidy and other tests for chromosome damage arising during mitosis or meiosis produced clear positive results.[46–62] In fact, the results from these studies demonstrates griseofulvin to be a potent aneugen in somatic and germ cells. This effect may lead to loss of chromosomes and altered gene expression.[24] Griseofulvin is an antimitotic agent and this may account for its teratogenic effects. The mechanism is unclear, as unlike some other teratogens and antimitotic agents it does not disrupt microtubules, but it does appear to bind to tubulin or to microtubule-associated proteins.[35,63–65] More recent evidence suggests that the drug may bind to tubulin at a site which it shares with paclitaxel, and that the two may work in a similar manner by kinetically suppressing microtubule dynamics which would explain its antimitotic effects and its ability to inhibit the proliferation of some types of tumour cells.[66]

Studies in mice and rats demonstrated that griseofulvin was carcinogenic. It caused liver tumours in these mice and thyroid tumours in rats.[67] Administration in the diets of mice resulted in hepatotoxicity, disruption of the normal liver architecture, and lesions that appeared to be liver tumours.[68] Parenteral administration to infant mice resulted in a high incidence of liver tumours.[69] Taken together, the data demonstrate that griseofulvin is carcinogenic in animals but the mechanism is unclear although it might be related to its antimitotic effects.[24,70] The International Agency for Research on Cancer concluded that griseofulvin was hepatocarcinogenic in mice and that there was inadequate evidence to assess its carcinogenicity in humans. It concluded that griseofulvin was possibly carcinogenic to humans.[71,72]

The induction of aneuploidy is regarded as a critical step in the process of carcinogenesis.[73–80] With the results of carcinogenesis assays in animal studies, this confirms griseofulvin's status as a carcinogen. Some may argue that it should therefore be possible to determine the underlying mechanism for griseofulvin-induced aneuploidy, and therefore a threshold dose.[81,82] Unfortunately, as griseofulvin gives different responses depending on the test system chosen, this may be difficult to achieve.[83]

Griseofulvin may cause allergic skin reactions when given topically, and some of these may be severe suggesting a possible occupational risk to exposed individuals.[84–88]

11.3 Amphotericin B and Other Polyenes

Amphotericin B (Figure 11.2) is not well absorbed from the gastrointestinal tract and it is only used orally for local treatment for oral infections. It is used in veterinary medicine for systemic fungal infections and is usually given intravenously or by intrathecal administration. It is poorly soluble in water and so most formulations make use of sodium deoxycholate, which then permits the formation of a suspension. In human and veterinary medicine, the limiting factor to systemic treatment with amphotericin B is nephrotoxicity.[1,2,7]

Nephrotoxicity associated with amphotericin B administration in humans is dose related and it may be severe and result in acute renal failure. The incidence of acute renal failure may be as high as 49 to 65%. It is accompanied by an increase in serum creatinine. Mortality may be high and is increased by the co-administration of other nephrotoxic drugs. Risk factors include cumulative dose, abnormal baseline renal function, concomitant nephrotoxic drugs such as cyclosporin, and infusion rates.[89–99] However, rapid infusion rates are also associated with severe hyperkalaemia and potentially fatal arrhythmias.[100–106] In one case, a woman given 5 mg/kg bw/day amphotericin B instead of the

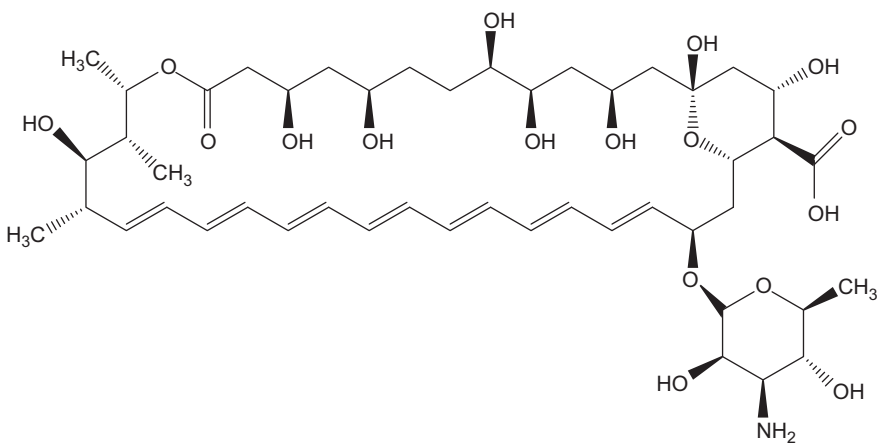

Figure 11.2 Structural formula of amphotericin B.

usually prescribed 0.5 to 0.8 mg/kg bw/day dose, developed cardiac arrhythmias, acute renal failure and anaemia. The error was noted after only two of the higher and incorrect doses and drug therapy was suspended but she died six days after admission for cryptococcal meningitis; she had a history of proliferative glomerular nephritis from lupus erythematosus.[101] Children are susceptible to the adverse renal effects of the drug.[107–111]

The mechanism or mechanisms that lie behind amphotericin B nephrotoxicity remain unclear. The drug causes vasoconstriction and it interacts directly with epithelial cell membranes which leads to a decrease in glomerular filtration rate and in renal blood flow. Mean renal blood flow may decrease by over 50% and it may take several months before this returns to normal, and in decreasing blood flow it may also lead to ischaemic injury.[89,92,99,112] In rats, administration of amphotericin B resulted in loss of tubular cells and decreases in glomerular filtration rate with increases in serum creatinine. The nephrotoxicity was exacerbated by other nephrotoxic drugs such as aminoglycoside antibiotics but a slight protective effect was given by verapamil, theophylline, flucytosine and loading with sodium chloride.[98,113–115] In rats, there appears to be a temporal relationship with administration and the development of nephrotoxicity.[116]

Experimental studies suggested that amphotericin B was less nephrotoxic when administered as a lipid formulation.[117,118] However, this was not seen in dogs given amphotericin B in a fat emulsion.[119] Amphotericin B is now routinely supplied and administered as a lipid formulation and this does appear to reduce its nephrotoxicity, but other adverse events, including hepatotoxicity, anaemia and thrombocytopenia may occur, albeit with low frequencies.[91,120–126]

Similar problems do not appear to have occurred through the clinical use of natamycin (pimaricin) but this drug is of low toxicity in animal studies.[127,128] It is not teratogenic in humans when given vaginally for susceptible infections.[129] Natamycin was not clastogenic in studies in mice but it is cytotoxic to human lymphocytes and pig hepatocytes. In the former, it induced micronuclei and chromosomal aberrations and, at the highest concentrations tested, it produced sister chromatid exchanges.[130–132] In human medicine, its use is largely restricted to the treatment of keratitis, while in veterinary medicine it is used for topical treatment of companion animals. Natamycin is used as a preservative for human foods for cheese and sausage products (E 235).

Nystatin is used therapeutically in veterinary medicine as an otic product for companion animals. There is very little toxicity data either in humans or in animals available for this drug but it does appear to be maternotoxic, at least when formulated as a liposomal product, at doses of 3.0 mg/kg bw/day in rats and rabbits. However, it was not teratogenic and it had no effects on reproductive parameters at doses below the maternotoxic dose.[133] It was not ototoxic in a chinchilla model.[134]

Natamycin is the only polyene antifungal drug authorised for food animal use in the EU (Table 11.1) and in view of its low percutaneous and gastrointestinal absorption, its use on individual cattle or horses and its low toxicity, it was considered that no maximum residue limit (MRL) was required on public health grounds.[135]

11.4 The Azoles

The azole drugs comprise the imidazoles and the triazoles. They are used against a broad range of fungal infections in animals and humans. In veterinary medicine they are largely used for skin and ear infections, frequently in combination with other drugs such as antibiotics, anti-inflammatory drugs and chlorhexidine, but in human medicine they are additionally used for the treatment of systemic fungal infections.[7] Clotrimazole is one of the older antifungal drugs in use in veterinary medicine while posaconazole is one of the newer additions.[1,2,7,9–13,136–139] As noted earlier in this Chapter, the azoles exert their effects on fungi by inhibition of the synthesis of fungal steroids.[1,2,7,14–21] These effects become important in some aspects of their toxicity in mammals, and in other aspects of some of their pharmacodynamic effects. The structures of clotrimazole, enilconazole and posaconazole are shown in Figure 11.3.

The laboratory animal toxicity profiles for the azole antifungal agents are not well documented in the literature. Enilconazole has a low to moderate acute toxicity with LD_{50} values in the rat being in the region of 400 to 600 mg/kg bw. The dermal LD_{50} value was over 4000 mg/kg bw. In repeat dose studies, the main target organ was the liver where vacuolation and fatty change was noted along with centrilobular swelling. The no-observed effect level (NOEL) was 2.5 mg/kg bw/day in the dog and 5 mg/kg bw/day in the rat. In multigeneration studies in rats there was an increase in the duration of gestation, a decrease in the numbers of live young and an increase in the numbers of stillborn pups. In studies in rabbits, the main effects were on maternal bodyweights, and a decrease in litter size and pup survival. At the highest dose used, 20 mg/kg bw/day, a decrease in litter size and an increase in resorptions were seen. There was no evidence of a teratogenic response. Enilconazole was tested in a number of assays for genotoxicity including those for mutations and clastogenicity but all the tests gave negative results. The drug was not carcinogenic in rats or mice.[140]

Posaconazole was also of low acute toxicity in rodents. It was tested in repeat dose studies in mice, rats, dogs and monkeys, and the main effects noted were indicative of phospholipidosis and those on steroidogenesis. The overall NOEL was 1 mg/kg bw/day. Some embryo- and foetotoxicity was noted at maternotoxic doses and in fertility studies, the main effects were an increase in the numbers of resorptions. There was no evidence of teratogenic effects. The drug was not genotoxic and although there was a slight increase in the incidence of adrenal tumours but no firm evidence of a carcinogenic effect.[141]

Comparable data for other drugs in the group are not available.

11.4.1 Steroidogenesis Inhibition

The azole antifungal drugs, as already noted, exert their pharmacodynamic effects through the inhibition of the synthesis of fungal steroids. However, they have also been found to have similar effects on mammalian steroidogenesis.[142] Studies *in vitro* showed that ketoconazole and other azoles decreased the

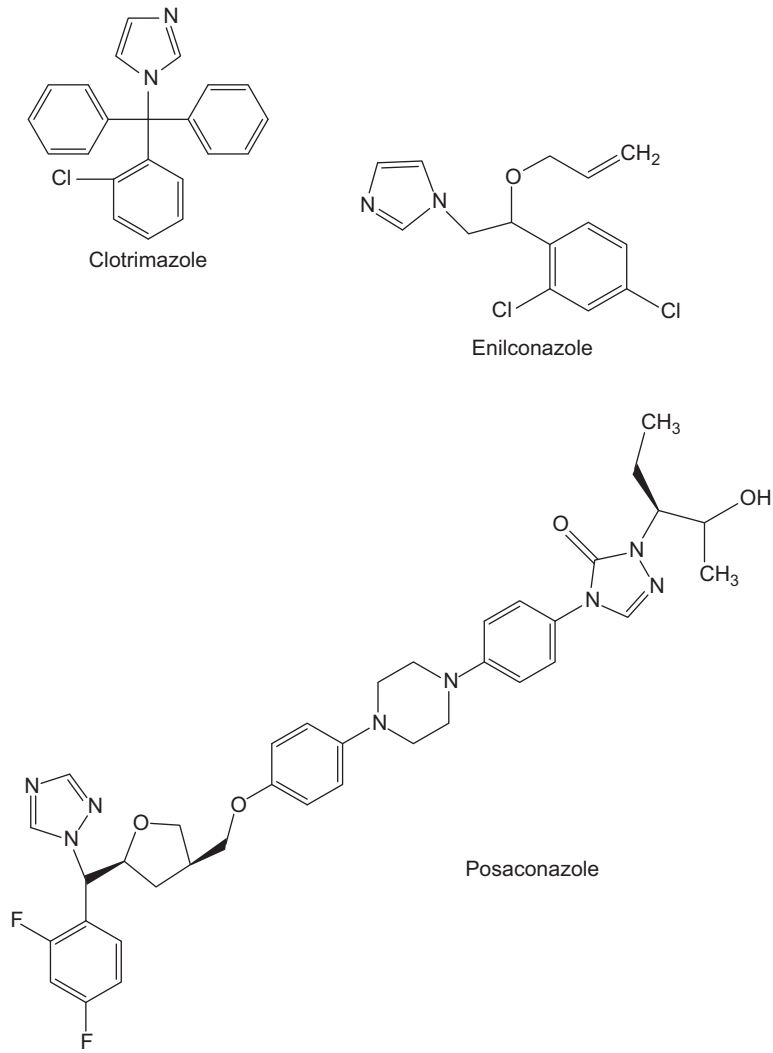

Figure 11.3 Structural formulae of clotrimazole, enilconazole and posaconazole.

synthesis of oestradiol and testosterone, while increasing the concentration of progesterone suggesting that the conversion of progesterone to other hormones was inhibited.[142] Similar findings were made *in vitro* using mouse cultured follicles.[143] Studies of ketoconazole and other azole antifungals in rats showed that ketoconazole induced testicular and epididymal atrophy in rats, accompanied by reductions in the numbers of sperm and serum testosterone, with degeneration of the seminiferous tubules and depletion of germ cells. When given to pregnant rats, ketoconazole and other azoles reduced the anogenital distance and reduced testicular testosterone concentrations.

These agents also caused a marked frequency of post-implantation loss, and increased the incidence of late and very late resorptions.[144,145] In human patients treated orally with ketoconazole, testosterone serum concentrations fell markedly but returned to normal 8 to 24 hours after drug administration. With long term administration, a transient fall in testosterone occurred although this persisted in one patient.[146] Ketoconazole and enilconazole also inhibited aldosterone biosynthesis in a human adrenocortical cell line.[147] Ketoconazole showed some activity in the male pubertal onset assay although the effects were not as marked as with testolactone, an antineoplastic drug and an inhibitor of steroid aromatase activity.[148] When tested in a study designed to detect endocrine effects using the draft OECD Guideline No. 407 design, ketoconazole produced a reduction in the weights of the accessory sex organs and epididymis, a reduction in retention of spermatids in the seminiferous tubules, a decrease in testosterone and increases in oestradiol, luteinising hormone and follicular stimulating hormone.[149]

These effects of the azole antifungal drugs have led to ketoconazole being considered as an anticancer agent in men with androgen independent prostate cancer. High dose ketoconazole can induce prolonged responses, especially when given in combination with other agents and notably the immunostimulatory cytokine granulocyte-macrophage colony stimulating factor.[150–154] Initial low dose ketoconazole therapy, rising to higher doses, may also be effective.[155]

The azole drugs also have adverse effects on pregnancy and some of these effects, if not all, may be related to their effects on steroidogenesis. Ketoconazole was found to reduce implantation rates, reduce serum progesterone levels and to increase ovarian weights in rats.[156] High doses of ketoconazole given to pregnant rats resulted in skeletal abnormalities such as short or bent ribs and fused rib cartilage, and misshapen carpal bones. Other effects reported have included cleft palate, misshapen limbs and discontinuous ribs.[157,158] Some of these effects are ameliorated by prednisone.[159] Similar effects have been produced by itraconazole and fluconazole while enilconazole appeared to induce neurobehavioral effects.[160–163] In fact, fluconazole led to hypoplasia, agenesis and fusion of the branchial arch in rat embryos, an effect probably not associated with hormonal inhibition.[163]

In view of its effects on adrenal steroidogenesis, ketoconazole has also been examined in the treatment of Cushing's syndrome. The drug was efficacious in the management of this condition and led to normalisation of urinary cortisol levels, while other symptoms such as hypertension and hypokalaemia reduced.[164–166] Fluconazole has also proved effective in the treatment of Cushing's syndrome.[167]

In a two-year rat carcinogenicity study with fluconazole, there was a decreased incidence of mammary fibroadenomas in females and adrenal phaeochromocytomas in males. These effects are also thought to be due to inhibition of steroidogenesis, through the inhibition of aromatase.[168]

Whether all of these effects are really due to effects on steroidogenesis or due to some other mode of action remains to be demonstrated. However,

the azole antifungal drugs have now earned the label of endocrine disrupting agents.

11.4.2 Hepatotoxicity

Itraconazole, fluconazole and ketoconazole have been shown to be hepatotoxic in rats and rabbits. Ketoconazole produced a dose-related increase in hepatic enzymes in the rabbit and histopathology revealed cloudy swelling, ballooning degeneration and necrosis. At 80 and 160 mg/kg bw this was extensive.[169] In rats, ketoconazole also resulted in hepatotoxicity with elevations of hepatic enzymes, glutathione depletion, hepatic covalent binding and activation of flavin-containing monooxygenases.[170] Itraconazole was not hepatotoxic after single intraperitoneal doses of up to 200 mg/kg bw but repeat doses over 14 days of 0, 10, 50 and 100 mg/kg bw/day produced hepatic necrosis, bile duct hyperplasia and biliary cirrhosis. Fluconazole produced only mild degenerative hepatic changes when given over 14 days in a similar manner suggesting that in rats at least, itraconazole was significantly more hepatotoxic.[171]

In humans, treatment with itraconazole, ketoconazole or fluconazole is associated with hepatotoxicity. This may be mild or severe, and with itraconazole and fluconazole, there have been resulting fatalities.[172–187] Liver toxicity in these circumstances may be asymptomatic with the only evidence being elevated liver enzymes, or it may be observed as acute hepatocellular, cholestatic or mixed hepatocellular-cholestatic reactions.[172–187] In rats, hepatic phospholipidosis has been observed following ketoconazole administration, while in dogs posaconazole led to phospholipidosis in neurons of the central nervous system, dorsal root ganglia and myenteric plexus.[188–190] Phospholipidosis has been observed with a number of amphiphilic drugs including phentermine, chlorphentermine, chlorpromazine, fluoxetine, tamoxifen, aminoglycoside antimicrobials and amiodarone.[191–199] Its development depends on a number of factors including inhibition of phospholipases, disruption of phospholipid metabolism and effects on the cell membrane bilayer. It is not usually regarded as a toxic effect in itself, but it can have adverse consequences. For example, with amiodarone, phospholipidosis mainly occurs in the lungs and this may underlie the subsequent inflammatory response and pulmonary fibrosis seen in animal models and in humans.[197–199]

11.4.3 Other Effects

In general, the imidazole and triazole antifungal drugs are regarded as safe and of low toxicity, and the effects described in 11.4.1 and 11.4.2 are frequently rare or asymptomatic.[140,141,200–204] Questions have been raised over the genotoxicity of these drugs but the tests on individual examples are negative, and there is no convincing evidence of an effect.[205–207] Ketoconazole has been found to cause prolongation of the QT interval in the guinea pig but this has not been found to be a major clinical issue, either with this drug, or with others in the class.[208] Miconazole has been reported to cause cardiorespiratory toxicity and possible

anaphylaxis.[209] The azole drugs are associated with numerous drug–drug interactions.[210,211]

11.5 Conclusions

The antifungal drugs used in veterinary medicine are also used in human medicine and all are associated with some form of toxicity. With amphotericin B this may be severe, and even fatal, and is relatively common. There are still question marks over the claimed greater safety of lipid formulations. The azole drugs are less toxic but toxicity may occur following higher doses and with prolonged administration. Much of their activity is attributable to their effects on steroidogenesis, and this is summarised in Figure 11.4.

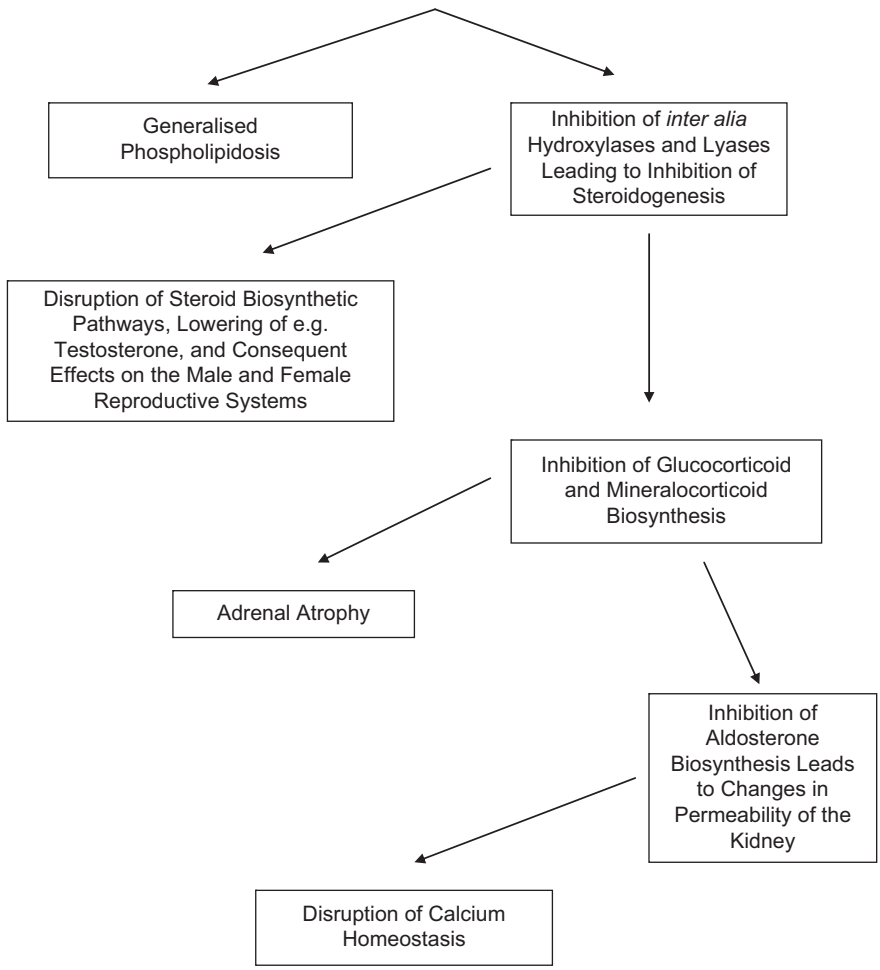

Figure 11.4 General Effects of the Azole Antifungal Drugs.

In general, it is unlikely that any of these effects will result from occupational or other exposures during veterinary use or as a result of exposure to residues in food of the limited number of drugs authorised for food animal use (Table 11.1). Clearly, griseofulvin is teratogenic and possibly carcinogenic but exposure during normal veterinary use is unlikely to deliver a sufficiently high dose to provoke adverse effects. It is not authorised for use in food animals in most jurisdictions and this seems a prudent precaution in view of its toxicological profile.

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CHAPTER 12

Antiparasitic Drugs

12.1 Introduction

Some antiparasitic drugs, notably the macrocyclic lactones, have already been addressed in Chapter 6. This chapter will examine the toxic effects of other compounds that are widely used, or have been used, as antiparasitic drugs in animals. In Europe and elsewhere, the main anthelmintic drugs are the benzimidazoles and levamisole. The major benzimidazole drugs are thiabendazole, albendazole, fenbendazole, flubendazole, mebendazole, oxfendazole, oxibendazole, triclabendazole and albendazole sulphoxide (ricobendazole, albendazole oxide). Febantel and netobimin are two prodrugs that *in vivo* are converted to fenbendazole and albendazole, respectively. Levamisole is the levo (*l*) isomer of (*dl*)-tetramisole, a mixture of the levo and dextro isomers. Tetramisole has been superseded by levamisole. These and some other antiparasitic drugs are discussed in this chapter.

12.2 Individual Drugs or Groups of Drugs

12.2.1 The Benzimidazoles

These drugs are based on the common structure of benzimidazole (1*H*-benzimidazole), which may be regarded as a fused ring system formed from benzene and the heterocyclic compound imidazole (Figure 12.1). The anthelmintic benzimidazoles are typified in chemical structure by albendazole (Figure 12.2). In addition to the major members of the group mentioned above, others, which are rarely or no longer used, include parbendazole, cambendazole and luxabendazole.[1–3] Some benzimidazoles, and notably albendazole, mebendazole and thiabendazole are used as anthelmintic drugs in human medicine.[4–7]

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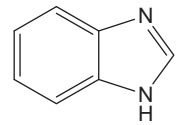

Figure 12.1 Chemical formula of benzimidazole.

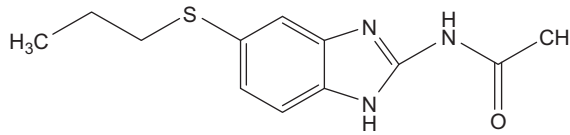

Figure 12.2 Chemical formula of albendazole.

The benzimidazole drugs have low toxicity in general. They are of low acute toxicity, give negative results in the majority of tests for genotoxicity, and are not carcinogenic.[8–12] When given therapeutically to non-pregnant animals, the benzimidazoles produce very few adverse effects, even when administered at doses several times those recommended. They are well tolerated by cats, cattle, sheep, pigs and pigeons.[13–18] Most reports suggest that these drugs are also safe in pregnant livestock including cattle, horses, sheep and pigs, but netobimin and albendazole have resulted in adverse pregnancy outcomes in sheep, while mebendazole led to an equivocal outcome in this species.[8–10,14,17,19–21] Administration of albendazole to pregnant sheep approximately 4 weeks after mating resulted in hemimelia and amelia.[21]

Several of these compounds have produced evidence of teratogenic effects, embryotoxicity and foetotoxicity in experimental animals when given at sufficiently high doses at sensitive periods of gestation. Parbendazole was teratogenic in rats (and sheep),[22] and albendazole is teratogenic in rats in some studies.[23,24] Oxfendazole, cambendazole and mebendazole produced evidence of embryotoxicity in rats.[25–29] Oral doses of albendazole up to and including 10 mg/kg bw/day given through days 6 to 15 of gestation had no effects on development in rats but doses of 20 or 30 mg/kg bw/day resulted in reduced ossification, malformations and foetal deaths.[30] The metabolites of albendazole can cross the placentas of rats and sheep and reach the foetus.[30,31] Doses of 10 or 14 mg/kg bw on day 10 of gestation caused skeletal anomalies in rats.[32] The prodrug netobimin produced foetal anomalies in sheep when given on day 17 of gestation and resulted in an increased incidence of resorptions and skeletal anomalies in this species and in rats.[33–35] Thiabendazole resulted in reductions in limb length when given to pregnant mice.[36] These effects generally occurred at doses which caused severe maternal toxicity and when given to pregnant mice at lower doses (up to 200 mg/kg bw/day), there were no adverse effects on pregnancy outcomes.[37] In contrast, fenbendazole has not produced similar effects when tested in a number of species, including pigs.[15]

The benzimidazoles are converted *in vivo* to the sulfoxide and sulfone metabolites; the prodrugs febantel and netobimin are first converted to their benzimidazole counterparts namely fenbendazole and albendazole, respectively. Ricobendazole is the sulfoxide of albendazole, while oxfendazole is the sulfoxide of fenbendazole. There is evidence to suggest that the sulfoxide metabolite is the active embryotoxic, foetotoxic and teratogenic agent. For example, when fenbendazole, its 6-hydroxy derivative or fenbendazole sulfone were given to rats on days 8 to 15 of gestation, there were no adverse effects on the offspring. However, when oxfendazole was given in the same manner it caused 80% embryolethality and skeletal malformations.[9] Albendazole sulfoxide on the other hand was not teratogenic in CF1 mice when given at doses of up to 200 mg/kg bw/day on days 1 to 3 of gestation.[38] In this particular case, it may be argued that while this may be a sensitive period for early embryogenesis, it is not a sensitive period for organogenesis in the mouse and so while this may demonstrate preimplantation safety of albendazole sulphoxide in this species, it fails to address overall developmental toxicity.

The benzimidazoles exert their anthelmintic effects (and in the case of thiabendazole and the chemically related pesticide benomyl (and related compounds), their antifungal effects) by binding to tubulin and acting as mitotic spindle poisons in a manner similar to that of some antineoplastic agents. They may also disrupt proton transfer across cell membranes and they may cause perturbations of parasite metabolism.[2,25,39–46] The effects on tubulin, and subsequent effects on mitosis through disruption of the spindle may explain some of the reproductive effects seen with these agents, and some of the effects seen in genotoxicity studies. As noted earlier, the genotoxicity profiles of these compounds are generally negative. However, clastogenicity, possibly arising from nondisjunction events, and accompanied by aneugenic effects, have been observed.[47,48]

Albendazole, thiabendazole and mebendazole are also used in human medicine for the treatment of helminthiasis. Thiabendazole is frequently associated with anorexia, nausea, vomiting and dizziness at therapeutic doses. It may also cause diarrhoea, drowsiness and headache. It has resulted in erythema multiforme, hallucinations, sensory disturbances and Stevens–Johnson syndrome. Mebendazole is without significant toxicity although it may cause abdominal pain and diarrhoea. Like mebendazole, albendazole is well tolerated and only occasionally results in abdominal pain, diarrhoea, nausea, dizziness and headache. Very rarely, it may cause signs of hepatotoxicity including increases in liver enzymes, jaundice and cholestasis and it is usually recommended that its use be avoided in patients with cirrhosis.[7,49] Albendazole has been reported to cause pseudomembranous colitis and dystonia in children.[50,51] The most serious adverse reactions, although very rare, are blood dyscrasias following albendazole use. Albendazole has been reported to have induced pancytopenia and aplastic anaemia in treated patients.[52,53] In one case of pancytopenia, the patient died.[54] It was speculated that pre-existing liver disease resulted in a higher concentration of albendazole sulphoxide in plasma, which may have caused haematotoxicity. The kinetic behaviour of albendazole is dose dependent and is affected by liver disease in humans.[55–57]

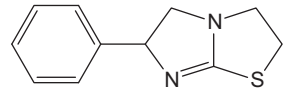

Figure 12.3 Chemical formula of levamisole.

12.2.2 Levamisole

Levamisole (6-phenyl-2,3,5,6-tetrahydroimidazo[2,1-*b*][1,3]thiazole; Figure 12.3) is widely used in cattle, sheep and pigs as an antinematodal drug. It was also used widely in dogs until the advent of ivermectin and it may still be used in those breeds that are sensitive to the toxic effects of the macrocyclic lactones (see Chapter 6).[3,58] The drug has cholinergic activity and it acts on the neuromuscular system of nematodes causing tonic paralysis. It also inhibits mitochondrial function by disrupting the fumarate reduction process.[59–61] It is not widely used as an anthelmintic in humans. However, it has been recognised to have immunomodulatory effects and so has been used in the treatment of rheumatoid arthritis. It also has beneficial effects in the treatment of some human cancers, and notably colon cancer, but its mode of action here is unknown.[62–72]

Levamisole is of moderate acute toxicity with oral LD_{50} values in the mouse, rat and rabbit being 205–285, 286–1095 and 458 mg/kg bw, respectively. In repeat dose studies in rats, the main adverse effects were a general reduction in organ weights and body weights, but with an increase in liver weights. In dogs given levamisole orally for 28 days the main adverse effects were ataxia and convulsions at the highest dose given (20 mg/kg bw/day). One animal died at this dose. However, no adverse effects were noted in a 90 day study in dogs with doses of up to 6 mg/kg bw/day. Severe toxicity occurred in a one year dog study with doses of up to 20 mg/kg bw/day. After eight weeks, all the animals given this dose, and one dog in the group given the next lower dose (5 mg/kg bw/day) developed haemolytic anaemia with reductions in haematocrit, haemoglobin and red blood cell counts. These animals were removed from the study and their haematology returned to normal but the anaemia returned when they were again administered levamisole. It also caused thrombocytopenia in dogs. The drug was not carcinogenic in rodents. However, it did produce positive results in two tests (chromosomal aberrations and sister chromatid exchanges) using human lymphocytes. It was not teratogenic in animals.[73,74] Further studies in dogs suggested that this species is uniquely susceptible to the haemolytic effects of levamisole.[75]

In humans treated with levamisole, the major adverse effects reported were haematologic including reversible leukopenia, agranulocytosis and thrombocytopenia. However, the haemolytic effects seen in dogs are not observed in humans. The agranulocytosis is observed in all continuous dosing regimens with doses ranging from 50 to 200 mg per day and treatment periods from 3 days every alternate week to continuous daily therapy. Agranulocytosis caused

by levamisole is dose-dependent.[73,76–78] Following cessation of levamisole therapy the marrow rapidly returns to normal.[76] The incidence of thrombocytopenia is estimated to be 0.03% while agranulocytosis may be up to 6%.[75] The mechanisms that lie behind the haematotoxic effects of levamisole are unclear but they may involve an immune effect and anti-levamisole antibodies. In a series of patients who developed agranulocytosis, complement-dependent granulocytotoxic antibodies were detected in serum. These were not found in patients who did not develop agranulocytosis.[79–82] Agranulocytosis with levamisole therapy might be more common in patients with rheumatoid disease. It also appears to be more common in women.[83] Hepatotoxicity, vasculitis, cerebral demyelination and psychosis are rare adverse effects with levamisole and they may occur in combination with 5-fluorouracil therapy.[84–90]

Recent observations have included agranulocytosis and other haematologic abnormalities among cocaine users.[91–95] This has been attributed to cocaine being adulterated with levamisole. Analysis of cocaine samples in the USA suggests that up to 88% were contaminated with levamisole.[96] Other adverse effects associated with levamisole-contaminated cocaine include cutaneous vasculitis, purpura and necrosis of the skin and ears.[92,93,97–101]

12.2.3 Salicylanilides

As the name suggests, the salicylanilides are derivatives of salicylanilide, the amide formed from aniline and salicylic acid (Figure 12.4). The main compounds are closantel, oxyclozanide, rafoxanide and niclosamide. Niclosamide is used in human medicine for infestations caused by various cestodes.[7] In veterinary medicine oxyclozanide and rafoxanide are used for the treatment of fluke infections caused by *Fasciola hepatica*. Closantel is also used for this condition but it is also active against *Haemonchus contortus* and certain arthropods of sheep and cattle. However, its main use is as a flukicide. The salicylanilides are uncouplers of oxidative phosphorylation in mitochondria and they prevent the synthesis of ATP. They share this property with several other compounds including pentachlorophenol, 2,4-dinitrophenol, salicylic acids, thiocarbazates and aromatic amines. Their flukicidal activity also appears to be dependent on reaching the mature flukes, probably in the bile ducts.[102–111]

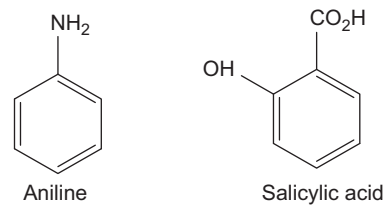

Figure 12.4 Chemical formulae of aniline and salicylic acid.

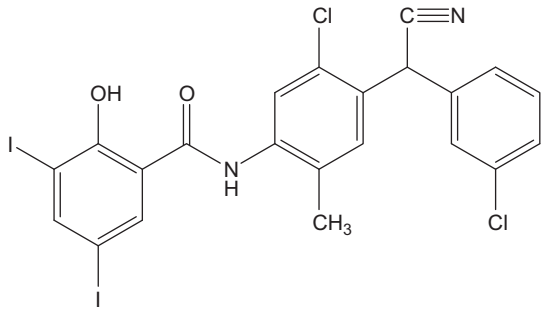

Figure 12.5 Chemical formula of closantel.

Closantel (Figure 12.5) is of moderate oral toxicity with LD_{50} values of 300–450 mg/kg bw in the mouse and 260–340 mg/kg bw in the rat. In repeat dose studies in rats, the relative weights of male gonads were increased at the highest dose used (40 mg/kg bw/day in the diet for 13 weeks). A cystic distention of the epididymis was seen in some low dose animals and in 70% of those given the highest dose. A number of high dose animals had spermatic granulomas with round cell infiltration, oedema and fibrosis. There was fatty deposition in the centrilobular region of the livers. Elevated liver enzymes occurred in repeat dose studies in dogs, and as with rats, there were fatty deposits found in the livers. A range of genotoxicity studies gave negative results. Closantel was not carcinogenic in mice or rats. However, the spermatic granulomas seen in repeat dose studies in rats were also noted in the rat carcinogenicity study. Despite this, there were no effects on fertility in a rat reproductive study and there was no evidence of teratogenic effects in studies in rats, rabbits and sheep.[112,113]

Closantel is normally well tolerated by human patients.[112] There is a report of blindness in a woman given veterinary closantel for gynaecological reasons after physicians wrongly identified it as a human drug.[114] Although no similar effects were noted in toxicology studies, optic neuropathy and blindness with retinal degeneration have been reported in a dog and in sheep and goats, usually following overdosing with the drug.[115–121]

Rafoxanide and oxyclozanide (Figure 12.6) are also of low toxicity in laboratory animal toxicology studies and they were not genotoxic, carcinogenic or teratogenic.[122–125] However, rafoxanide did produce evidence of optic neuropathy in a 13 week oral toxicity study in dogs and in dogs given 3 to 11 oral doses of 100 mg/kg bw/day. Vacuolation was observed in the optic nerves and central nervous system, with vacuolation in the submeningeal white matter, cerebrum, cerebellum, mid-brain and medulla.[122,123] Rafoxanide was not tested in a repeat dose study in dogs. However, it did cause amaurosis in sheep with a status spongiosus affecting parts of the brain and the optic nerves, with lesions in the retina.[126] Consequently, the optic pathology may be a class effect in some mammalian species, and possibly, following the report of blindness in a woman following closantel administration, in humans. Niclosamide has not been widely used in veterinary medicine.

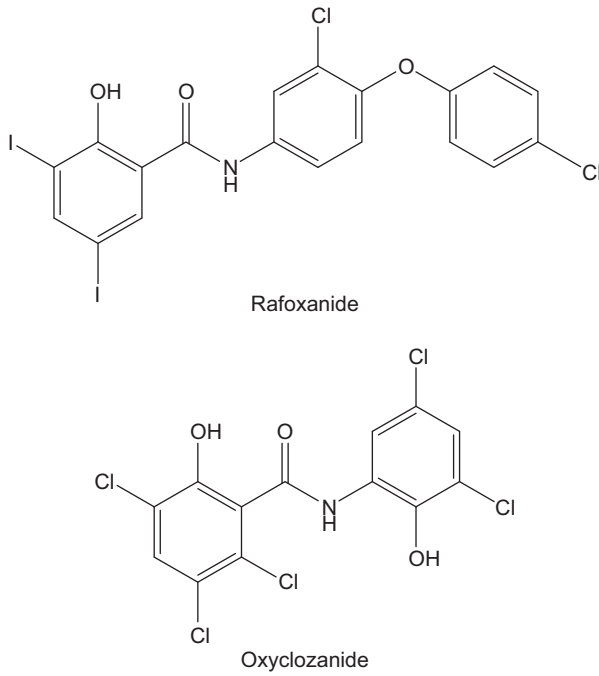

Figure 12.6 Chemical formulae of rafoxanide and oxyclozanide.

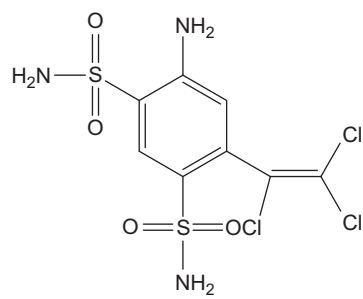

Figure 12.7 Chemical formula of clorsulon.

12.2.4 Clorsulon

Clorsulon (4-amino-6-trichloroethenyl-1,3-benzenedisulphonamide; Figure 12.7) is a benzenesulphonamide which is used as a flukicide against *F. hepatica* and other flukes in sheep and cattle, and against other ruminant parasites.[107,127–130] The drug is thought to enter the parasite through ingestion of red blood cells to which it is bound.[131] It exerts its effects through the inhibition of parasite phosphoglycerate kinase.[103,104,132–133]

Clorsulon has very low acute oral toxicity after oral administration to mice and rats with LD_{50} values being in excess of 10 000 mg/kg bw. After intraperitoneal administration, the LD_{50} values were between 678 and 938 mg/kg bw in these species. In repeat dose studies in rats and dogs, the main effects were a reduction in thyroid weights in rats and haemosiderosis in liver and spleen with bone marrow hyperplasia and extramedullary haematopoiesis in dogs. In a 13 week repeat dose oral study in rats, epithelial hyperplasia of the kidney and urinary bladder hyperplasia occurred with clorsulon administration. There was a significant increase in thyroid weights due to thyroid follicular cell hyperplasia. Similar effects were noted in a 54 week rat oral repeat dose study. The compound had no effects on reproductive performance in rats and it was not teratogenic in mice and rabbits. It was not mutagenic in several tests for genotoxic activity but it produced positive results in two *in vivo* studies, namely, a bone marrow micronucleus test and a test for chromosomal aberrations, both in mice. Despite this, it gave negative results in carcinogenicity studies in mice and rats.[134] Clorsulon is a weak inhibitor of carbonic anhydrase and it produces significant increases in urinary pH, urinary volume and urinary sodium concentrations. It decreases renal reabsorption of sodium and results in increased excretion of sodium, potassium, carbonate and water. It is not known if this phenomenon contributes to the urinary bladder and renal epithelium hyperplasia although this is plausible. The urinary bladder epithelial cell hyperplasia reverses on drug cessation.[135]

There are no reports of human exposure to clorsulon and subsequent adverse effects.

12.2.5 Tetrahydropyrimidines

The major representative drugs are pyrantel, morantel and oxantel (Figure 12.8). Oxantel is a more recent member of the group. Pyrantel and oxantel are used in human patients. Pyrantel is used in the treatment of pinworm, roundworm and hookworm infestations and is valued because of its very low human toxicity.[3] Oxantel is used for the treatment of whipworm (trichuriasis) for which pyrantel is ineffective. Morantel is closely related in chemical structure to pyrantel (see Figure 12.8) but it does not appear to have been extensively used in human medicine.

In veterinary medicine, the main tetrahydropyrimidine drugs are pyrantel and morantel. Pyrantel is used widely in horses as the pamoate (embonate; a naphthoic acid derivative) salt and it is active against a range of roundworms. As the tartrate and citrate, it is used in cattle, sheep and pigs in some countries. However, in the European Union (EU), the maximum residue limit (MRL) only covers the use in horses. Morantel, usually as the citrate or tartrate is used to treat parasitic infestations in a number of species. Pyrantel, in combination with ivermectin is used to treat susceptible parasites in dogs and in combination with praziquantel (see section 12.2.7), parasites in cats and dogs.[3,58] Oxantel, in combination with pyrantel and praziquantel is used to treat parasitic disease in

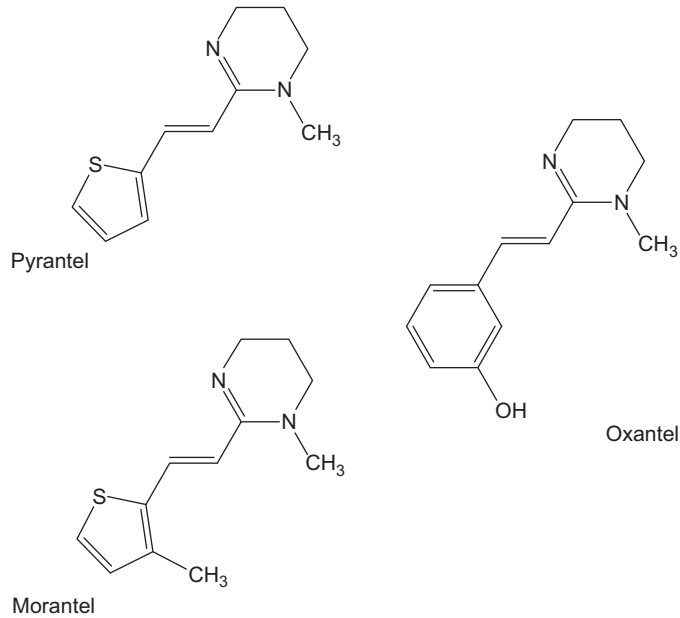

Figure 12.8 Chemical formulae of pyrantel, morantel and oxantel.

dogs.[136] At the time of writing in 2012, oxantel does not have EU MRLs and so it may not be used in food animals.

The tetrahydropyrimidines exert their effects on parasites by inducing marked activation of nicotinic receptors producing spastic paralysis. They are also potent agonists of acetylcholinesterase receptors. Pyrantel and morantel are estimated to be 100 times more potent than acetylcholine in this respect.[3,7,137–139]

Pyrantel and morantel are poorly absorbed after oral administration and most of the dose remains in the gastrointestinal tract and this explains their effectiveness against gut parasites and their low mammalian toxicity after oral administration. With pyrantel, only 6% of an oral dose was excreted in the urine with the remainder in the faeces. In dogs, urinary excretion accounted for around 15% of the orally administered dose. Absorption of morantel as the tartrate appeared to be slightly higher. Approximately 27% of the administered dose was excreted in the urine of mice and up to 43% of an oral dose of 30 mg/kg bw in dogs. There also appeared to be reasonable absorption in cattle, pigs and sheep.[140,141]

Pyrantel pamoate has low acute oral toxicity with LD_{50} values in the range of 2000 mg/kg bw in the rat, mouse and dog. However, the tartrate salt, probably because of better gastrointestinal absorption, is more acutely toxic after oral administration with an LD_{50} in mice of 175 mg/kg bw. Neither the pamoate nor the tartrate produced any major effects in repeat oral dose studies in rats. The major effects in studies in dogs were slight elevations in liver enzymes and

diarrhoea. It produced no major effects in a rat reproduction and lactation study and there was no evidence of teratogenic effects in studies in rats and rabbits. It was only tested in limited genotoxicity studies but nevertheless gave negative results and it provided no evidence of carcinogenicity in a 93 week study in rats and in a 2 year study in dogs (which is probably inadequate to assess carcinogenicity).[140,142]

Morantel produces similar metabolites to pyrantel, which is not surprising in view of its chemical similarity. The LD_{50} values for the tartrate and citrate salts were of the same order of magnitude as the tartrate salt of pyrantel described above. These were 179 to 260 mg/kg bw in mice and 551 to 586 mg/kg bw in rats. As with pyrantel there were no major effects seen in repeat dose studies in rats, while in dogs in a 2-year study the main adverse effects were vomiting and elevated absolute and relative liver and adrenal weights. It had no effects on fertility in rats and was not teratogenic in studies in mice, rats and rabbits. Unlike pyrantel, it was tested in a battery of studies for genotoxic activity although several of these were marred by poor experimental design. Nonetheless, it produced no evidence of genotoxic effects and it gave negative results in a rat carcinogenicity study.[141]

In humans, these drugs may produce toxic effects but only at very high doses. The usual adverse events are headache, dizziness, rash and fever. Gastrointestinal disturbances may occur.[7] There has been a single and recent case of massive proteinuria in a patient (4 year old male) treated with pyrantel pamoate for oxyuriasis (pinworms).[143] The condition resolved on cessation of treatment and was not present at 3 and 6 month follow-ups. Although the authors suggested that nephrotic syndrome was induced by pyrantel there is no conclusive proof of this and, under most circumstances, these drugs are very safe in human patients. Adverse reactions are also rare in animal patients and reports have been limited to diarrhoea and vomiting although isolated deaths have occurred in dogs and cats.[144] There are isolated reports of contact dermatitis with morantel.[145,146]

In the EU, morantel appears in Table 1 (allowed substances) of Regulation (EU) No 37/2010 with numerical MRL values. However, pyrantel appears in the same Table with "no MRL required" in view of its use being restricted to horses and its rapid metabolism.

12.2.6 Pyrazinoisoquinolones

These drugs are derived from pyrazinoisoquinoline and specifically from 1,3,4,6,7,11*b*-hexahydro-2*H*-pyrazino[2,2-*a*]isoquinoline (azaquizole, Figure 12.9) and the two major members of this group are praziquantel and derquantel (Figure 12.10). Praziquantel has been used for many years in human and veterinary medicine but derquantel is a relative newcomer to veterinary medicine.

Praziquantel has been used for many years in human medicine for the treatment of diseases caused by cestodes and trematodes. It is the drug of

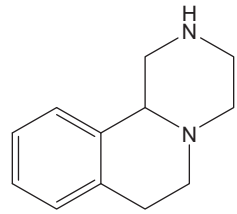

Figure 12.9 Chemical formula of 1,3,4,6,7,11*b*-hexahydro-2*H*-pyrazino[2,2-*a*]isoquinoline (azaquizole).

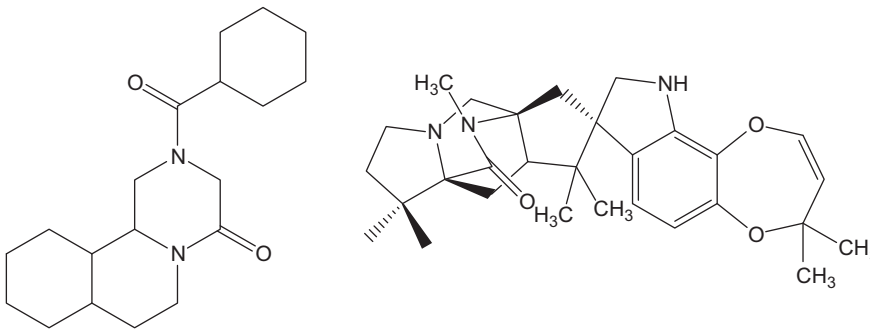

Figure 12.10 Chemical formulae of praziquantel and derquantel.

choice for the treatment of schistosomiasis but it is also used for a number of other parasitic conditions including liver fluke.[7,147–150] In animals, praziquantel is also used in diseases caused by cestodes and trematodes; it is highly effective against *Taenia* spp. and *Echinococcus granulosus* in farm animals. In companion animals it is employed in the treatment of diseases caused by a variety of parasites, frequently in combination with febantel or pyrantel.[58,107,150]

Praziquantel is regarded as a very safe drug in humans and in animals.[149–151] The drug is rapidly metabolised after oral or intravenous administration to rats, dogs or monkeys. For example, 15 minutes after oral or intravenous administration to monkeys the proportions of metabolites in serum were 99% and 50% respectively. Similar findings were made in rats and dogs. It is highly bound to serum (more than 70%) in all species studied. It is of low acute oral toxicity in mice and rats with LD_{50} values in excess of 2000 mg/kg bw. Apart from vomiting in a 90 day oral repeat dose study in dogs it was virtually non-toxic in this study and in repeat dose studies in rats. It had no effects on fertility in rats, and it was not embryotoxic, foetotoxic or teratogenic in rats and rabbits. It produced no toxic effects, including tumour induction, in studies where rats and hamsters were given the drug orally for 104 or 80 weeks respectively.[152–154] Although the genotoxic safety of praziquantel has been

questioned, particularly in respect to co-exposure to environmental mutagens such as benzene or the bladder carcinogenesis associated with schistosomiasis in humans,[155–157] the drug has been tested in a range of studies for genotoxic potential and has given uniformly negative results.[152–154,158–161] In 1995, a structure–activity assessment concluded that praziquantel had the potential to act as a non-genotoxic carcinogen.[162] However, in view of the lack of any evidence of potential preneoplastic effects in short-term repeat dose studies, and the lack of any carcinogenic effect in longer term studies in rats and hamsters, this seems unlikely. In fact, aside from its effects on parasites, which are mediated through an almost instantaneous tetanic reaction of the muscles of the parasite, possibly through a calcium dependent mechanism,[163] praziquantel is almost devoid of biological activity. In the EU, as the drug is rapidly metabolised and concentrations of drug residues are well below the ADI, it appears in Table 1 of Regulation (EU) No 37/2010 as ''no MRL required'' for use in sheep (including sheep producing milk for human consumption) and horses.[152,164,165]

Derquantel is structurally related to praziquantel, but the molecule is more complex and is in fact a spiroindole compound (Figure 12.10). It is also known by a number of synonyms, including 2-deoxyparaherquamide. The drug is relatively new and has been developed initially at least for the control of parasites in sheep. It is a nicotinic acetylcholine receptor antagonist and it is more toxic than praziquantel. No mortality occurred in rats with acute oral doses of up to 1000 mg/kg bw but doses of 350 mg/kg bw produced jerky movements, piloerection and reduced somatomotor activity. In dogs, acute doses produced injection of the sclera, mydriasis, ptosis and relaxed nictitating membranes. In a neurobehavioral study in dogs, similar signs were seen at the lowest dose used, 1 mg/kg bw. Doses of 2 and 20 mg/kg bw were lethal in horses.[166,167]

In repeat dose studies in rats, the main signs of toxicity were associated with CNS effects. Thyroid follicular hypertrophy also occurred. In a one year study in rats, the main effects were lowered thyroid weights and elevated liver and kidney weights. Hypospermia and cataracts also occurred. However, the most pronounced effect was bile duct hyperplasia. In a second 1-year study with lower doses of derquantel, bile duct hyperplasia occurred in controls but there appeared to be an increased incidence in treated rats. In repeat dose studies in dogs, there were no notable findings. Derquantel had no effects on reproductive performance and it was not teratogenic. It was tested in a battery of studies for genotoxic potential but it gave negative results. No carcinogenicity studies were performed but in view of the negative genotoxicity studies, these are not necessary and there is no reason to consider derquantel to be a carcinogen.[166,167]

Unlike praziquantel, derquantel was assigned numerical MRL values in the EU.

There have been few adverse drug reactions in animals reported with praziquantel and, as noted earlier, it is well tolerated also in humans. Similarly, there have been few reports in humans exposed to praziquantel during veterinary use. The few that exist include non-specific signs following exposure

including headache, dizziness, epiphoria, nausea, vomiting and abdominal pain. Eye irritation occurred after ocular contact.[168] There is currently inadequate information to assess in-use safety of derquantel.

12.2.7 Monepantel

Monepantel is a new antinematodal drug intended for use in sheep. It is classed as an amino-acetonitrile derivative and its chemical name is *N*-[(1 *S*)-1-cyano-2-(5-cyano-2-trifluoromethylphenoxy)-1-methylethyl]-4-trifluoromethylsulfanyl-benzamide. Its chemical structure is shown in Figure 12.11.[169–171] Monepantel is specific for the ACR-23 protein which is in the nematode-specific subfamily of nicotinic acetylcholine receptors.[172]

Monepantel has low acute oral toxicity in rats with the LD_{50} value in excess of 2000 mg/kg bw. Dietary administration of monepantel for 13 weeks at concentrations of up to 6000 ppm (equivalent to 959 mg/kg bw/day) produced no significant signs of toxicity. However, there were elevations in liver enzymes and increases in plasma cholesterol concentrations. Liver weights were increased at the highest dietary level. The only histological findings were in the liver where centrilobular fatty change was seen. There was also increased focal necrosis and lymphoid cell infiltration in females given 600 and 6000 ppm monepantel in the diet. In a 4 week study in rats given dietary monepantel at concentrations of up to 12 000 ppm equivalent to 1044 and 1017 mg/kg bw/day for males and females respectively, the major effects were an increase in serum cholesterol, increases in serum triglycerides, increases in phospholipids and increased globulin. Increased absolute and relative liver weights occurred along with centrilobular hypertrophy in the liver and follicular cell hypertrophy in the thyroid. Similar effects were noted in rats given doses of up to 12 000 ppm for 3 months. A 52 week feeding study was conducted in rats with dietary concentrations of up to 12 000 ppm monepantel. There were no major signs of toxicity during the study except for reductions in body weights in animals given the two highest concentrations in the diet. Again, there were effects on serum lipids including increased serum cholesterol concentrations. There were increases in absolute and relative liver weights but no significant histological changes.

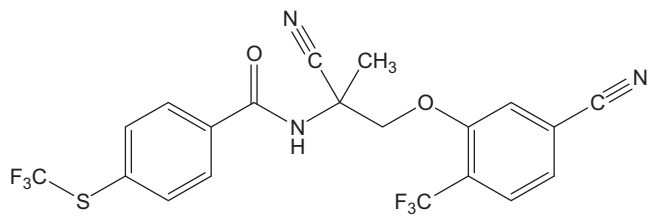

Figure 12.11 Chemical formula of monepantel.

In dogs given diets containing up to 40 000 ppm (1217 mg/kg bw/day) monepantel for 4 weeks, there were no major signs of toxicity except for an increased severity, but no increased incidence, of thymus involution at the highest dietary level. When given to dogs for up to 13 weeks in the diet at concentrations of up to 30 000 ppm (963 and 1176 mg/kg bw/day for males and females respectively) there were no signs of treatment related effects except for a reduction in body weights in males given the highest dietary concentrations. The major findings were increased serum alkaline phosphatase and, at termination, increased absolute and relative liver weights. Histopathological changes were minimal and consisted of slight hepatocellular hypertrophy in males given the highest dietary level and in females at all doses. In a 52 week dietary study in dogs given diets containing up to 3000 ppm monepantel, there were no treatment related clinical signs or deaths. There were no adverse effects on ophthalmoscopy and no haematological effects of note except for a reduced activated partial thromboplastin time in males given the two highest dietary levels (300 and 3000 ppm). Elevations of alkaline phosphatase occurred in all dogs at all dietary levels.

At termination, increased absolute and relative liver weights occurred in all test groups. In males given the highest dietary concentration of monepantel liver hypertrophy was seen. This hypertrophy was probably related to a proliferation of smooth endoplasmic reticulum. In the jejunum, dilated Lieberkühn glands were associated with enlarged goblet cells, which may have been due to increased mucous secretion.

Monepantel was tested in a range of studies for genotoxicity but gave negative responses. It was not carcinogenic in mice and rats, and produced no evidence of embryotoxic, foetotoxic or teratogenic effects in rats, rabbits and sheep. It had no effects on reproductive performance in rats. It was also tested in a number of safety pharmacology studies including a test for cardiovascular and respiratory effects in the dog, behavioural effects in rats, a charcoal gut motility study in rats and tests for effects on immune responses. It had no effects on these, nor did it produce any evidence for sensitizing activity.[173,174]

From these data, it can be concluded that on the basis of the evidence thus far available, monepantel is of low mammalian toxicity. Data available also show that it is safe for clinical use in sheep.[174]

12.2.8 Piperazine

Piperazine (1,4-diazacyclohexane; Figure 12.12) has been used for many years as an anthelmintic in human and veterinary medicine and it is widely regarded as a safe and effective drug.[3,7,58] The salts used, normally the hydrochloride and the citrate show a relationship between solubility and toxicity, with the less soluble salts being the less toxic. For example, the acute oral LD_{50} values for piperazine phosphate, citrate, hydrochloride and hexahydrate in rats were 22 350 mg/kg bw, 13 200 mg/kg bw, 6200 mg/kg bw and 7000 mg/kg bw equivalent to 9500, 5280, 4360 and 3100 mg/kg bw when expressed in terms of

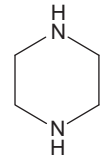

Figure 12.12 Chemical formula of piperazine.

piperazine base. Regardless, piperazine is of a low order of acute toxicity in rats. It also had a low order of acute toxicity in mice.[175]

No major signs of toxicity were seen in repeat dose studies in rats or dogs, including a 52 week study in rats with oral doses of up to 1200 mg/kg bw/day and a 13 week dietary study in dogs with levels of up to 3692 mg/kg feed (approximately 122 mg/kg bw/day). It had no significant effects on reproductive performance in rats nor was it teratogenic. However, in rabbits piperazine phosphate produced cleft palate and umbilical hernias at maternotoxic doses with the main effects being noted at the highest dose used, 500 mg/kg bw/day.[175]

A number of genotoxicity studies have been conducted with piperazine including some with *Salmonella typhimurium* tester strains in the presence and absence of metabolic activation, a study using mouse lymphoma cells, a test for chromosome aberrations and a mouse micronucleus test. Negative results were obtained in these studies and so there is no evidence that piperazine is genotoxic.[175–177] As a secondary amine, there is concern that piperazine may react with nitrite to yield carcinogenic nitrosamines. This has been investigated in several studies where mice were given nitrite and piperazine in the drinking water or nitrite and piperazine were given orally to mice. There was no evidence of carcinogenic activity. Studies in humans where piperazine was given orally produced *N*-mononitrosopiperazine. However, it is thought that the carcinogenic agent arising from nitrite and piperazine is *N*,*N*′-dinitrosopiperazine. The former is water soluble while the latter is lipophilic and may pass through cell membranes and into cells. In a host-mediated assay in mice with piperazine and nitrite both mono- and dinitrosopiperazine produced positive results while piperazine gave a negative response.[175,178,179] Overall, the carcinogenic risk from ingestion of piperazine or piperazine residues in food of animal origin is probably exceedingly low.

Piperazine is weakly neurotoxic and it has neuromuscular blocking activity but is 500 to 3000 times less potent than *d*-tubocurarine.[138,180] In humans, in high doses or when given to paediatric patients it may induce neurological effects which may be severe. These include an unusual form of cerebellar ataxia characterised by rolling of the eyes, hypotonia, hypereflexia and dysmetria. This condition has been referred to as "worm wobble."[181–190] It may also induce seizures or worsen an existing epileptic condition.[191–194] It is mildly hepatotoxic.[195] These effects are not restricted to humans and similar adverse reactions have been reported in canine and feline patients treated with

piperazine.[196–203] In animals it produces muscle tremors, ataxia, depression and incoordination, signs similar to those noted in humans with piperazine toxicity.[203] At 100 mg/kg bw intravenously in rats, piperazine citrate resulted in severe bradycardia, elongations of the QT interval and atrioventricular block. Three out of 7 rats treated with this dose died.[204] In rats and mice, piperazine potentiated the effects of chlorpromazine, but not prochlorperazine and significantly increased the acute toxicity of the former.[205]

Piperazine is a respiratory sensitizer and it has resulted in occupational asthma in industries where the compound is present in industrial work-place air.[206–208] IgE antibodies against conjugates between human serum albumen and piperazine have been demonstrated in two asthmatic subjects occupationally exposed to piperazine (and *N*-methylpiperazine).[209] Skin sensitisation has been reported.[210,211] Moreover, some patients sensitised to ethylenediamine may cross react with piperazine.[212] These reactions are rare and generally confined to industrial settings with high occupational exposures. It is extremely unlikely that occupational or user exposure during veterinary use would elicit these reactions.

A case of purpura in a 10 year old girl treated with piperazine has been reported.[213]

Considering that millions of doses of piperazine are given annually to humans and to animals, then the numbers of adverse reactions are extremely low especially when it is recognised that the drug has been commercially available for over 50 years. The same may not be true of simple substituted piperazines such as benzylpiperazine and trifluoromethylphenylpiperazine, which are increasingly being used as designer "party pills" for recreational use.[214–218]

12.2.9 Diethylcarbamazine

Diethylcarbamazine (*N*,*N*-diethyl-4-methyl-1-piperazinecarboxamide; Figure 12.13) a piperazine derivative, is an anthelmintic drug that in humans is used for the treatment of filariasis.[7] In veterinary medicine it is used mainly, indeed almost exclusively for the prevention and treatment of heartworm (*Dilofilaria immitis*) in dogs.[58]

There are few toxicity data available for diethylcarbamazine. In rats given 1000 mg/kg bw diethylcarbamazine by the intraperitoneal route, the main effect

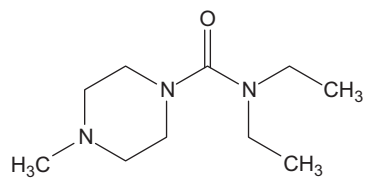

Figure 12.13 Chemical formula of diethylcarbamazine.

was a sudden fall in heart rate. This was less pronounced at 750 mg/kg bw and did not occur at all with a dose of 500 mg/kg bw. The drug may inhibit calcium ATPases in cardiac myocytes.[219] It has not been extensively tested in genotoxicity studies, or at least these are not well documented in the open scientific literature. One study suggested that it may be clastogenic as it produced positive results in the mouse micronucleus test and in a metaphase analysis for chromosome aberrations.[220] It was not teratogenic in rats or rabbits.[221] In the rat, administration of diethylcarbamazine for 12 days resulted in hypertrophy of Leydig cells of the testis and vacuolation of Sertoli cells. Mitochondria in spermatids showed vacuolation.[222] However, the mechanism is unknown.

In treated dogs, the main adverse reaction following diethylcarbamazine treatment is severe, and similar to hypovolaemic shock. It may be accompanied by tissue damage and hepatic injury. The reaction, which can be partly prevented by diazepam, appears to be an anaphylactoid response related to parasite burden, and specifically to circulating microfilariae and may be an allergic reaction to proteins released by dead parasites.[223–229] In humans it can result in a similar effect in the treatment of onchocerciasis and loiasis.[7]

12.2.10 Nitroxynil

Nitroxynil (4-hydroxy-3-iodo-5-benzonitrile; Figure 12.14) is a fasciolide with activity against *Fasciola hepatica*; it is also active against *Haemonchus contortus*.[105,107,230,231] Nitroxynil is used in pheasants, red legged partridges and other game birds for the treatment of syngamiasis caused by the nematode *Syngamus trachea* (gapeworm), a common parasite of many farmed and wild avian species.[232–236]

Nitroxynil is similar in structure to the herbicides ioxynil and bromoxynil (Figure 12.15). These compounds are uncouplers of oxidative phosphorylation and nitroxynil appears to exert its effects on parasites in this way.[237]

Nitroxynil is moderately acutely toxic with oral LD_{50} values in rats from 200 to 450 mg/kg bw for the *N*-methylglucamine salt and 170 mg/kg bw for the *N*-ethylglucamine salt. Major signs of toxicity included ataxia, sedation, prostration and hyperpnoea. In repeat dose studies in dogs, deaths occurred at doses of 10 mg/kg bw/day. In a 13 week repeat dose study in rats with oral

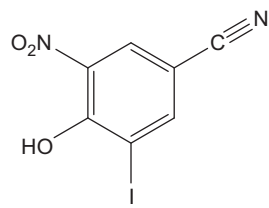

Figure 12.14 Chemical formula of nitroxynil.

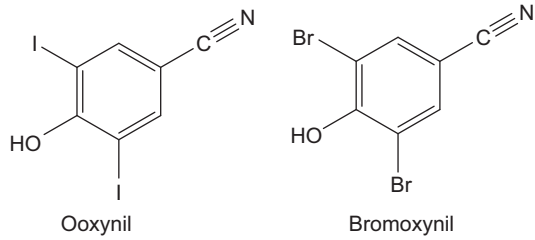

Figure 12.15 Chemical formulae of ioxynil and bromoxynil.

doses of up to 32 mg/kg bw/day, there were minor changes in thyroid hormone concentrations in plasma of male rats at all doses used and in females given 10 or 32 mg/kg bw/day. There were also minimal changes in the thyroid with changes in the height of the epithelium and deceases in colloid. A further study was conducted in rats to define a NOEL in male rats based on changes in the thyroid and thyroid hormone levels. This was identified as 0.5 mg/kg bw/day. There were no major effects on reproductive performance in rats and there was no evidence of teratogenicity in three studies in rabbits at doses of up to 3 mg/kg bw/day over the sensitive period of organogenesis. It was not genotoxic in a range of studies and there was no evidence of carcinogenicity except for increases in thyroid adenomas and carcinomas in both rats, which are not surprising as nitroxynil possesses an iodine atom and is goitrogenic in rats.[125,238] It shares this thyroid disrupting effect with ioxynil which also contains iodine, but not with bromoxynil which is bromine substituted.[239–241]

Nitroxynil has not been used in human medicine and there are no reports of toxicity associated with its manufacture, use, abuse or misuse.

12.2.11 Halofuginone

Halofuginone (Figure 12.16) is a halogenated derivative of febrifugine, a quinazolinone alkaloid obtained from the plant *Dichroa febrifuga*. It has a diverse range of pharmacological activity. It is an inhibitor of collagen type I gene expression and has been investigated for antitumour activity, for its effects on wound healing, in muscular dystrophy, and as an inhibitor of angiogenesis and specifically for its antitumor effects in this respect.[242–255] In veterinary medicine it is used as the lactate for its antiprotozoal activity especially in the treatment of *Crytosporidium parvum* in non-ruminating calves. It is also active against other protozoal parasites especially species of *Eimeria* in poultry (see also Chapter 9).[256–261]

Halofuginone is of relatively high acute toxicity in rats and mice with oral LD_{50} values of 30 and 5 mg/kg bw when tested as the lactate or hydrobromide salts.[262] The acute inhalation LC_{50} was 53 μg L^{-1} in the rat while the acute dermal LD_{50} was 16 mg/kg bw in the rabbit. In 4 week toxicity studies in mice the main effects were on haematological parameters with alterations in cell volume, mean cell volume, and mean cell haemoglobin at doses of 0.16 and

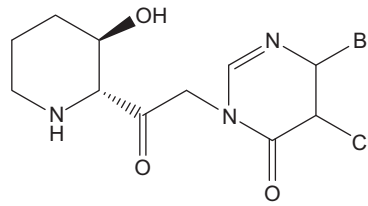

Figure 12.16 Chemical formula of halofuginone.

0.35 mg/kg bw. In a 13 week study in the rat there were no haematological abnormalities but females given the highest dose of 0.7 mg/kg bw/day showed fat deposition in the liver and hepatocyte vacuolation with a decrease in glycogen in periportal cells. In a 13 week repeat dose toxicity study in dogs there was a significant decrease in mean cell volume in the high dose group (0.134 mg/kg bw/day) but no other haematological changes. Decreases in mean cell volume and mean cell haemoglobin concentration occurred in dogs given 0.16 mg/kg bw/day for 26 weeks but not at lower doses. Halofuginone had no effects on reproductive performance in mice, but in dogs doses of 0.067 and 0.134 mg/kg bw/day significantly reduced testicular size and a reduction in fertility was reported. In a three generation study in rats, halofuginone at 0.063 and 0.126 mg/kg bw reduced the body weights of parental F_0, F_1 and F_2 animals but it had no effects on reproductive performance. It was not embryotoxic or teratogenic when administered to pregnant rats or rabbits but it was maternotoxic. The drug was tested in a range of genotoxicity studies and negative results were obtained in most of these. However, it gave positive results in the Ames reversion assay with *Salmonella typhimurium* strains TA 1538 and TA 98 in the presence and absence of metabolic activation. It was not carcinogenic in mice or rats.

There are no reports of halofuginone toxicity in human or animal patients.

12.2.12 Nitroimidazoles

The nitroimidazoles are antiprotozoal drugs, with antimicrobial activity. The main nitroimidazole used in human medicine is metronidazole, whereas in veterinary medicine it is dimetridazole. Ronidazole and ipronidazole have also been used. The chemical structure of these drugs is similar to that of the nitrofurans except the nitrofuran heterocyclic ring is replaced with that of the nitroimidazole ring. The chemical structures of the nitroimidazoles are shown in Figure 12.17.

In veterinary medicine, the main uses of the nitroimidazole group is the treatment of trichomoniasis in cattle and histomoniasis in poultry, especially in turkeys and game birds.[263–267] Ipronidazole and other nitroimidazoles are also effective in the treatment of swine dysentery caused by infection with the spirochaete *Brachyspira hyodysenteriae*.[268–271] Metronidazole is used for a

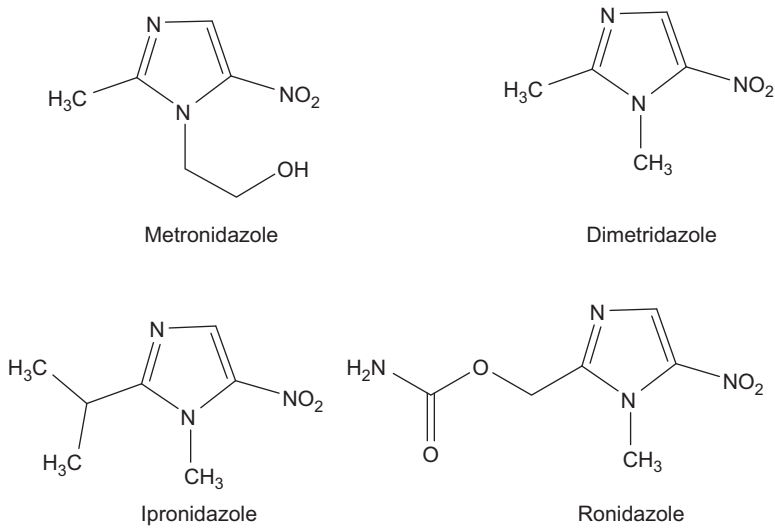

Figure 12.17 Chemical formulae of the nitroimidazoles.

variety of bacterial and protozoal infections in companion animals.[272,273] In human medicine, the main uses of metronidazole (Flagyl) are in the treatment of parasitic and microbial diseases caused by protozoa and anaerobic bacteria.[274] It has also been used as a radiosensitiser for the radiotherapy of malignant tumours possessing hypoxic cells.[275–280]

Dimetridazole and ronidazole are generally of low acute toxicity with oral LD_{50} values in the range 1800 to 2500 mg/kg bw in rodents. Ipronidazole appears to be slightly more acutely toxic with oral LD_{50} values of approximately 950 mg/kg bw in mice and rats. With dimetridazole, the main finding in repeat dose studies in dogs was mild testicular atrophy. Hepatomegaly occurred in dogs given repeat doses of ipronidazole and testicular hypoplasia occurred with ronidazole in repeat dose studies in dogs. All three compounds produced some evidence of neurotoxicity with ataxia, fine muscle tremors and dilatation of pupils among the signs noted at the higher doses. The compounds did not affect reproductive performance in rodents, but ipronidazole resulted in some degeneration in the testes. None of these compounds was teratogenic in rodents or rabbits but they did produce evidence of maternotoxicity at higher doses. Ronidazole was foetotoxic at higher doses.[281]

The most concerning aspect of the results of studies with these compounds were their genotoxicity profiles. Dimetridazole was genotoxic in the Ames *Salmonella* reversion assay, in a bacterial fluctuation study and in a test for gene conversion in *Saccharomyces cerevisiae* strain D4. However, it gave negative results in a range of other studies including those for clastogenicity and unscheduled DNA synthesis. Ipronidazole gave positive results in a range of *in vitro* tests for gene mutation, while ronidazole gave similar results to

dimetridazole but was also positive in a mouse bone marrow cytogenetic assay. Dimetridazole gave equivocal results in carcinogenicity studies with rats and there was no clear evidence of a carcinogenic response. Ipronidazole resulted in increased incidences of pulmonary adenomas, with pulmonary hyperplasia in mice. There was an increased incidence of benign mammary tumours in rats given ipronidazole. Ronidazole produced a small increase in benign and malignant lung tumours in mice and increases in benign mammary tumours in rats. In all of these studies the increased incidence of neoplasms was small and it tended to be dose related.[281–292]

Similar equivocal results have been obtained with metronidazole in genotoxicity studies.[288,293–304] A study in mice with metronidazole led to an increased incidence of malignant lymphomas and lung tumours, while a study in rats produced an increased incidence of mammary and liver tumours. There was also an increased incidence of Leydig cell and pituitary tumours in male rats.[305,306] There was no evidence for a carcinogenic effect of metronidazole in 771 women who had been given metronidazole for the treatment of vaginal trichomoniasis.[307] Moreover, when the study was followed-up some years later, and after adjustment for the effects of smoking, there was still no evidence for a carcinogenic effect.[308] A retrospective cohort study of 328 846 children whose mothers had been treated with metronidazole during pregnancy concluded that there was no increase in cancer risk associated with *in utero* exposure to the drug.[309] In a study of 12 000 people treated with metronidazole, there was no excess incidence of cancer associated with its use.[310] Despite this evidence, the National Toxicology Program's twelfth report classified metronidazole as a chemical "reasonably anticipated to be a human carcinogen",[311] which, on the evidence available to date, might be considered to be excessive.[312–314]

Metronidazole, at least in part, is subject to reductive metabolism by rat liver microsomes and it is believed that short-lived highly reactive metabolites may be responsible for the genotoxic effects,[315–317] a phenomenon that might then be extended to other nitroimidazoles. Unfortunately, like the nitrofurans (see Chapter 8), these studies have proved inconclusive. Although the nitroimidazoles are reactive, or are converted to reactive metabolites which then bind covalently to cellular macromolecules, it has not been possible to prove that the bound residues cannot be released as potentially toxic materials following ingestion and digestion of food of animal origin.[318–329] As a consequence, no MRLs were established for these drugs in the EU and they were prohibited for use in food animals. They have since been prohibited for food animal use in several other countries. The downfall of the nitroimidazoles in food animal use is probably linked with their mode of action. They are thought to be metabolised initially by reduction of the nitro group and subsequently to reactive moieties that cause single stranded DNA breaks in the target pathogens.[330]

At high doses or with prolonged treatment in human patients, metronidazole induces a cerebellar ataxia with other central abnormalities. Occasionally, an encephalopathy may develop.[331–342] The ataxia usually resolves on drug cessation.[343,344] The neurotoxicity is similar to the effects seen in toxicology studies. It is also similar to neurological effects seen in feline patients treated with

metronidazole or ronidazole.[345–347] Metronidazole can produce an effect similar to that of disulfiram when alcohol is ingested, which is exacerbated in combination with disulfiram.[348,349] Sudden death has occurred due to the interaction of metronidazole with alcohol.[350]

Metronidazole is a contact sensitising agent and it has resulted in contact dermatitis in patients and in those occupationally exposed to it.[351–357] It has also resulted in serum sickness and fixed drug eruptions.[358–365]

12.2.13 Imidocarb

Imidocarb (Figure 12.18) is a urea (carbanilide) or diamidine derivative used in the treatment of babesiosis caused by infection with *Babesia*, a protozoal parasite. The major babesial disease in Europe is bovine babesiosis or redwater fever. It is caused by *Babesia divergens* that is spread by the tick *Ixodes ricinus*. The organism can spread to immunocompromised humans and cause serious disease. The parasite lives inside erythrocytes that eventually rupture and lead to the typical red-coloured urine that gives the disease its common name. It also leads to abortion in pregnant cows. The treatment of choice is a single subcutaneous injection of imidocarb dipropionate at a dose of 0.85 mg/kg bw for treatment and 2.15 mg/kg bw for prevention.[263] In the United States, dogs may be infected by *Babesia canis* which is spread by the brown dog tick, *Rhipicephalus sanguineus*. Canine and equine babesiosis can also be treated with imidocarb.[263,366–369] The mechanism of action is not clear but imidocarb inhibits cholinesterase.[370–372]

Imidocarb is of low to moderate acute oral toxicity in rodents with LD_{50} values for the dipropionate in the range 550–700 mg/kg bw in mice and 1000–2000 mg/kg bw in rats. In a repeat dose toxicity study in mice, all of the animals given the two highest doses (63 or 130 mg/kg bw/day) died within three to four days of the commencement of treatment, as did 50% of those given the next lowest dose of 31 mg/kg bw/day. The main signs of toxicity were lethargy and a slowing of respiratory rate. In rats given 5 mg/kg bw/day for 30 days, there were no effects on electrocardiographic, ophthalmoscopic or urinary parameters. However, increased leukocytes were noted in blood samples taken from three rats. In a further study in rats, animals were given doses of up to 1500 mg/kg bw/day for three months. This dose proved lethal to all animals in this dose

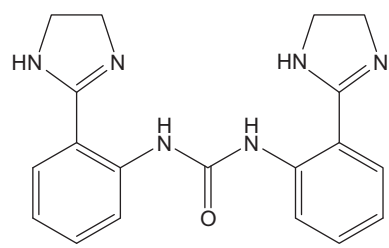

Figure 12.18 Chemical formula of imidocarb.

group. Animals given 250 mg/kg bw/day or less survived, but all treated rats had lower body weights than control animals. At termination, there were reductions in prostate weights, increased adrenal and thyroid weights in males and increased adrenal weights in females. Another study in rats made use of dietary administration with achieved doses of 26, 75 and 420 mg/kg bw/day in males and 32, 100 and 550 mg/kg bw/day in females. No signs of toxicity were seen in these animals but one high dose male died, as did one control female. Mild cholestasis occurred in the livers of high dose animals. Dogs given 80 mg/kg bw/day imidocarb in a 90 day study died or were humanely killed. These animals had signs of an anticholinesterase effect. Animals given 5 or 20 mg/kg bw/day survived until the end of the study period. The main changes found at termination included increases in the absolute and relative weights of the kidneys, adrenals and thyroid. In the high dose animals that died, livers showed haemorrhagic necrosis, fatty change and hepatocyte vacuolation. Monkeys given 5 mg/kg bw/day imidocarb showed no signs of toxicity and no compound related changes on gross examination.[373]

Imidocarb was foetotoxic in rats at a dose of 135 mg/kg bw/day in a study of reproductive performance. A dose of 45 mg/kg bw/day had no adverse effects. A dose of 14 mg/kg bw on the first day of pro-oestrus and then again immediately after mating, had no effects on fertility in dogs. Imidocarb was foetotoxic in rats and rabbits, but not teratogenic. It was tested in a wide range of genotoxicity assays and it gave negative results in the majority of these, although it produced a positive result in a test for chromosomal aberrations in human peripheral lymphocytes *in vitro*. However, the overwhelming evidence is that the drug is not genotoxic. A long-term toxicity/carcinogenicity study in rats was marred by poor survival of the experimental animals. Only 9 of 65 male animals given the highest dose of 240 mg/kg bw/day survived until termination. Survival was better in high dose females. At termination, high dose males had a higher incidence of cutaneous fibromas and high dose females had a higher incidence of mammary fibroadenomas. This study was generally poorly reported and the results of the histopathological findings were re-evaluated. This re-evaluation revealed that rats of each sex given the highest dose had an increased incidence of fibrosarcomas that was not statistically significant. Overall, there was no compelling evidence for a carcinogenic effect of imidocarb in rats.[373]

There are no reports of effects in humans with imidocarb and no evidence of extensive use in human medicine. In veterinary use, animals overdosed with imidocarb accidentally or experimentally showed signs of an anticholinesterase effect with excessive salivation, serous nasal discharge and dyspnoea. Hepatocellular necrosis was reported in cattle.[374–377] In dogs and horses given large overdoses of imidocarb, deaths occurred and the main findings were hepatic necrosis.[378,379]

The major problem for imidocarb and its veterinary use is the length of the product's withdrawal period. In the EU, the MRL values for imidocarb were established at 300, 50, 2000 and 1500 $\mu g\,kg^{-1}$ for bovine muscle, fat, liver and kidney, respectively, and 50 $\mu g\,kg^{-1}$ for bovine milk. These MRLs, which are in

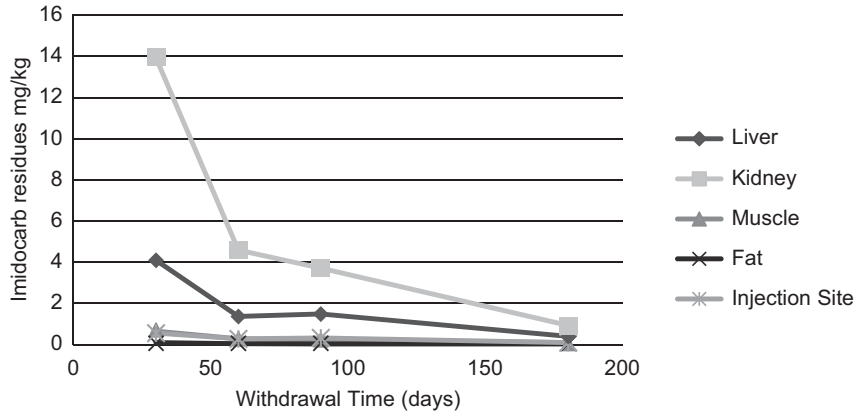

Figure 12.19 Depletion of imidocarb in cattle tissues.

no way remarkable, mean that the product containing imidocarb has a very long withdrawal period, *i.e.* the time that must elapse between treatment of the animal and when it may safely be slaughtered for human consumption. Taking the UK as an example, there are approximately 145 products intended for use in cattle that have a short to medium meat withdrawal period (1 to 35 days) and the average withdrawal period is 17 days.[380] There is then around 33 products that are long acting depot injection formulations, oral boluses or products with a longer withdrawal period. For these, the average withdrawal period is 71 days. However, the pharmacokinetics of imidocarb are such that residues depletion, and notably residues depletion to below the respective MRL values for muscle, fat, liver and kidney, is very slow. This can be seen from Figure 12.19[381] and the withdrawal period for this product is 213 days.

This does present farmers with some particular difficulties. It means that once treated, cattle can then not be sold on for human consumption for over seven months. Should an animal sustain an injury that requires humane slaughter, it cannot enter the human food chain until the withdrawal period had elapsed. This means that treating animals for the prevention of babesiosis cannot be undertaken lightly as there are practical and economic considerations to be considered.

12.2.14 The Trypanocidal Drugs – Isometamidium and Diminazene

These drugs are used in areas of the world where diseases caused by trypanosomes are endemic. Hence, they are not used in most areas or Europe or North America. Consequently, EU MRLs have not been established for these drugs. However, they play a major role in animal welfare and food economics in those parts of the world where trypanosomiasis is a problem, such as in many parts of Africa.

Isometamidium (as the chloride) is a phenanthridinium derivative (Figure 12.20) for the treatment of disease caused by *Trypanosoma congolense*, *T. vivax*, *T. brucei* and *T. evansei*. It is used mainly in cattle, but also in sheep, goats, buffalo, horses, donkeys, camels and dogs.[382–384] *In vivo* it is converted in part to ethidium (homidium) a compound that in its own right has been used to treat trypanosomiasis. Ethidium is a DNA intercalating agent that is also used in combination with a fluorescent moiety as a biological stain. In fact ethidium (Figure 12.21) belongs to a class of compounds that bind to DNA,[385,386] and specifically to the minor groove of DNA. Some of these compounds have therapeutic properties but they may also be genotoxic.[387,388] Binding of ethidium to DNA can cause changes in its topology and result in mutagenic effects.[387,389–401]

Isometamidium resulted in frameshift mutations in *Salmonella typhimurium* strains TA 1537, TA 1538 and TA 98 in the presence of metabolic activation.

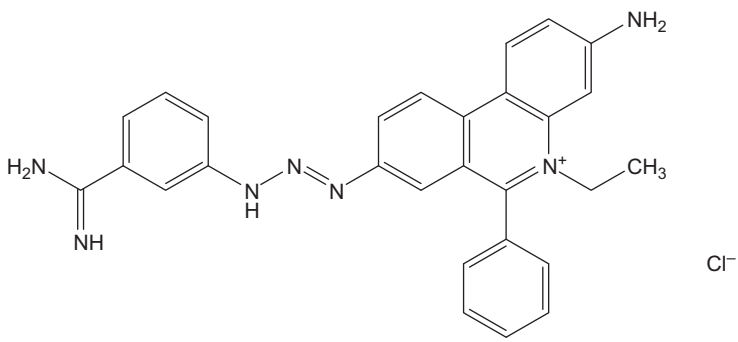

Figure 12.20 Chemical formula of isometamidium.

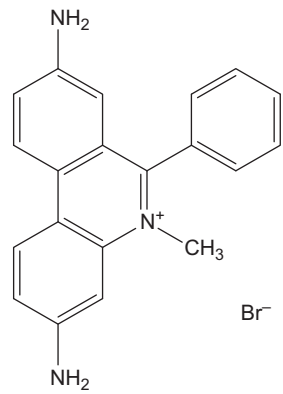

Figure 12.21 Chemical formula of ethidium.

It induced significant increases in the numbers of chromosomal aberrations in an *in vivo* cytogenetic assay in rats.[386,402] A further *Salmonella* reversion assay also gave positive results. However, the compound gave negative results in an *in vitro* cytogenetics assay with cultured human lymphocytes, in a test for gene mutation in mouse lymphoma L5178Y cells, in an *in vitro* cell transformation test, in an *in vivo* cytogenetics assay with rat bone marrow and in two nuclear anomaly assays in the rat. Another rat bone marrow cytogenetics assay gave equivocal results. The data suggest that overall, despite some of the tests mentioned above no longer being considered valid, that isometamidium is weakly genotoxic, probably as a result of conversion to the more reactive metabolite.[403]

Isometamidium was moderately toxic with an oral LD_{50} value of 455 mg/kg bw in the rabbit. In a short repeat dose study in the dog with a total dose of 20 mg/kg bw/day, intravenous administration resulted in vomiting, ataxia, defecation, lacrimation and salivation within 1 to 5 minutes of administration. At necropsy, the main findings were pigment deposition in the liver and kidney. In an extremely limited study using one cynomolgus monkey given daily doses of 2 mg/kg bw/day for 10 days and one cynomolgus monkey and one rhesus monkey given a single injection of 2 mg/kg bw followed by nine daily injections of 4 mg/kg bw/day intravenously, some hepatic necrosis was evident at necropsy and there were changes in the bone marrow. In more conventional repeat dose studies, rats were given gavage doses of 50, 225 or 1000 mg/kg bw/day isometamidium for 13 weeks. Immediately after dosing, all animals showed salivation, hair loss and respiratory distress that was severe in those in the two highest dose groups. A number of high dose males and females died by week 3 and the remainder were humanely killed. Caecal distention was the main effect found at necropsy. There was mild hyperplasia of the caecal mucosa and some animals showed increased splenic weights. No carcinogenicity studies have been conducted with isometamidium.[402,403] In mice, the acute toxicity of isometamidium was reduced by administration of atropine.[404]

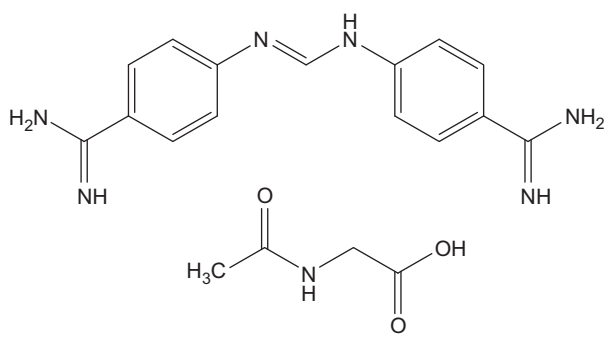

Figure 12.22 Chemical formula of diminazene aceturate.

Diminazene aceturate (Figure 12.22) is a dibenzamidine derivative which is active against a range of trypanosomes and other species in a number of animals including cattle. It is also an effective treatment for babesiosis.[405–413] The toxicity of diminazene is poorly documented. Unlike isometamidium, it is not a DNA intercalating agent. However, it does bind to trypanosome DNA but the genotoxic significance of this, if any, is not clear.[414–422] It induced respiration deficient petite mutations in yeast in a manner similar to that of isometamidium by binding to yeast mitochondrial DNA.[423–425] It was not genotoxic in the mouse micronucleus test.[426] In more recent studies, diminazene gave negative results in the *Salmonella* reversion assay, the mouse bone marrow micronucleus test and a forward mutation assay in hamster V79 cells at the HGPRT locus.[427] However, diminazene induced micronuclei in L5178Y mouse lymphoma cells,[428,429] while in a study which used binucleate and mononucleate lymphocytes in the cytokinesis block micronucleus assay, diminazene induced micronuclei in both cell types and the data suggested that the drug was a potent aneugen.[430]

Diminazene is known to be a chromatin-decondensing agent which affects the distal chromatin block of the long arm of the Y chromosome in human lymphocytes and, as already described, disrupts the curvature of DNA by binding to its minor groove. In doing so, it also disrupts the cell cycle at the G2 phase resulting in polyploidy.[431–433] This activity in disrupting mitosis is probably responsible for its activity against trypanosomes.[434]

Like isometamidium, it has not been tested in rodent carcinogenicity bioassays. It has been tested in teratogenicity studies in rats and although it produced maternotoxicity and embryotoxicity, there was no evidence of teratogenic effects.[427,435]

In humans, diminazene is known to cause pyrexia, nausea, vomiting, paralysis and pains in the soles of the feet. A study of 99 patients who had been treated with diminazene 12 to 109 months earlier for trypanosomiasis were traced and subjected to a medical examination. The patients had each received three doses of 5 mg/kg bw at one or two day intervals. No adverse effects were noted in any of these patients.[436] Signs of toxicity including ataxia, hippus and nystagmus were seen in a dog treated therapeutically with diminazene aceturate which was also given a similar drug, phenamidine isethionate.[437] The effects were caused by an overdose arising from the combination of the two agents. Signs of neurotoxicity had been observed in repeat dose studies with diminazene in dogs. These were accompanied by haemorrhagic necrosis in the brain stem and cerebellum that were thought to have been due to necrosis of capillaries and arteries.[427]

To investigate the potential harmful effects of isometamidium on consumer safety, a single calf was treated with a combination of 45 mg [^{14}C]-isometamidium plus 73 mg of unlabelled drug (1 mg/kg bw isometamidium). Tissues from the calf had no detectable radioactivity and when these were fed to rats, there were no discernible effects and no adverse findings on gross examination at necropsy.

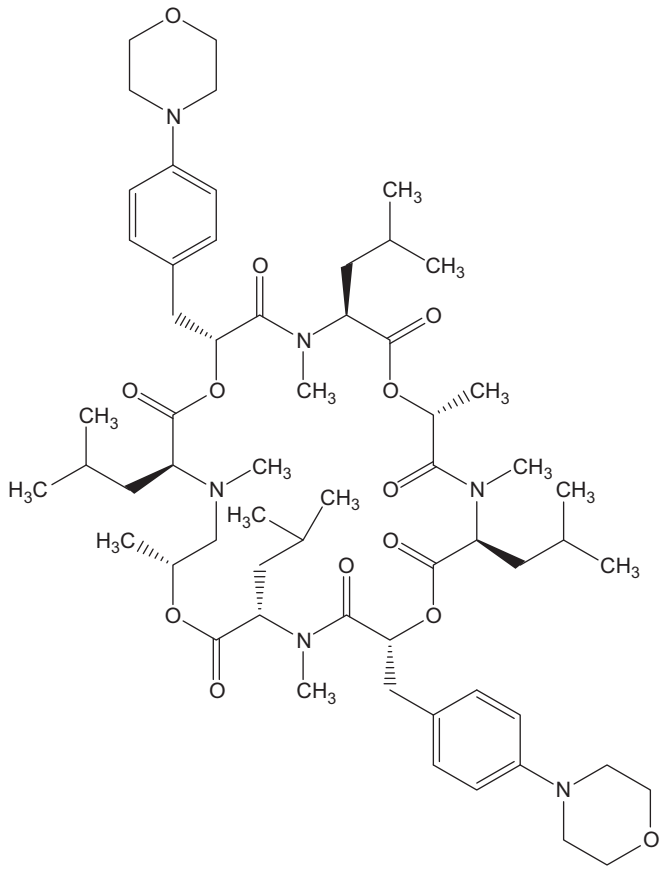

Figure 12.23 Chemical formula of emodepside.

12.2.15 Emodepside

Emodepside (Figure 12.23) is a member of a new class of anthelmintic drugs, the cyclooctadepsipeptides.[439] The drug paralyses parasites through activity at the neuromuscular junction and through actions on SLO-1, a gene that encodes for calcium activated potassium channels in the parasite. This results in hyperpolarisation of the body wall muscle.[440–444] It is used in combination with toltrazuril for the treatment of certain parasitic diseases in dogs and cats. It is effective against certain nematode species including *Toxocara canis* and against some species of coccidia including *Isospora canis*.[445–448]

There is very little in terms of toxicity data in the published literature. Emodepside has low acute oral toxicity in rats and mice but where toxicity was observed clinical signs included ataxia, piloerection, decreased motility and dyspnoea. Repeat dose studies in rats and mice for up to 17 weeks

resulted in reduced weight gain and signs of neurotoxicity including ataxia. In rats the NOEL was in the region 0.73 to 1.11 mg/kg bw/day and in mice 10.5 to 16.8 mg/kg bw/day. In dogs given oral doses of up to 20 mg/kg bw/day for 4 weeks, the major effect was a reduction in food intake in females. At doses of 10 or 20 mg/kg bw/day, vomiting and tremors/ataxia occurred in males, and females had tremors/ataxia with staggering and incoordination. The NOEL was 5 mg/kg bw/day. In reproduction studies in rats, emodepside produced evidence of maternotoxicity. Moreover, some pups had uncoordinated gaits and protruding eyes. The lowest NOEL was 0.8 mg/kg bw/day. It was not teratogenic in the rat or rabbit and the overall NOEL for teratogenicity studies was 0.5 mg/kg bw/day. Emodepside produced no evidence of genotoxic effects in a range of studies.[449] Carcinogenicity studies with emodepside have not been conducted but on the basis of its chemical structure and the results of genotoxicity studies, it would not be expected to be carcinogenic.

There are no reports of adverse events in exposed humans. It has been reported to have induced a morphea-like lesion (localised scleroderma) with alopecia in a cat given a topical treatment of emodepside plus praziquantel.[450] The lesion resolved after about 60 days following treatment with minoxidil for the alopecia.

12.3 Conclusions

The antiparasitic drugs are valuable tools in the therapeutic arsenal required to treated animals infected with helminths, flukes, and protozoa and with other organisms. Some of these drugs are undoubtedly potentially toxic, but usually at doses that exceed the therapeutic dose ranges for each drug. The main concern must be consumer safety since few of these drugs appears to pose a major user safety issue, or at least they do not generate reports of adverse effects as a result of occupational exposure. As is evident from Table 12.1, the establishment of MRLs in the EU has considered many aspects of the toxicity of these drugs when calculating ADI values on which the MRLs are based.

Perhaps the most controversial issue involved in the consideration of MRLs for these agents was the decision to prohibit the use of the nitroimidazoles in food animals. It can be argued that the evidence in terms of genotoxicity and carcinogenicity is hardly compelling and one of these drugs, metronidazole, is still widely and routinely used in human medicine. Moreover, there is ample evidence from studies of bound residues to support the argument for safety. On the opposite side of that argument is the view that while there are clear benefits to the human patient from treatment with metronidazole, that person also carries the burden of any risk. The same benefits apply to the veterinary patient but here potential risks are born not only by the patient but also by consumers. In the circumstances, the precautionary approach is probably reasonable.

Table 12.1 NOEL and ADI Values Forming the Basis of EU MRLs for Antiparasitic Drugs.

Drug	*Basis for NOEL and (NOEL)*	*Safety Factor*	*ADI (mg/kg bw/day)*
Albendazole	Teratogenicity in rats and rabbits (5 mg/kg bw/day)	1000[a]	0.005
Ricobendazole	As albendazole[b]	1000	0.005
Netobimin	As albendazole[b]	1000	0.005
Oxfendazole	Hepatic vacuolation in rat carcinogenicity study (0.65 mg/kg bw/day)	100	0.7
Fenbendazole	As oxfendazole[c]	100	0.7
Febantel	As oxfendazole[c]	100	0.7
Mebendazole	Effects on haematology and blood chemistry in 13 week oral dog study; teratogenic effects in rats (2.5 mg/kg bw/day)	200[d]	0.0125
Oxibendazole	Differences in haematocrit in a 98-day rats study (30 mg/kg bw/day)	500[e]	0.06
Thiabendazole	Based on a range of end-points – effects on liver, spleen, thyroid, reproductive toxicity and teratogenicity (10 mg/kg bw/day)	100	0.1
Levamisole	Haemolytic anaemia in dogs (1.25 mg/kg bw/day)	200[f]	0.006
Closantel	Testicular effects in a 13 week study in rats (2.5 mg/kg bw/day)	100	0.03
Oxyclozanide	Brain cell vacuolation in brains of dogs in a 13 week oral study (5 mg/kg bw/day)	200[g]	0.03
Rafoxanide	Vacuolation in the optic nerves and CNS in a 13 week dog study (0.4 mg/kg bw/day)	200[h]	0.002
Niclosamide	N/A[i]	–	–
Clorsulon	Urinary bladder hyperplasia in rat 13 week study. No clear NOEL (LOEL 0.2 mg/kg bw/day) [j]	200	0.001
Pyrantel	Slight haematological changes in rats and hepatic changes dogs in 2 year studies (3 mg/kg bw/day)	100	0.012[k]
Morantel	Minor hepatic changes in 2 year dog study (1.2 mg/kg bw/day)	100	0.012
Oxantel	N/A[l]	–	–
Praziquantel	No details given; based on rat 4 week oral study (33 mg/kg bw/day)	200[m]	0.17
Derquantel	Effects on nictitating membranes in 90 day dos study (0.1 mg/kg bw/day)	100	0.001
Monepantel	Increased alkaline phosphatase and increased liver weights in 1 year dog study (3 mg/kg bw/day)	100	0.03
Piperazine	Minor changes in liver enzymes 13 week toxicity study in dogs (25 mg/kg bw/day)	100	0.25

Table 12.1 (*Continued*)

Drug	*Basis for NOEL and (NOEL)*	*Safety Factor*	*ADI (mg/kg bw/day)*
Diethylcarbamazine	N/A[i]	–	–
Nitroxynil	Effects on thyroid hormone levels and thyroid morphology in a 90 day rat study (0.5 mg/kg bw/day)	100	0.005
Halofuginone	Effects in 3-generation rat study and teratogenicity study in rabbits	100	0.0003
Dimetridazole	N/A[n]	–	–
Ronidazole	N/A[n]	–	–
Ipronidazole	N/A[n]	–	–
Metronidazole	N/A[n]	–	–
Imidocarb	Hepatic necrosis, fatty change and vacuolation of hepatocytes as marginal effects at all dose levels in 90 day dog study (LOEL 5 mg/kg bw/day)	500[o]	0.01
Isometamidium[16]	Effects on the caecum of rats in a 13 week toxicity study (50 mg/kg bw/day)	500[p]	0.1
Diminazene[16]	Effects including foci of softening in the brain and testicular atrophy in a 9 month dog toxicity study (20 mg/kg bw/day)	200[q]	0.1
Emodepside	N/A[i]	–	–

[a]Safety factor of 1000 used to take account of the severity of the effect.
[b]As albendazole, ricobendazole and netobimin are interrelated metabolically.
[c]As fenbendazole, oxfendazole and febantel are interrelated metabolically.
[d]Normal safety factor of 100 increased by a factor of 2 due to inadequacies in the dog study.
[e]Safety factor increased by a factor of 5 due to induction of polyploidy.
[f]Safety factor increased by a factor of 2 due to concerns over human susceptibility to levamisole-induced haemolytic anaemia.
[g]Safety factor increased by a factor of 2 due to the small difference between the NOEL and the therapeutic dose which caused side-effects in target animals.
[h]Safety factor increased by a factor of 2 due to the severity of the effects.
[i]Not used in food animals in the EU or in most other countries.
[j]Marginal effects were seen at the lowest dose so lowest effect dose level (LOEL) identified and safety factor increased by a factor of 2.
[k]Recognised that the metabolism of pyrantel and morantel are similar so ADI derived for morantel was also used for pyrantel.
[l]Not used in food animals, at least in the EU.
[m]Safety factor increased by a factor of 2 as the repeat dose toxicity study which formed the basis of the ADI was of only 1 month duration.
[n]Concerns over mutagenicity and carcinogenicity so ADI cannot be established. Prohibited for use in food animals in the EU and in some other countries.
[o]No clear NOEL and so LOEL used with an increased safety factor of 5.
[p]Drug not used in the EU so no MRL required. Values here are taken from the evaluation by the Joint FAO/WHO Expert Committee on Food Additives (JECFA).
[q]A marginal pharmacological effect was seen at the lowest dose in the rat study and this, with the limited extent of the data available meant that the safety factor was increased by a factor of 5.
[r]The safety factor of 200 compensates for the inadequacies in the design of the dog study from which the NOEL was derived.

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CHAPTER 13

Some Other Pharmacologically Active Drugs

13.1 Introduction

In addition to the major classes of drugs discussed elsewhere in this book, a variety of other agents are used in companion and farm animal veterinary medicine. Some of these drugs may be used only infrequently while others may either be in more regular use or may pose sufficient hazard to human health if used improperly. The drugs that fall into the latter category, more frequent use or potential hazard to human health, will be discussed in this chapter.

13.2 Opiates and Synthetic Opiates

13.2.1 Etorphine

A number of opiates are used in companion and farm animal practice and of these, one of the most interesting from a toxicological and pharmacological viewpoint is etorphine. Etorphine (Figure 13.1), often in combination with other drugs such as xylazine, acepromazine or thiopentone, has been used as an analgesic or immobilising agent or as a capture drug for wildlife. It was originally synthesised from oripavine but it can also be derived from thebaine.[1–3] It induces catatonia and it is used to immobilise a wide range of game species including giraffes, various deer species, muskoxen, elephants, bears, buffalo and wildebeest.[4–16] Although it has a good safety profile, in horses and donkeys it causes a dramatic rise in blood pressure and heart rate, with muscle tremors although this can be controlled to an extent with thiopentone.[17–19] Fatalities may occur following use in deer, and it has led to hyperventilation, hypoxaemia

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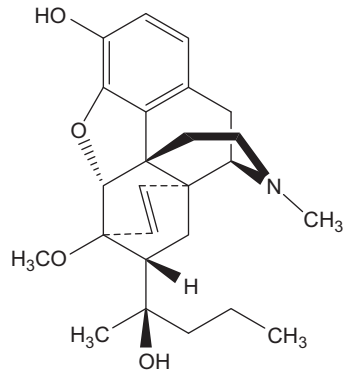


Figure 13.1 Chemical formula of etorphine.

and metabolic acidosis in the rhinoceros. Its use in pigs and wolves has had mixed results.[20–23] The drug is also used in small animal medicine as a sedative.[4,24–26]

The product itself is supplied as an aqueous solution, which also contains acepromazine (*Large Animal Immobilon*) or with methotrimeprazine (*Small Animal Immobilon*). Its action can be reversed by the antagonists naloxone and nalorphine, but also with the specific antagonist, diprenorphine (*Large* and *Small Animal Revivon*).[27–29] Depending on the species and the nature of the test, etorphine is 1000 to 80 000 times more potent than morphine.[4] In humans, the lethal dose through accidental injection is estimated to be between 30 and 120 μg.[30]

There have been reports of accidental self-injection of etorphine which were successfully treated with the use of the antagonists.[31–35] These incidents involved skin penetration with a wet needle rather than the delivery of any significant amount of drug and the main signs and symptoms reported including "feeling very ill" and a dramatic fall in blood pressure.[33,32,35] Following one incident where a wet needle injury resulted in the death of a veterinarian, the UK's expert independent advisory body, the Veterinary Product's Committee (VPC), recommended the suspension of the product licence for this product.[36,37] The VPC subsequently went on to make a number of recommendations for strengthening the labelling warnings and the use of naloxone or nalorphine as reversing agents or, if not available, the use of the animal reversing agent *Revivon*. It also made recommendations about the safe use of the product as some of the wet needle incidents had arisen while carrying loaded syringes inappropriately, for example, in a pocket. It recommended that rather than being affixed to the syringe, the needle itself should be inserted into the animal and the syringe then connected while the needle used to withdraw the product from its vial is safely disposed of. The drug is then administered and the syringe and needle disposed of safely. It also recommended that the drug only be used in the presence of an assistant capable of administering the

antagonist, that this assistant be properly briefed on what to do should an accident occur and that a stock of naloxone or *Revivon* be available whenever the drug was to be used. Cardiac massage may also be needed.[38] However, it should be noted that diprenorphine itself has agonist properties and so it should only be used in humans if drugs such as naloxone are not immediately available.[39] In a case of intentional self-injection with *Large Animal Immobilon*, cardiorespiratory collapse occurred. The patient was resuscitated with reversing agent and provided with haemodynamic support.[40]

At low oral doses (25 μg, 50 μg and 100 μg per person) in humans, etorphine produced pupillary constriction and euphoria.[41] It was found to control cancer pain at 1 $\mu g\,kg^{-1}$ bw intramuscularly. In a study of 32 cancer patients, the therapeutic oral dose for pain relief was between 50 and 400 μg, with 100 μg the most commonly used dose. The main side effect was sedation.[43]

It has been suggested that etorphine is a drug suitable for transdermal delivery.[44] However, it is poorly absorbed through the skin. Despite this, a number of adverse effects have been reported following skin contamination (or possibly, injection).[45–48] Some of these may arise from psychological stress because of the publicity and "fear factor" surrounding the drug.[49]

In animals, etorphine has produced effects suggestive of an ability to induce drug dependence.[50] Concern had been raised over its abuse potential.[51,52] Animal studies also suggest that etorphine may enhance contact hypersensitivity to other chemicals.[53] As etorphine has now largely been replaced in veterinary medicine by other drugs or combinations of drugs, these fears are likely to remain unrealised.

13.2.2 Butorphanol

Morphine produces its major affects in the central nervous system through its actions on μ receptors. Along with other μ-opioid (μ-subtype) agonists, it induces profound analgesia as well as drowsiness, mood changes, depression, nausea and reductions in gastrointestinal activity. Morphine is relatively specific for the μ receptors, while etorphine, which is non-selective, can act on others including κ and δ receptors. All the opiates can produce a wide range of effects, but these effects are determined by the receptors on which they act and their specificity.[54–56] Acute opioid toxicity can be attributed to a variety of effects and it results in sedation and, at high doses, coma. Respiratory rate is depressed and cyanosis frequently occurs. Blood pressure may decline dramatically. These effects conspire to produce hypoxia and eventually capillary damage and shock. Death is usually due to respiratory failure and its complications.[54]

Butorphanol (Figure 13.2) is a mixed receptor agonist with partial μ receptor agonist activity and κ actions.[54] It is of low toxicity and is considered to have a low potential for abuse.[57] It is used in veterinary medicine for sedation, immobilisation and analgesia for laboratory, companion and exotic animals such as zebra; it is also used in horses.[58–63]

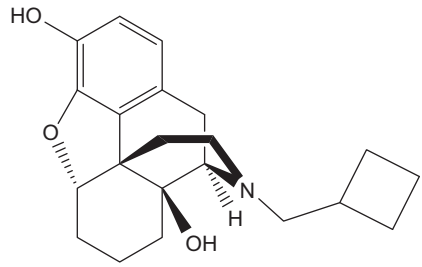


Figure 13.2 Chemical formula of butorphanol.

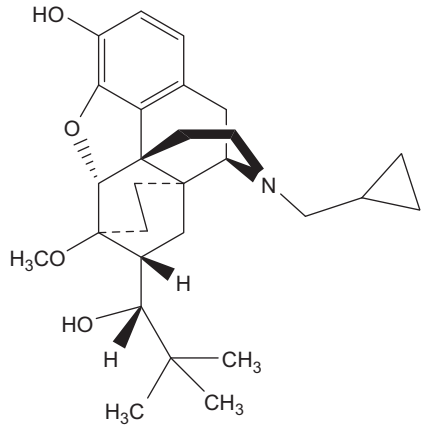


Figure 13.3 Chemical formula of buprenorphine.

Intramuscular injection of a small amount of a mixture of detomidine and butorphanol by a 36 year-old male produced no major effects and the patient recovered uneventfully.[64] However, when a veterinarian contaminated his hands with detomidine and butorphanol he developed acute poisoning, probably as a result of the combined effects of both agents. Although this drug combination is believed to be poorly absorbed through skin, the affected individual had dermatitis, a common condition in veterinarians, which probably facilitated skin absorption. The patient recovered with supportive care.[65]

13.2.3 Buprenorphine

Buprenorphine (Figure 13.3), like etorphine is also a derivative of oripavine. In humans, it produces analgesia and other CNS effects that are similar qualitatively to those of morphine although it is significantly more potent than this drug. It is a κ antagonist and a μ partial agonist/antagonist, with some δ

antagonist activity.[54,59,66–68] In veterinary medicine it is used for its analgesic effects and it is one of the most commonly used opioid analgesics in companion animal medicine, probably due to its pharmacological effects being of longer duration than those of morphine.[59,69–71]

There have been no reports of adverse health effects in humans due to the use of buprenorphine in veterinary medicine or because of the misuse or abuse of veterinary formulations. However, buprenorphine, like other opioids can produce respiratory depression and it may have some, albeit low, potential to affect the QT-interval.[72–74] Buprenorphine, with naloxone, is used in the treatment of opioid addiction in humans.[75–80] This has occasionally led to toxicity, especially in paediatric patients. Overdosage with buprenorphine in children produced lethargy, miosis, vomiting, respiratory depression, agitation or irritability, pallor and coma. Doses of 2 to 4 mg produced clinical effects that persisted from 2 to 24 hours or more. Fractions of a dose of buprenorphine and naloxone in a child resulted in emesis and drowsiness but there were no other clinical effects.[81–84] In one study, ingestion of buprenorphine/naloxone accounted for the largest number of unintentional ingestions among patients younger than 3 years. The affected patients showed drowsiness, lethargy, miosis and depression.[85] Higher doses of buprenorphine in adults have been associated with hepatic injury and nephrotoxicity.[86–88] Fatalities have occurred due to "snorting" of buprenorphine with alcohol consumption.[89] There are no indications based on organ systems and antibody measurements in experimental animals, that buprenorphine is immunotoxic.[90]

Clearly, the normal clinical use of buprenorphine in veterinary medicine is associated with low risks to humans but it does possess the potential for toxicity especially if inadvertently ingested by young children or if subjected to misuse or abuse.

13.2.4 Fentanyl

Fentanyl (Figure 13.4) is a synthetic compound without much apparent structural similarity to the opiate drugs. It is a potent agonist for the μ receptor, although other receptor populations may be involved in its actions.[91] In human

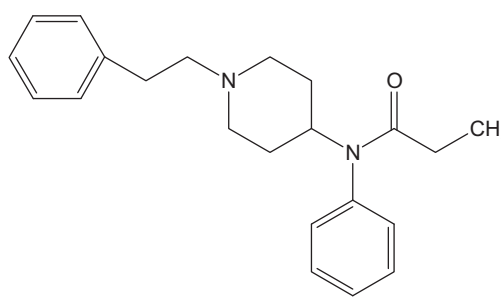


Figure 13.4 Chemical formula of fentanyl.

medicine, it has been used for the control of severe pain including breakthrough pain that is a significant problem in the control of cancer pain.[54] In companion animal medicine, it is used to induced neuroleptanalgesia in combination with droperidol.[59]

In humans, rats and dogs, fentanyl produces respiratory depression and declines in blood pressure; it also may decrease oxygen partial pressure and increase that of carbon dioxide, at least in rats.[74,92,93] At high doses in dogs, fentanyl produces convulsions and toxicity arising from effects on metabolism.[94,95] In humans, fentanyl is frequently administered as a transdermal patch for the treatment of chronic pain and these patches may occasionally result in life-threatening toxicity and fatalities as a result of abuse or misuse.[96] There is no doubt that fentanyl is subject to abuse, and this is exacerbated or least facilitated by fentanyl patches which contain more drug than is actually required to ensure sufficient dermal absorption.[97] Thus patches have been known to be ingested and even inserted rectally.[98–105] In one incident, the patch was used, rather like a tea bag, to produce an infusion for oral ingestion.[97] The abuse of patches can result in fatalities.[97,98,102] Overdoses of fentanyl in the Chicago and surrounding areas resulted in 342 deaths between April 2005 and December 2006.[106] Pulmonary oedema and congestion are typical post-mortem findings and ethanol may be a significant risk factor in fentanyl-associated deaths.[107] Occasionally, fatalities associated with fentanyl abuse are suicides rather than accidental occurrences.[108,109]

There is also concern over occupational exposure to fentanyl. Fentanyl contamination and urinary excretion has been noted in pharmaceutical industry employees although no sings of toxicity were reported.[110] Exposure and toxicity may occur in health care workers.[111,112] There is also concern over exposure to fentanyl in operating rooms, and the phenomenon of second-hand exposures to fentanyl, with ensuing addiction in health care workers, and especially physicians, anaesthetists and surgeons where there is a recognised problem of drug abuse.[112–116]

In clinical use in humans, fentanyl is regarded as a safe drug but toxicity may occur occasionally although somnolence is the major effect reported.[117–120] Despite widespread use of intrathecal administration, fentanyl has not been reported to produce extensive toxicity by this route.[121] It is metabolised by way of cytochrome P450 3A4 and drugs which inhibit this can potentiate fentanyl toxicity in patients.[122,123] Fentanyl precipitated serotonin toxicity due to a drug interaction with the selective serotonin reuptake inhibitor, paroxetine.[124] As a result of concerns over reproductive effects of fentanyl in healthcare workers and patients, it has been subjected to a number of teratogenicity studies, either alone or with nitrous oxide. It produced no evidence of teratogenic effects in studies in mice and rats.[125–127]

In veterinary medicine, the drug is given intravenously, intramuscularly or subcutaneously to control pain in companion animals. The fentanyl transdermal patch is not authorised or approved but the human product may be used off-label in cats and dogs. If the patch is prescribed to companion animals it is recommended that it is applied and removed by the veterinarian to prevent

client exposure, and that steps be taken to prevent abuse by the animal owner *e.g.* collection of patches from the home.[59]

13.3 Euthanasia Agents

The major euthanasia agents used in veterinary medicines are the barbiturates and these have already been discussed in Chapter 5. When formulated for euthanasia, these products, and indeed other products used for this purpose, are not normally sterile unlike other parentally, and especially intravenously, administered formulations as this is considered unnecessary as the patients concerned will not recover from the effects of the drug and so the secondary effects of bacteria and other extraneous agents are irrelevant. The products are often frequently formulated with a dye to distinguish them from other similar but sterile formulations. Euthanasia agents differ from all other veterinary medicinal products in pharmacovigilance terms in that lack of efficacy, that is failure to cause death of the patient, is a reportable adverse event (lack of expected efficacy).

As described in Chapter 1, veterinarians, along with other healthcare workers, are considered to be at higher risk of suicide than other professional groups. Veterinarians may suffer from higher incidences of depression and they may have major concerns about their choice of career while supportive structures for advice and counselling are poor.[128] Veterinarians also have access to means of suicide including firearms and potentially toxic drugs, and euthanasia agents are an obvious choice. Barbiturates intended for euthanasia have been implicated in suicides, including those by veterinarians.[129] However, barbiturates are also a popular means of suicide by physicians.[130]

Embutramide, mebenzonium iodide and tetracaine hydrochloride (Figure 13.5) are the components of a product known as *Tanax* or T-61. This is an effective euthanasia agent, as embutramide induces deep anaesthesia, mebenzonium causes curariform paralysis of muscles, including those involved in breathing, and tetracaine relieves pain at the injection site as well as being toxic in its own right, at least at the concentrations used in this product.[131,132] Tanax has been used in suicide attempts, including those by veterinarians, either by injection or by ingestion; some of these attempts were successful.[129,133–135] The product is formulated in dimethylformamide and this may induce severe liver toxicity, especially after ingestion.[136,137] However, in two veterinarians who attempted suicide with this product, one by injection and the other by ingestion, there were no signs of severe liver toxicity following 14 days of *N*-acetylcysteine administration although it was not clear as to how efficacious this agent was.[138] Both patients recovered.

13.4 Neuroactive Steroids

The neuroactive steroids or neurosteroids are a class of steroids structurally related to progesterone, which have anaesthetic properties.[139,140] In fact progesterone itself was shown to have anaesthetic and sedative properties in the

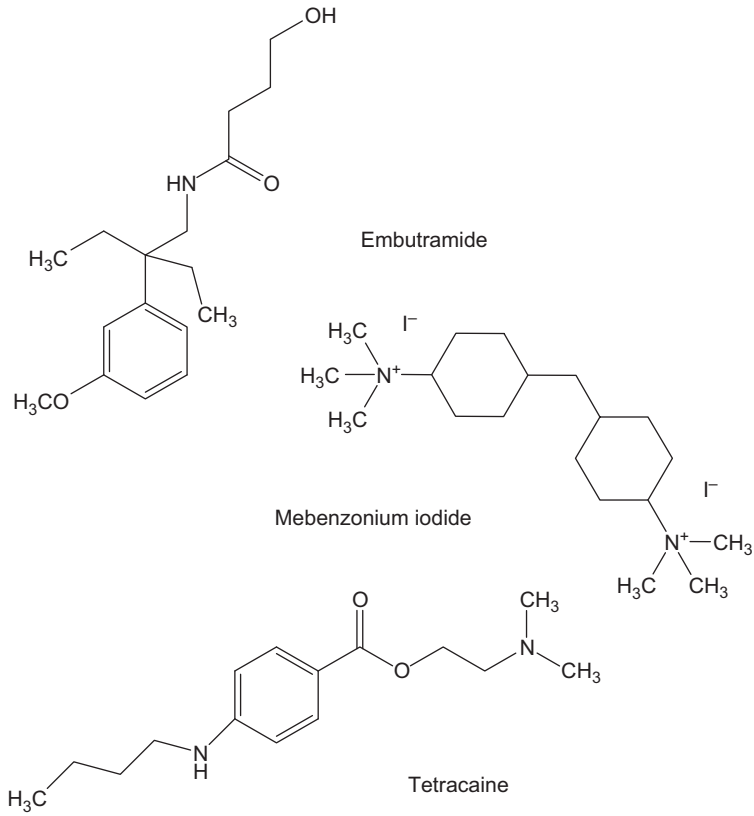


Figure 13.5 Chemical formulae of embutramide, mebenzonium iodide and tetracaine hydrochloride.

early 1940s.[141] Some of its metabolites, such as allopregnalone and pregnalone, also have activity. Unlike other steroids, these do not interact with cytosolic hormonal steroid receptors but instead are though to target γ-aminobutyric acid A ($GABA_A$) receptors and chloride channels in the central nervous system.[139,140] The activity of the endogenous neuroactive steroids spurred on an effort to discover synthetic analogues that could be used for clinical purposes.[142,143] One product which was developed was known as Althesin in human medicine and Saffan in veterinary medicine. It is a combination of two neuroactive steroids, alfaxolone and alphadolone acetate (Figure 13.6).

Saffan is an injectable anaesthetic for use in cats although it has also been used in other small animals. In cats, it may produce oedema of the ears and paws following administration.[144,145] However, the most serious effects were laryngeal and pulmonary oedema which were occasionally severe and resulted in the deaths of affected cats.[146–149] This appears to be due to the release of histamine or a histamine-like substance caused by the solubilising agent Cremophor EL, a polyethoxylated castor oil derivative used in the formulation.[147]

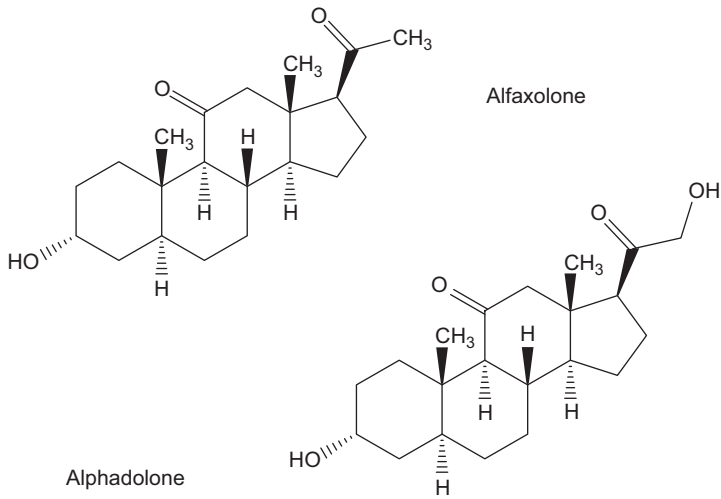


Figure 13.6 Chemical formulae of alfaxolone and alphadolone.

In humans, the product produces hyperpnoea on administration and apnoea following overdose. It also produces marked depression in cardiopulmonary function in humans and in other animals.[150–152] There are no reports of adverse reactions in humans following accidental self-injection or through any other routes of exposure and Saffan has largely been replaced by other injectable anaesthetics and notably by propofol (Chapter 5).

13.5 Sedative Agents

A number of sedative drugs are used in veterinary medicine, including drugs used prior to anaesthesia, for relieving stress, or in combination with analgesics. The major groups are discussed below.

13.5.1 α_2-Receptor Adrenergic Agonists

The imidazole drugs are used in veterinary medicine for sedation, either alone or in combination with other agents. The most important of these agents in veterinary medicine are detomidine, medetomidine, romifidine (Figure 13.7) and dexmedetomidine. These drugs contain the imidazole heterocyclic ring. Dexmedetomidine, which is also used in human medicine, is the *S* isomer of medetomidine. All of these agents are structurally related to the human drug clonidine (Figure 13.7). Xylazine (Figure 13.7) is not an imidazole drug and the imidazole ring is replaced by a dihydro-1,3-thiazine moiety. All of these drugs are α_2-receptor adrenergic agonists.

In humans, infusion of clonidine causes a rise in blood pressure and heart rate, possibly as a result of activation of post-synaptic α_2-receptors in smooth

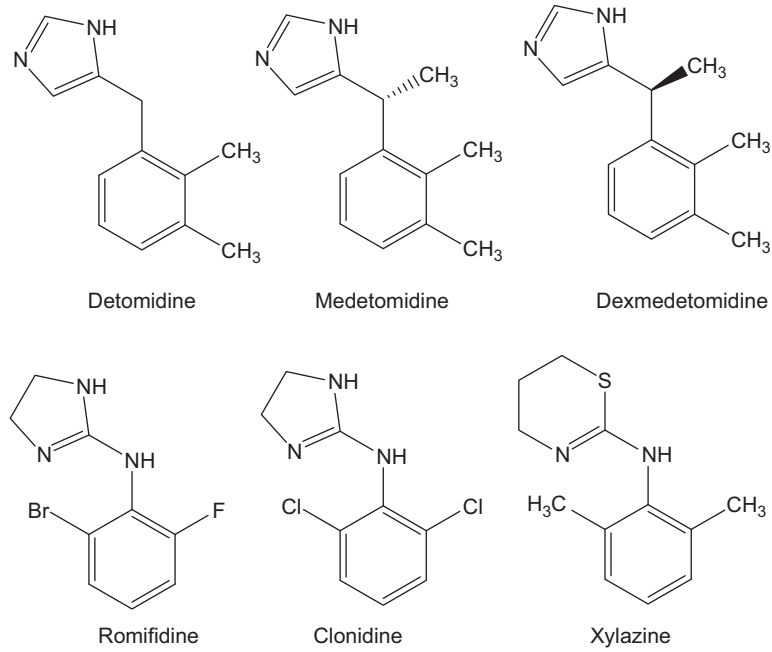


Figure 13.7 Chemical formulae of detomidine, medetomidine, romifidine, dexmedetomidine, clonidine and xylazine.

Table 13.1 Major veterinary therapeutic uses of the α_2-receptor adrenergic agonists.

Drug	*Major use*	*Species*
Detomidine		
Injection	Sedation, mild analgesia	Cattle, horses
Oromucosal gel	Sedation	Horses
Medetomidine	Sedation	Cats, dogs
Dexmedetomidine	Sedation	Cats, dogs
Romifidine	Sedation	Horses
Xylazine	Sedation	Cattle, horses, cats, dogs

muscle.[153] This is followed by a hypotensive response. The initial hypertension is not seen after oral administration. The major use of clonidine in humans has been in the treatment of hypertension although it has a range of other uses.[154] Clonidine and dexmedetomidine have been used prior to anaesthesia to increase haemodynamic stability, to induce sedation and to prevent excessive secretion.[155,156] In fact one of the major adverse effects of clonidine in humans is dry mouth and sedation.[154] In veterinary medicine, these drugs are used mainly as sedatives and mild analgesics (Table 13.1).[59,157–161] They are also used for other purposes, often in combination with other drugs. For example,

xylazine has been used in combination with etorphine as a capture drug.[9,10,15,16] Medetomidine is also used as an antifouling agent.[162–164]

In general, these agents have a safe history of use and human adverse drug reactions following exposure during veterinary use are rare. Acute poisoning in a veterinarian resulted from dermal exposure to a product containing detomidine and butorphanol. The affected individual had dermatitis of the hands and this almost certainly facilitated absorption of the drug. He recovered after supportive treatment.[65] Another patient suffered no major effects after intramuscular injection of a small amount of detomidine and butorphanol product.[64] (See also section 13.2.5.) However, concern has been expressed at the relatively large quantities of these drugs being used and the possibilities for human exposure, especially in zoos, and the availability of the α_2-adrenergic antagonist atipamezole has been recommended for these circumstances.[39] Other exposures to these drugs have resulted in eye irritation, bradycardia, somnolence and some non-specific effects.[165]

In humans, signs of toxicity following exposure to xylazine include depression, syncope, bradycardia and hypotension.[166,167] Most cases of poisoning, some involving farmers, arise from intentional self-administration. Patients usually fully recover with supportive treatment.[168–171] In one case, a 34-year-old man injected himself with an estimated 15 mg/kg bw xylazine. He was discovered 30 minutes later comatose and with apnoea. His blood pressure was 120/70 mm Hg and his heart rate 60 beats/minute. After two days in hospital he developed sinus tachycardia with multifocal premature ventricular contractions that were brought under control with lignocaine. The coma and depression lasted 60 hours. Owing to the marked respiratory depression noted, the author concluded that he would probably have died without medical intervention.[168] A veterinary nurse who accidentally injected himself with xylazine experienced hypotension, bradycardia and coma. He eventually made a full recovery with supportive care.[172] A 39 year old woman admitted to hospital with symptoms of faintness, blurred vision and tiredness was also found to have sinus bradycardia and a blood pressure of 130/90 mm Hg. She was found to have a urinary concentration of 1674 $\mu g\,L^{-1}$ xylazine and a serum concentration of 30 $\mu g\,L^{-1}$.[173] Systemic toxicity has been reported after ocular exposure to xylazine.[174] Severe toxicity may occur after inhalation exposure, a route of drug abuse with xylazine.[175,176] The drug has been implicated in suicides and in homicides.[176–178]

Atipamezole (Figure 13.8) referred to above is used to reverse the effects of these drugs in animals.[179–183] It has also been suggested as a treatment for poisoning by the formamidine insecticide amitraz. Amitraz is also an α_2-adrenergic receptor agonist with some structural similarities with clonidine (see Chapter 7) and it should be effective not only for the treatment of the effects of amitraz, but also for the reversal of the effects of the drugs discussed in this section. However, the drug has not been assessed for safety or effectiveness in humans.[184]

Xylazine generally has high toxicity in animal studies with oral LD_{50} values in the rat of 130 mg/kg bw and an intravenous LD_{50} value in the dog of 20–25.

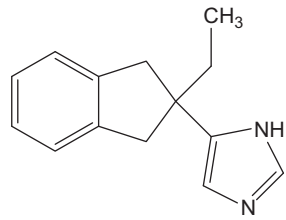


Figure 13.8 Chemical formula of atipamezole.

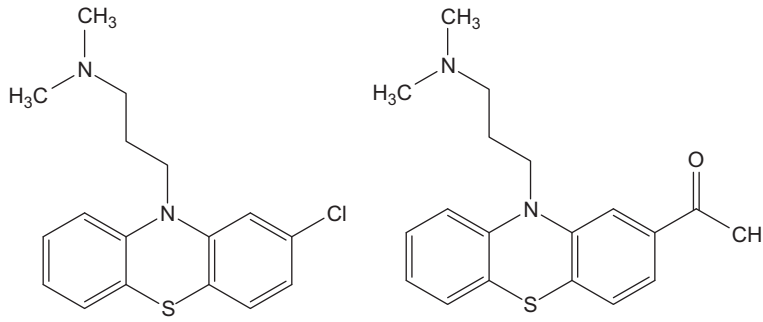


Figure 13.9 Chemical formulae of chlorpromazine and acepromazine.

The minimum lethal dose in the horse is between 15–28 mg/kg bw. Therapeutic doses range from 0.01 to 0.5 mg/kg bw intravenously and 0.05 to 2 mg/kg bw intramuscularly in a range of mammalian species. It gave negative results in a range of genotoxicity studies except for tests with *Salmonella typhimurium* strains TA 1535 and TA 1538 where weak positive responses were observed. Negative results were noted with strains TA 100, TA 98 and TA 1537.[185]

13.5.2 Phenothiazines

The two phenothiazine drugs that have been used in veterinary medicine are chlorpromazine and acepromazine (Figure 13.9). Both have been used as tranquilizing agents and sedatives in large and small animals. Their use now is restricted to companion animals because of concerns over residues when administered to food animals.[161,186]

In human medicine, chlorpromazine is used mainly as a sedative, tranquilizer and antipsychotic drug. It is widely regarded as a safe drug but it can produce side-effects, some of them severe. Many of these effects are due to its anti-cholinergic effects and include slurred speech, dry mouth, constipation, urinary retention, dystonia, tardive dyskinesia and akathisia.[187–189] It can also, rarely,

produce leukopenia and agranulocytosis.[190–192] Acepromazine was used in human medicine, but its use now is largely restricted to veterinary medicine.

The phenothiazines can also produce photosensitisation in human patients treated with these drugs.[193–197] Contact dermatitis and photosensitisation have been reported in farmers exposed to chlorpromazine.[198,199] Acepromazine has been the subject of an attempted suicide in a veterinary lay worker and of a successful suicide attempt.[200–202] Deaths have occurred in children who had ingested acepromazine and imipramine.[203] Signs of phenothiazine toxicity occurred in a child aged 2.5 years soon after ingesting 3 to 4 tablets each containing 25 mg acepromazine intended for the treatment of a large dog.[204] In fact, serious toxicity in children following ingestion of phenothiazines is rare but where it does occur it is usually due to exposure to chlorpromazine.[205]

Chlorpromazine was used in food animals in the European Union (EU). When the information was reviewed in order to establish maximum residue limits (MRLs) for the drug, it was concluded that there were insufficient data to allow the identification of a no-observed effect level and no acceptable daily intake could be calculated. Hence, the drug was prohibited for use in food animals in the EU.[206] The Joint FAO/WHO Expert Committee on Food Additives (JECFA) considered MRLs for chlorpromazine and another phenothiazine drug propionylpromazine. It too considered the available data to be inadequate and was unable to establish MRLs for either compound.[207,208]

13.5.3 Butyrophenone Neuroleptic Agents

There are two butyrophenone compounds used in veterinary medicine, azaperone and fluanisone. These are closely related structurally to the human drug haloperidol (Figure 13.10). Azaperone is used to control aggressiveness and fighting in pigs.[209–213] It also has other uses including anaesthesia and use with other agents such as etorphine, butorphanol and medetomidine as a capture agent.[214–216] Azaperone and haloperidol have been found to be effective in controlling stress in roe deer.[217] Fluanisone, as a combination product with fentanyl (*Hypnorm*) is used as an anaesthetic for rats and other small rodents, and in rabbits.[218–223]

Haloperidol results in a number of effects in laboratory animals including catalepsy, ptosis, hypothermia and dystonias.[224–226] In humans it is used as an antipsychotic drug and it is associated with a range of adverse drug reactions including extrapyramidal effects such as dystonias, akathisia and pseudoparkinsonism. It can also lead to tardive dyskinesia. Other effects include, dry mouth, constipation and depression.[161,227] It may induce myocarditis and prolongation of the QT interval.[228,229] In children, accidental overdosage has resulted in disturbances of consciousness, tremors in the extremities, an oculogyric crisis (dystonic reaction with rotation of the eyeballs), dysarthria, drooling, akathisia, hyperreflexia and opisthotonos.[230] It has been associated with a number of fatalities.[231]

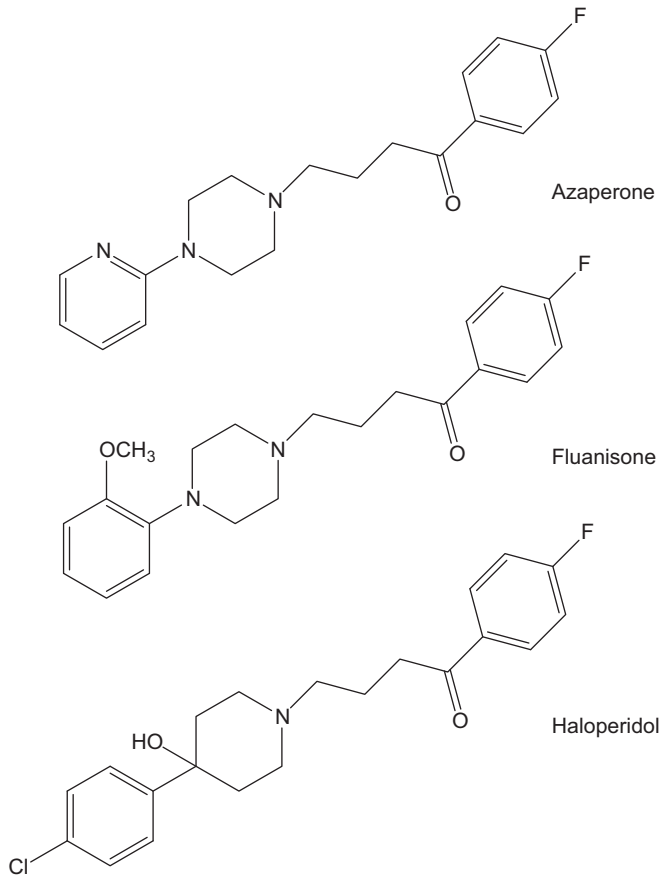


Figure 13.10 Chemical formulae of azaperone, fluanisone and haloperidol.

In humans treated with azaperone for psychosis (20 male patients), doses of 0.5 mg three times per day, increasing to 20 mg three times per day for up to 2 months revealed no adverse drug reactions up to a dose of 2 mg three times per day. At higher doses there was a dose related increase in sedation and at 20 mg three times per day patients complained of dizziness. There were no effects on haematology or blood biochemistry.[232] Occupational photoallergic dermatitis has been reported in a pig breeder occupationally exposed to azaperone.[233]

Azaperone is of moderate acute oral toxicity with LD_{50} values in the mouse, rat and guinea pig being 385, 245 and 202 mg/kg bw respectively but it is more toxic after intravenous administration (LD_{50} value of 38–42 and 28 mg/kg bw in the mouse and rat respectively). It produced no notable effects in repeat dose studies in rodents and dogs and it had no major effects in studies of reproductive performance or for teratogenic effects. Genotoxicity studies gave negative results, but some metabolites, notably azaperol, gave weak negative results in the Ames reversion assay with some *Salmonella* strains. It was not

carcinogenic in a study in rats and the main effect noted here, and in a 2 year study in dogs, was sedation.[232,234,235]

13.6 Carazolol

Carazolol is a β-adrenoceptor antagonist (β-blocker).[236–239] It is used for the prevention of sudden death due to stress in the transportation of pigs. Unlike most of the β-blocking agents used in human medicine such as propranolol, pindolol and acebutolol, carazolol is a carbazole derivative (Figure 13.11).

Carazolol is moderately toxic after single oral doses to mice and rats with oral LD_{50} values of 132–160 and 80–88 mg/kg bw respectively. However, it is of high acute toxicity after intravenous administration with LD_{50} values of 14 mg/kg bw in mice and 10 mg/kg bw in rats. In repeat dose studies in rats, but not in dogs, the major pharmacological effect was a reduction in heart rate. In pharmacological studies, the main effect in mice was the inhibition of fighting. In rabbits, isoprenaline-induced tachycardia was inhibited. In dogs, a similar effect was noted on isoprenaline-induced responses. Doses of 1 mg/kg bw intravenously caused cardiovascular depression with decreases in arterial blood

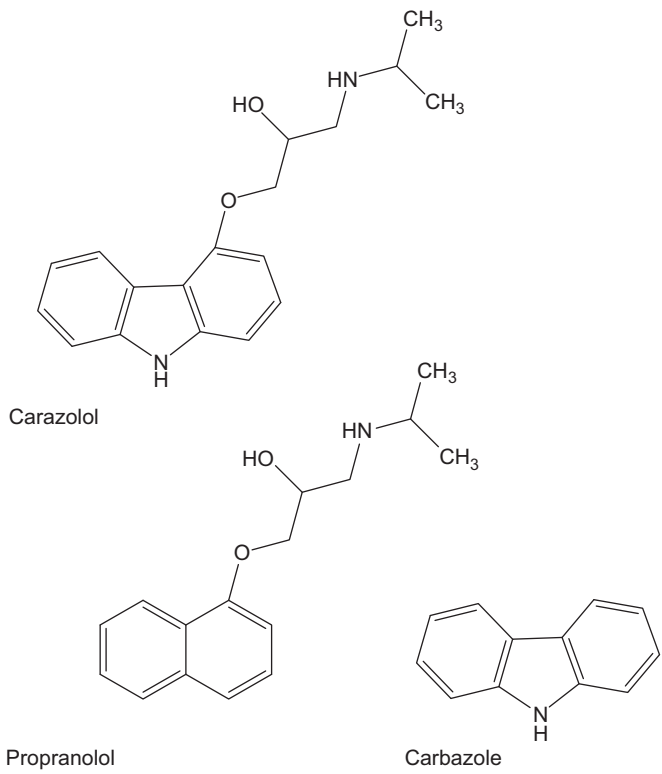


Figure 13.11 Chemical formulae of carazolol, propranolol and carbazole.

pressure, left ventricular pressure and cardiac output. Doses of 4, 16 or 64 mg/kg bw intravenously to dogs produced a dose dependent inhibition of isoproterenol-induced tachycardia and isoprenaline-induced increased cardiac output. At 64 mg/kg bw almost complete blockade occurred. The drug gave negative results in genotoxicity studies.[240]

In studies in healthy human volunteers, doses of 0.5 mg intravenously or 5 and 7.5 mg orally produced inhibition of heart rate and a significant lowering of blood pressure. An oral dose of 0.7 mg carazolol produced an increase in bronchial resistance in patients with chronic bronchitis. Significant falls in heart rate, blood pressure and pressure-rate product occurred in patients with sympathicotonic cardiovascular disease at rest or after exercise when given an oral dose of 2.5 or 5 mg carazolol, 3 times per day for 7 days. Doses of 5 mg per person, 3 times daily for 4 weeks produced a significant hypotensive effect in patients with essential hypertension. In a study of patients with angina pectoris, patients were treated with oral pindolol 5 mg or oral carazolol 2.5 or 5 mg, 3 times daily. From the results of these studies a no-effect level of 10.6 $\mu g\,kg^{-1}$ bw was derived for the pharmacological effects of carazolol in humans. In another study, patients with chronic bronchitis or asthma were given carazolol and the effects on respiratory function were measured. An overall no-effect level of 0.5 $\mu g\,kg^{-1}$ bw was determined. A further study in healthy volunteers determined the pressure-rate product and from the area under the curve for relative inhibition *versus* time, a no-observed effect level (NOEL) of 10 $\mu g\,kg^{-1}$ bw was derived. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) used this NOEL of 10 $\mu g\,kg^{-1}$ bw to calculate an acceptable daily intake of 0.1 $\mu g\,kg^{-1}$ bw when considering MRLs for carazolol in food of animal origin. The EU MRL values were also elaborated on the basis of an acceptable daily intake (ADI) of 0.1 $\mu g\,kg^{-1}$ bw.[241,242]

13.7 Clenbuterol

Clenbuterol is a selective β_2-adrenergic agent with pharmacological properties that are similar to those of structurally related agents such as salbutamol (albutamol) and terbutaline (Figure 13.12). In human medicine, these drugs are used mainly in the treatment of asthma. They have a number of adverse effects that arise from excessive activation of β-adrenergic receptors. These effects can be serious and include tachycardia, which if extreme, may result in myocardial necrosis. Hence, they may be particularly injurious to patients with cardiovascular disease after oral administration. To limit the possibility of systemic effects, the aerosol use of these drugs was developed. This delivers therapeutic doses of the drug to the bronchi but avoids high systemic exposure.[154]

In veterinary medicine clenbuterol is used for similar effects in horses with respiratory disease where it is given orally or by injection. It is also used as a tocolytic agent in cattle to delay delivery to prepare the birth canal, to act as an aid to obstetrical procedures, to relax the uterus for caesarean section, to delay and programme delivery, to facilitate the replacement of a prolapsed uterus and

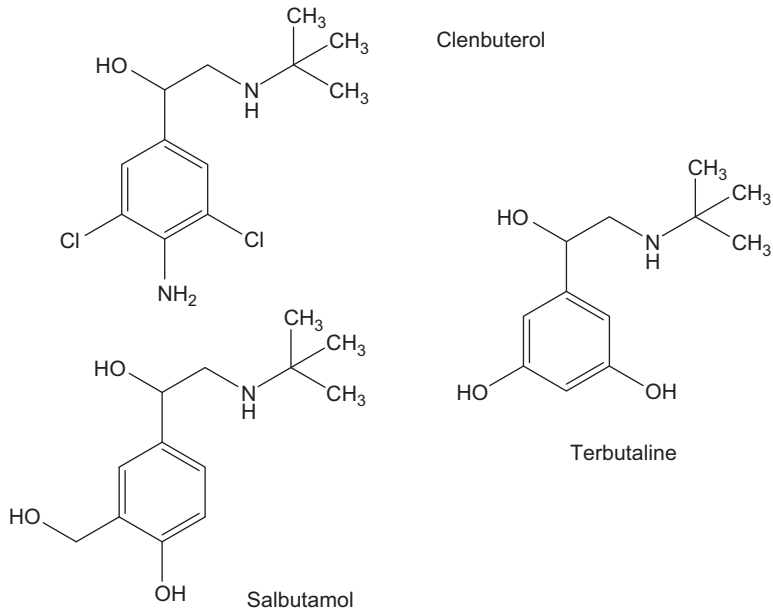


Figure 13.12 Chemical formulae of clenbuterol, salbutamol and terbutaline.

to facilitate embryo transfer.[160,243,244] Clenbuterol has been used in human medicine (*Spiropent*) but it has been replaced by other drugs.[245,246]

However, clenbuterol also has had another use. This is the manipulation of carcass quality in food producing animals. The administration of clenbuterol (or cimaterol) to cattle and pigs results in an effect commonly referred to as repartitioning where muscle mass increases and adipose tissue is reduced.[247–249] Due to concerns over residues in food of animal origin as well as concerns over the safety of clenbuterol, its growth enhancing use was prohibited in the EU and in the USA.[250,251] Regardless, the illegal use of clenbuterol as a growth promoter has continued in some countries.[250–255] Not surprisingly, clenbuterol is a common drug included in residue surveillance plans for several countries, including EU countries (see Chapter 3). As a result of the illegal use of clenbuterol in food animals, presumably without any controls over dose, duration of dosing and withdrawal periods, there have been several outbreaks of poisoning involving people who have eaten meat or offal from illegally treated animals. In one case in Catalonia, Spain in 1992, 113 people were affected. Of these, 50 were found to have had nervous symptoms, tachycardia, muscle tremors, myalgia and headache. Symptoms began from 15 minutes to 6 hours after consuming veal liver, and they persisted for up to 6 days. Clenbuterol was detected in 47 urine samples. There were no fatalities.[256] In Portugal, over 50 people were affected in one episode and symptoms included tachycardia, tremors of the extremities, nausea, headaches and dizziness. Ingested lamb and beef were implicated in this instance.[257] In 15 affected patients in Madrid,

symptoms of poisoning appeared 30 minutes to 2 hours after the ingestion of veal liver. Patients presented with tremors, palpitations, anxiety, malaise, nausea and pruritus. Tachycardia was observed in all patients. The concentration of clenbuterol in the veal sample was 500 ppb.[258] In France, 22 patients were affected 1 to 3 hours after eating contaminated veal.[259] Similar episodes have occurred elsewhere in Spain, in Italy, and in China.[260–267]

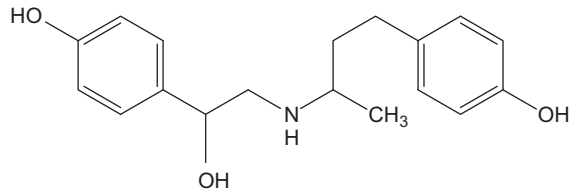
In recent years, interest has spread to the illegal use of clenbuterol in humans for improvement of sporting ability.[268–272] These misuses have led to toxicity, including myocardial infarction.[273–275] It has also raised the possibility of contaminated food compromising the outcome of drug tests in sport.[276] There have been several cases where heroin and other drugs have been adulterated with clenbuterol and instances of poisoning have originated from these.[276–281] Patients who have deliberately taken clenbuterol for other reasons, including those who have swallowed the veterinary products, developed tachycardia, hypokalaemia, hypomagnesia and, in one case, atrial fibrillation.[282,283]

There has been a report of the development of tardive dyskinesia in a man treated with clenbuterol. This was controlled with reserpine treatment.[284] A case of contact dermatitis has been reported following exposure to intermediates in the manufacture of clenbuterol.[285]

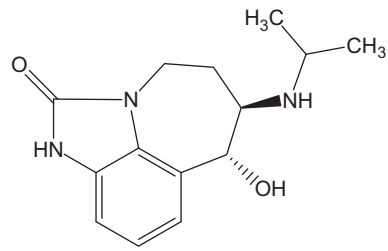
Clearly, if misused or abused, clenbuterol has the capacity for serious exaggerated pharmacological and toxicological effects. Clenbuterol is recognised as a serious hazard when the veterinary drug is misused.[286] In fact, many of the adverse reactions are predictable from studies in animals, from knowledge of its pharmacological effects and from the recognition of its activity as a potent β-adrenergic blocking agent.[287]

The other main β-adrenergic agents authorised for carcass quality improvement are ractopamine and zilpaterol (Figure 13.13). These are approved in a number of countries including the USA where they are permitted in cattle, pigs and turkeys (ractopamine)[288–292] or cattle (zilpaterol),[293–296] but they are not permitted in the EU.

In 1992, JECFA considered the toxicity and residues data available for ractopamine. The compound has low acute oral toxicity in mice (LD_{50} values of 3547 and 2545 mg/kg bw in male and female mice) but somewhat higher toxicity in rats (oral LD_{50} values of 474 and 367 mg/kg bw in male and female rats). In repeat dose studies in dogs, the main effects were bradycardia for which an NOEL could not be identified. However, in monkeys, the drug caused tachycardia as might be expected from its pharmacology and an NOEL of 0.5 mg/kg bw/day was identified although another value, 0.125 mg/kg bw/day, was identified in another study. The drug had no major direct effects on reproductive performance, while foetal anomalies were only observed at doses that were clearly maternotoxic. It was not genotoxic. There were limited studies in humans following exposure to oral and aerosol forms. There was no clear evidence of bronchodilator activity and the only significant pharmacological effect was 15 to 20 mm Hg rise in systolic blood pressure after to 30 to 45 mg orally. JECFA was unable to identify an NOEL on the basis of the data it had available.[297]



Ractopamine



Zilpaterol

Figure 13.13 Chemical formulae of ractopamine and zilpaterol.

At a later meeting of JECFA, a substantial amount of new data were provided on the safety of ractopamine, including further data in humans.[298] At this meeting, JECFA concluded that the most appropriate study was one in humans for cardiac effects and it identified an NOEL of 67 $\mu g\,kg^{-1}$ bw on the basis of changes in electromechanical systole, left ventricular ejection time and maximum velocity of circumferential fibre shortening. As the study was conducted in humans, JECFA used a safety factor of 10 to calculate the ADI of 1 $\mu g\,kg^{-1}$. The subsequent JECFA MRL was then advanced into the Codex Alimentarius system. The EU in the form of the European Commission, which has long opposed the use of growth promoters, predictably opposed the Codex MRL (see Chapter 3). In addition, it referred the toxicological aspects to the European Food Safety Authority and its FEEDAP Panel (see Chapter 9). In the ensuing report, FEEDAP criticised the JECFA evaluation for a number of reasons. The major criticism was that JECFA had used a study in humans which was a pilot study which was not designed to identify an NOEL. It commented that the absence of a double-blinded study design would permit the introduction of bias, particularly from the placebo effect. It asserted that if an ADI is to be determined from a pharmacological study in humans, then the end-point must not only consider "clinically relevant ("adverse") effects in the consumer" but also "subjective discomfort even when occurring only for a short time". It finally concluded that the human study could not be taken as a basis for identifying an NOEL and therefore no MRL could be established. This view was supported by the Committee for Medicinal Products for Human Use (CVMP).[299]

The more cynical might claim that this is another example of the EU retrospectively attempting to find scientific reasons to justify a ban that it had already implemented for other reasons. While there is some truth in the

FEEDAP panel report, the reasons given are not compelling. The NOELs for a number of drugs have been chosen by the CVMP and by JECFA from pharmacological studies. Providing that these are not seen in isolation and that other aspects of safety are supported by relevant toxicology studies, then the decision to use a pharmacological end-point is justified. There was an adequate package of toxicology data in support of ractopamine. It seems likely, that in view of their low oral bioavailability and with the proper use of MRLs and withdrawal periods, ''residues of such compounds in edible tissues of properly treated animals would not likely represent a credible risk to consumers''.[289]

Isoxuprine (Figure 13.14) is a β-adrenergic agent used almost exclusively to treat navicular disease and laminitis in horses (navicular disease is an inflammatory and degenerative disease of the navicular bone of the limb while laminitis is major cause of lameness and disability in horses that is associated with ischaemia of the digital dermal tissues[300–303]). It has also been used as a tocolytic agent in horses, cattle, pigs, sheep and goats. Its major mode of action appears to be as a vasodilator but its effectiveness in the horse is the subject of some controversy. The doses used are considered insufficient to produce cardiovascular changes and its analgesic properties are open to question.[304–308]

In human medicine, isoxsuprine has been used for the treatment of Raynaud's phenomenon.[309] However, its major use has been as a tocolytic agent and for the prevention of preterm labour.[309–314] It has also been used in the treatment of cardiovascular disease.[315,316] Unfortunately, the use of isoxsuprine in humans is associated with the induction of pulmonary oedema.[317–322]

In the EU, when establishing the MRLs for isoxsuprine, the CVMP considered a portfolio of toxicology data that showed that the substance had low acute oral toxicity in rats and mice with LD_{50} values in the range 900 to

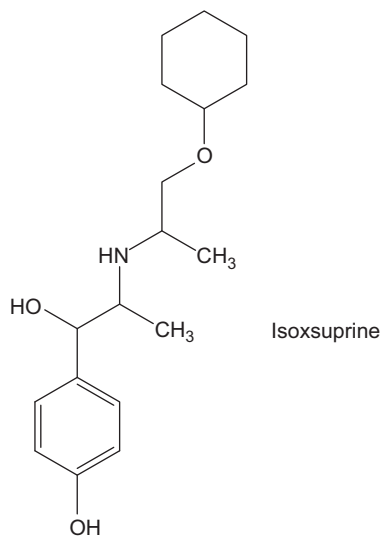


Figure 13.14 Chemical formula of isoxsuprine.

6000 mg/kg bw. In repeat dose studies in rats and dogs, the only significant effects were decreases in haemoglobin, packed cell volume and erythrocyte counts. It had no major effects on reproductive performance and was not a teratogen. The drug was not mutagenic in a range of genotoxicity studies. The NOEL of 20 mg/kg bw was identified from effects on maternotoxicity and foetotoxicity from a teratogenicity study in rats and a toxicological ADI (0.2 mg/kg bw) was calculated. However, a pharmacological NOEL of 0.2 mg/kg bw based on heart rate in dogs was lower than its toxicological counterpart and a pharmacological ADI was established (0.002 mg/kg bw). On the grounds that isoxsuprine is employed for infrequent treatments in individual animals, and that animals are unlikely to be sent for slaughter immediately after treatment and taking into account rapid depletion of residues, the CVMP considered that MRLs were unnecessary on public health grounds for use in cattle and horses. However, there was insufficient residues depletion data for use in pigs, sheep and goats.[323] Treatment of horses and cattle with isoxsuprine does result in residues and, in the case of pregnant cows, these may extend to the calf. However, as noted by the CVMP, these residues rapidly deplete.[324,325]

13.8 Non-steroidal Anti-inflammatory Drugs

A number of non-steroidal anti-inflammatory drugs (NSAIDS) are employed in veterinary medicine for a wide variety of purposes. The majority are used for their analgesic properties and in companion animals, the most common use is for the control of musculoskeletal pain and general anti-inflammatory and antipyretic properties.[69,326–328] Many of these drugs belong to the older class of NSAIDs, the non-selective cyclooxygenase (COX) inhibitors. Tepoxalin is an inhibitor of cyclooxygenase and lipoxygenase.[329] More recently introduced drugs are selective inhibitors of one of the isoforms of COX, COX-2. Some of the major NSAIDs used in veterinary medicine are shown in Table 13.2 and their chemical structures depicted in Figures 13.15, 13.16 and 13.17.

In 1971, John Vane demonstrated that drugs such as aspirin and indomethacin inhibited the synthesis of prostaglandins in guinea pig lung preparations. These drugs belong to the non-selective COX inhibitor class. That is, they inhibit both COX-1 and COX-2. COX-1 is a constitutive enzyme which catalyses the biosynthesis of cytoprotective prostaglandins from arachidonic acid in several organs including the stomach mucosa, kidneys, pancreas and brain as well as in thrombocytes, while COX-2 is an inducible enzyme in inflamed tissues. COX-1 is integral in the arachidonic acid cascade for the production of thromboxane A_2 and prostacyclin. It therefore follows that substances that inhibit COX-1 are likely to produce adverse effects through loss of cytoprotection while those that inhibit COX-2 are likely to have therapeutic effects.[330–335]

Of course, none of this was known in 1897 when aspirin was first synthesised or indeed even earlier where it had long been recognised that extracts of willow bark had anti-inflammatory properties.[333] Aspirin soon entered into clinical use and it was followed by other compounds such as indomethacin. The first adverse effects to become apparent were those on the gastrointestinal system.

Table 13.2 Non-steroidal anti-inflammatory drugs used in veterinary medicine.

Name	*Chemical name*	*Main species*
Nonselective COX inhibitors		
Carprofen	2-(6-chloro-9*H*-carbazol-2-yl)propanoic acid	Cattle, dogs, horses
Diclofenac	{2-[2,6-dichlorophenyl)amino]phenyl}acetic acid	Cattle, pigs
Tolfenamic acid	2-[(3-chloro-2-methylphenyl)amino}benzoic acid	Cats, cattle, dogs, pigs
Ketoprofen	2-(3-benzoylphenyl)propanoic acid	Cattle, horses, pigs
Flunixin meglumine (Banamine)	2-{[2-methyl-3-(trifluoromethyl)phenyl]amino}nicotinic acid	Cattle, horses, pigs
Phenylbutazone	4-butyl-1,2-diphenyl-3,5-pyrazolidinedione	Dogs, horses[a]
Meloxicam	4-hydroxy-2-methyl-*N*-(5-methyl-1,3-thiazol-2-yl)-2*H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide	Cattle, goats, horses, pigs, rabbits
COX and lipoxygenase inhibitor		
Tepoxalin	3-[5-(4-chlorophenyl)-1-(4-methoxyphenyl)-1*H*-pyrazol-3-yl}-*N*-hydroxy-*N*-methylpropanide	Dog[b]
Selective COX-2 inhibitors		
Cimicoxib	4-(4-chloro-5-(3-fluoro-4-methoxyphenyl)imidazole-1-yl)benzenesulfonamide	Dog
Robenacoxib	{5-ethyl-2-[(2,3,5,6-tetrafluorophenyl)amino]phenyl}acetic acid	Cats, dogs
Mavacoxib	4-[5-(fluorophenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]benzenesulfonamide	Dogs
Firocoxib	3-(cyclopropylmethoxy)-5,5-dimethyl-4-[4-(methylsulfonyl)phenyl]-2(5*H*)-furanone	Dogs, horses

[a]In the EU products must carry a warning that horses treated with phenylbutazone products may never be slaughtered for human consumption.
[b]Withdrawn in some countries.

13.8.1 Gastrointestinal Effects

The gastrointestinal effects of aspirin and other NSAIDs arise because of their inhibition of COX-1 and the subsequent loss of cytoprotection for the gastric mucosa in particular. This results in gastric and duodenal irritation and bleeding and is a problem in clinical use in humans and in other species. If left untreated it may prove to be fatal.[332–343] After the discovery of COX-2 in 1991, a search began for selective COX-2 inhibitors on the theoretical grounds that these should spare the gastrointestinal tract and as a result, a range of compounds were introduced into human and veterinary medicine,[344,345] as detailed in Table 13.2. However, it is still unclear if these new drugs are indeed free of significant gastrointestinal side effects and available data show that some may possess significant gastrointestinal toxicity.[332,344,346,347]

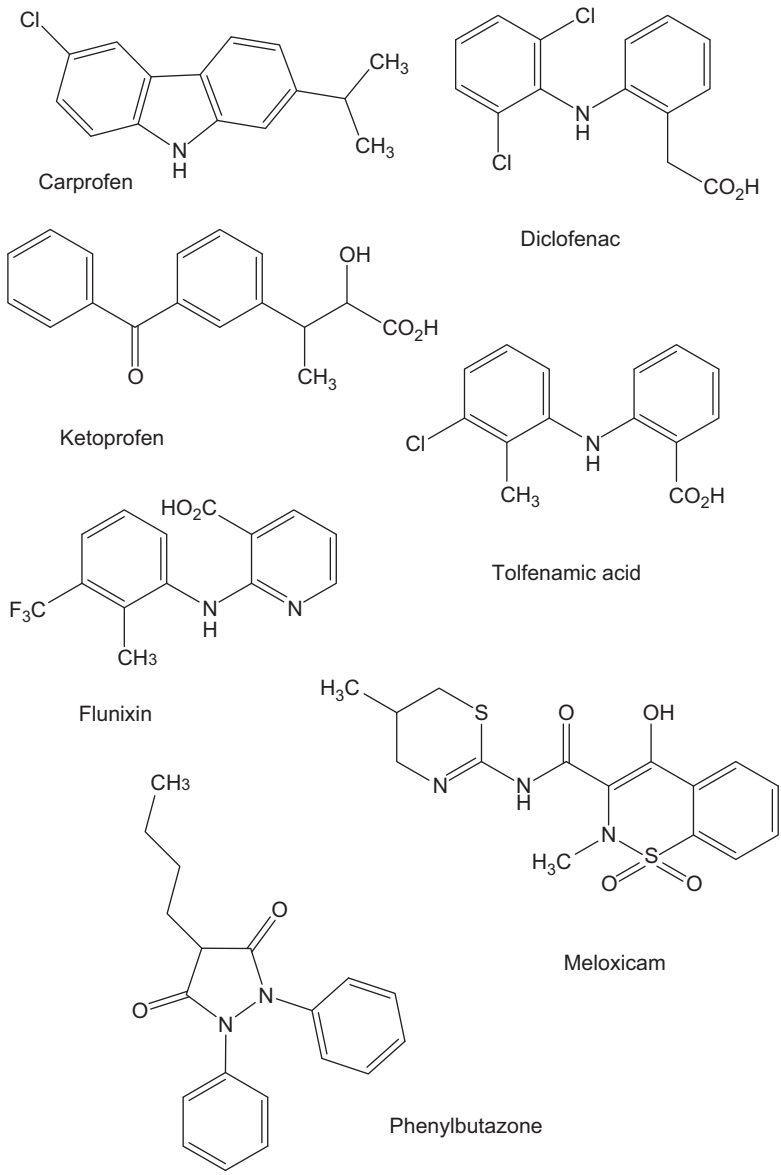


Figure 13.15 Chemical formulae of carprofen, diclofenac, ketoprofen, tolfenamic acid and flunixin meglumine, phenylbutazone and meloxicam.

13.8.2 Cardiac Effects

The first two COX-2 selective NSAIDs to be introduced into human clinical medicine were rofecoxib and celecoxib in 1999. Rofecoxib (*Vioxx*) soon became a best selling drug for the treatment of arthritis and other inflammatory with

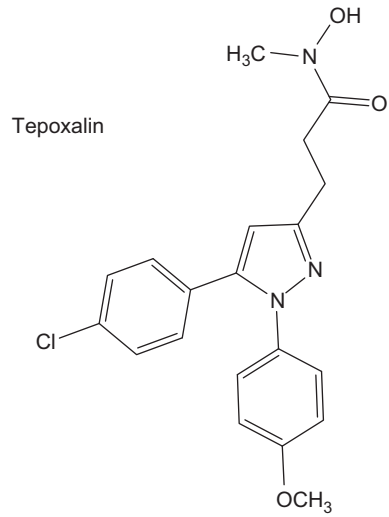


Figure 13.16 Chemical formula of tepoxalin.

global sales of approximately US $2.5 billion in 2003.[348,349] In September 2004, the manufacturer, Merck, announced its withdrawal.[350–352]

The first suggestions of adverse effects with rofecoxib came from the *Vioxx* GI Outcomes Research (VIGOR) trial in 2000, where the effects were compared with those of the non-selective drug naproxen, in a relatively large group of patients. This showed a 5-fold increased risk of myocardial infarctions, some with fatal consequences, in patients treated with rofecoxib when compared with those treated with naproxen.[353] Other studies have since confirmed these results and demonstrated increased morbidity and mortality in patients treated with this drug.[354–371] The *Vioxx* episode has become a modern study in how not to deal with a drug withdrawal and a developing crisis, and how to avoid bad publicity when action needs to be taken.[372–378]

Other studies have shown other COX-2 inhibitors to have qualitative similarities with rofecoxib even if the quantitative aspects are different.[379–383] Interestingly, the cardiovascular adverse effects (and nephrotoxicity, see next section) of COX-2 inhibitors had been predicted some years previously.[334,335,384,385] The drugs affect sodium balance and in turn, haemodynamics. Blood pressure is regulated by the kidneys through prostaglandins and as the kidneys are targets for the effects of COX-2 inhibitors, and so an interaction seems inevitable. It has been estimated that patients taking 25 mg rofecoxib once daily underwent a 20 mm Hg rise in blood pressure and this, and the lower activity on platelet aggregation, may increase the risk of cardiac effects significantly.

13.8.3 Nephrotoxicity

In the kidney, as noted in the previous section, prostaglandins regulate the vasoconstrictor effects of angiotensin II, and other hormones such as

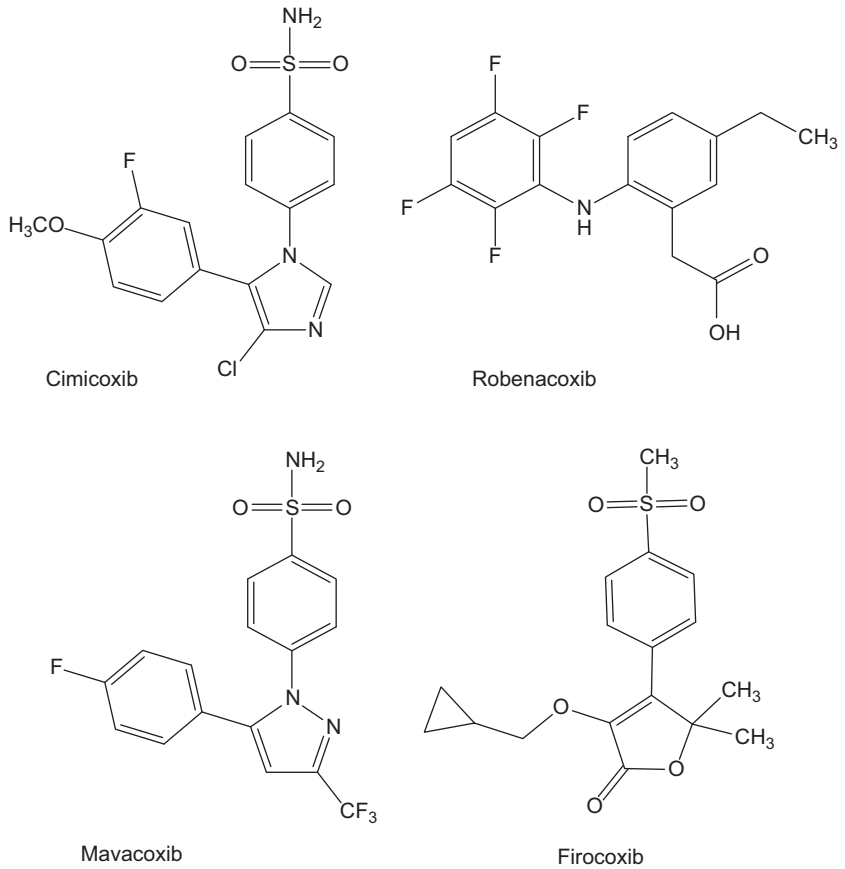


Figure 13.17 Chemical formulae of cimicoxib, robenocoxib, mavacoxib and firocoxib.

vasopressin. They also regulate glomerular filtration rate, vascular resistance and renin secretion. The COX enzymes are located throughout the kidney with COX-1 being found in collecting ducts, interstitial cells, endothelial cells and smooth muscle of pre- and postglomerular vessels, and COX-2 being distributed in endothelial and smooth muscle cells of arteries and veins and in renal podocytes.[386,387] Inhibition of prostaglandin synthesis in the kidney by COX inhibitors, including COX-2 inhibitors, is likely to affect renal function as well as cardiac function. Several non-specific COX inhibitors and COX-2 inhibitors are known to result in functional abnormalities in the kidney. These include fluid imbalances and disruption of electrolyte homeostasis. However, nephrotoxicity including acute renal failure, interstitial nephritis, nephrotic syndrome and renal papillary necrosis may also occur and many of these effects are exacerbated by other conditions such as congestive heart failure. They are also associated with the concomitant use of diuretics and a history of analgesic

abuse.[387–403] The evidence currently available suggests that the COX-2 inhibitors have similar risks to the non-selective COX inhibitors.[401,404]

13.8.4 Phenylbutazone

Phenylbutazone (Figure 13.15) is a NSAID that in the past was widely used in veterinary medicine across a range of species. It is an effective anti-inflammatory and analgesic that is still used widely in horses and dogs.[326,328,405] In dogs treated with phenylbutazone, pancytopenia has developed.[406] It has also been associated with the development of myelofibrosis and aplastic anaemia in dogs.[407,408] In humans, treatment with phenylbutazone or with the related drug oxyphenbutazone, has led to the development of aplastic anaemia.[409–416] The mortality from oxyphenbutazone and phenylbutazone due to aplastic anaemia has been estimated to be around 4 in 100 000 and 2 in 100 000 respectively, with the elderly being at higher risk.[413,417] Interestingly, the effect had been predicted for phenylbutazone some time earlier because of its similar chemical structure to other drugs known to cause aplastic anaemia, specifically to amidopyrine and other pyrazoles.[418]

Abuse of veterinary phenylbutazone is known to occur and ulcers and renal insufficiency has been reported in a horse trainer who used the drug.[419] Oral intake of around 17 g of a veterinary formulation for toothache by a racetrack worker resulted in grand mal seizures, coma, hypotension, respiratory and renal failure and hepatotoxicity. The patient recovered after six weeks of supportive intensive care and repeated dialysis.[420] Aplastic anaemia in humans has resulted from the use of veterinary phenylbutazone and from the use of a herbal medication adulterated with phenylbutazone.[421,422]

The majority of the NSAIDs are unlikely to offer any undue gastrointestinal, cardiovascular or renal risks to users of veterinary drugs, as exposures are likely to be insignificant and infrequent. MRLs and tolerances have been established for those members of the class used in food animals. Phenylbutazone is generally not permitted for use in food animals. As noted in Table 13.2, it is has no EU MRL for use in food animals and if used in horses, tissues from treated animals may not enter the human food chain.

13.9 Tropane Alkaloids

These drugs, which are based on the chemical structure of tropane, include atropine, scopolamine, hyoscyamine and cocaine (Figure 13.18). Of these, the most frequently used in veterinary medicine is atropine. Atropine is a competitive antagonist for the muscarinic acetylcholine receptor. It has a number of pharmacological effects including decreasing bronchial and other secretions. It is used in the treatment of organophosphorus compound poisoning in horses, cats and dogs, but it is also employed as an antiemetic, antiarrhythmic, bronchodilator and in the treatment of digitalis poisoning.[423–425] Atropine is found naturally in deadly nightshade (*Atropa belladonna*), Jimson weed (*Datura*

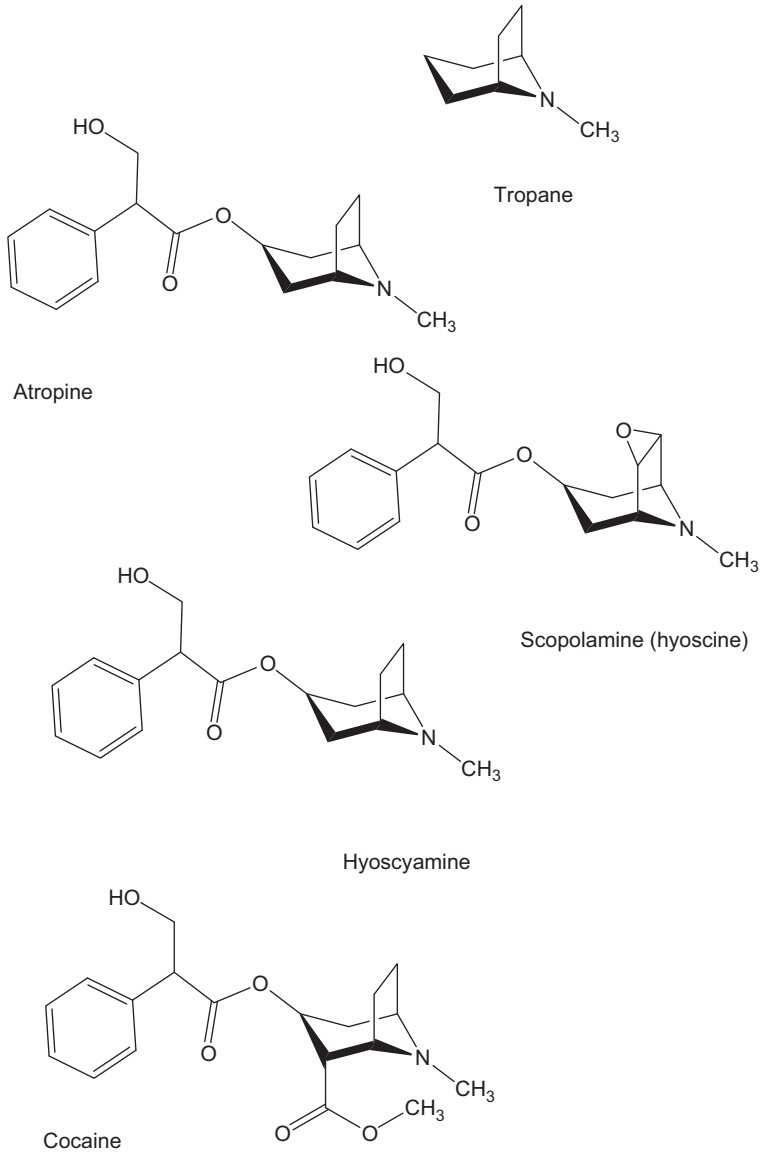


Figure 13.18 Chemical formulae of tropane, atropine and related drugs.

stramonium), mandrake (*Mandragora officinarum*) and in other members of the *Solonaceae*, a group which also includes tomatoes and potatoes. Ingestion of natural sources such as Jimson weed can induce toxicity, which in severe cases may be fatal.[426–431]

The use of atropine in human medicine has been associated with toxicity, including lethal toxicity, since the latter part of the nineteenth century.[432,433]

One of these cases describes some specific signs of atropine poisoning in humans including dilated pupils, dizziness, loss of use of legs, dry tongue, loss of pupillary reflexes, flushed face and loss of conscious. Although the patient had initially responded to supportive care and "a drachm of digitalis in some brandy" he eventually died.[433] In fact the signs "hot as a hare, red as a beet, blind as a bat, dry as a bone and mad as a wet hen" have been used to describe the effects of atropine toxicity in humans. These signs refer to some of the effects that it induces including fever, hot dry flushed skin, skin rashes, dilated pupils, blurred vision, increased intraocular pressure, tachycardia, tachypnoea, dry mouth, hyperactivity, hallucinations, muscular stiffness, convulsions and coma.[434] These effects, while not always seen together, are typical of atropine poisoning which as noted above, may be fatal in severe cases.[435–441] Some severe and fatal cases have involved relatively small amounts of drug, including drug delivered as an ointment or as drops, sometimes in infants.[434,437,438–446]

Treatment with atropine has resulted in rhabdomyolysis.[447] Exposure to atropine has resulted in allergic reactions, including contact dermatitis.[448–451]

Atropine is a widely used drug in human medicine and its history, relatively free from adverse events, suggests that while its therapeutic use may pose a significant hazard, it does not pose a significant risk. If the drug is misused or abused, severe toxicity may be expected and contamination with the skin may elicit contact dermatitis.

13.10 Local Anaesthetics

The major local anaesthetic used in veterinary medicine is lidocaine (xylocaine, lignocaine). The structurally related compounds bupivacaine and mepivacaine are also used. Tricaine mesylate (methanesulfonate, MS-222) is used for anaesthesia and euthanasia of fish, amphibians and reptiles. The choice of local anaesthetic frequently depends on the desired duration of anaesthesia. Bupivacaine is more lipid soluble and provides a longer period of anaesthesia than the less lipid soluble lidocaine. These agents are used for minor surgery and for the local control of pain in tissues such as the skin. They may therefore be used for topical anaesthesia and for anaesthesia by infiltration where subcutaneous injection allows regional anaesthesia by permeation into surrounding tissues. Other uses include intra-articular administration, epidural block and spinal block.[59,452] Tricaine mesylate is usually administered by immersion of the animal in an aqueous solution of the drug.[453–455] As mentioned above, it is used for anaesthesia and euthanasia of fish, amphibians and reptiles.[456–460] It is very soluble in fresh and sea water but it can markedly reduce the pH which may prove toxic to fish and so sodium bicarbonate is used to maintain a pH of 6.5 to 7.5. As a result of the slow rates of drug metabolism in poikilotherms, tricaine may be toxic to fish.[461]

In human use, lidocaine is one of the most important local anaesthetics, and particularly in dentistry but it has application in almost any circumstances

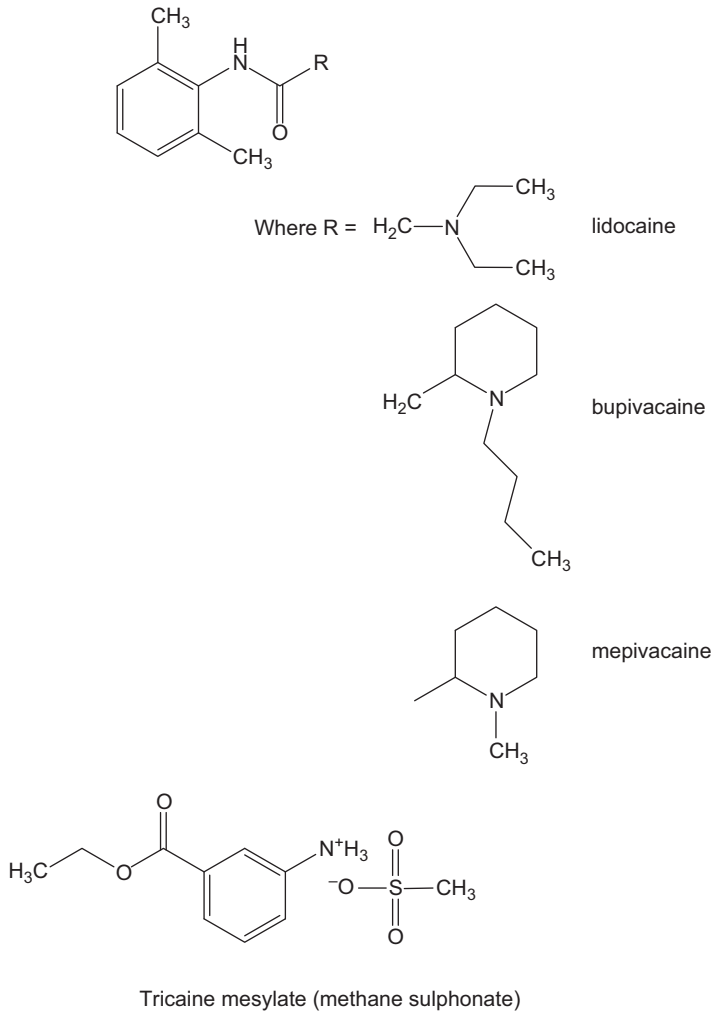


Figure 13.19 Chemical formulae of lidocaine, bupivacaine, mepivacaine and tricaine mesylate.

where an intermediate acting local anaesthetic is required.[462] In veterinary and human medicine, these drugs also have a number of other indications including use as antiarrhythmic agents.[463,464] The structures of these agents are shown in Figure 13.19.

These local anaesthetics block conduction of the nerve impulses by acting at the cell membrane. They achieve this by preventing the increase in the permeability of excitable membranes to sodium ions due to a direct effect on voltage-gated sodium ion channels.[465] Although these drugs are extremely safe and effective in normal clinical use, they may be toxic under some circumstances.

Under normal circumstances, lidocaine is not absorbed through the skin but permeability may be increased in neonates.[466] Systemic absorption leads to CNS symptoms including tinnitus, paraesthesia around the mouth and hypaesthesia as well as effects on cardiac function and physiology.[467] Systemic exposure may result in seizures, other neurologic disturbances and psychological effects.[468–475] Toxicity has been noted in a 3 year old infant after topical application of a eutectic mixture of 2.5% lidocaine and 2.5% prilocaine (EMLA) and after transoral/transpharyngeal topical spraying in an adult.[476,477] A death resulted from gargling with 20 mL of 4% lidocaine solution in preparation for oesophagogastroduodenoscopy and several deaths during liposuction have been attributed to excessive doses of lidocaine.[478,479] Allergy to lidocaine is rare but it has been reported as a systemic effect and as contact dermatitis.[480,481]

The numbers of daily injections of lidocaine for routine dentistry are enormous and added to the other uses of this and related compounds in medicine, then the safety of these drugs in emphasised. However, toxicity can occur and accidents such as self-injection with the drug during veterinary use are clearly best avoided.

13.11 Antiepileptic Drugs

Idiopathic epilepsy is a disease that occurs in many species including humans. It is a relatively common condition in dogs. Epilepsy, once it has developed, is a life-long condition and consequently, it can be an expensive disease for the pet owner to contend with. Consequently, the disease is best treated, wherever possible with cheap medications. Conveniently, there are a number of such medicines available, including primidone, phenobarbital (and other barbiturates) and potassium bromide. The most widely used drug in the treatment of canine idiopathic epilepsy is phenobarbital and, in cases of refractory epilepsy, supplementation with other drugs and notably with potassium bromide is indicated. The barbiturates have been discussed in Chapter 5. Other drugs used in the treatment of epilepsies in humans, including levetiracetam, may also be employed, especially where the response to phenobarbital and bromide therapy is inadequate.

13.11.1 Potassium Bromide

In 1857, Edward Sieveking presented 52 cases of human epilepsy to the Royal Medical and Chirurgical Society, now the Royal Society of Medicine, in London, and the chairman of the proceedings Sir Charles Locock commented that he had successfully treated women with ''hysterical'' epilepsy, in the majority of cases with ''menstrual, catamenial or uterine'' epilepsy, with potassium bromide. He had previously reasoned that on the basis of an earlier German report where 10 grains (about 0.7 g) resulted in impotence and anaphrodisiac effects, and as masturbation was thought to be one of the causes of

epilepsy, that the substance would be useful in treating non-epileptic women affected by "sexual excitement." He went on to try the drug in patients with epilepsy and he obtained satisfactory results.[481–484] Potassium (and sodium or ammonium) bromide then became the standard treatment for epilepsies in humans and continued until the introduction of phenobarbital in 1912 and phenytoin in 1938.[482–485]

Oral potassium bromide, either as a monotherapy or in combination with phenobarbital, has been used for the treatment of epilepsy in veterinary medicine for many years.[486–492] The drug is very safe and effective and reports of toxicity in treated dogs are rare. Polyphagia may occur in up to 25% of treated animals, and polydipsia and polyuria are rare. Sedation, ataxia and effects on limbs, which in severe cases may progress to quadriplegia and a condition that resembles myasthenia gravis may occur. Affected animals are suffering from bromism, a dose dependent neurotoxicity, which can be treated by dose reduction (or drug withdrawal) and the administration of chloride and loop diuretics to facilitate bromide excretion.[491,493,494] A survey of veterinarians favoured the use of potassium bromide for its clinical efficacy and cost to dog owners while a survey of dog owners were satisfied with its clinical performance.[487,495]

Perhaps the major advantage as far as human safety is concerned is that potassium bromide is not very toxic. While it is true that bromism can develop in humans after oral administration, the fact that it takes several months to achieve steady state kinetics means that inadvertent or deliberate ingestion of a dog's medication will not result in toxicity. Studies in volunteers given 1 mg/kg bw/day (about 60 to 70 mg) for 8 weeks revealed no signs of toxicity. Doses of 4 or 9 mg/kg bw/day for 12 weeks to volunteers produced only marginal effects on the thyroid.[496,497]

13.12 Substances with Hormonal Activity

A number of drugs used in veterinary medicine have hormonal activity. These may be naturally occurring, endogenous agents or their synthetic analogues. A number of these agents are discussed below.

13.12.1 Insulin

Like their owners, cats and dogs are living longer and suffering from life-style diseases, mainly obesity or diseases associated with aging. One of these conditions is diabetes mellitus and it is treated with injections of one of the analogues of insulin. This may be porcine insulin or its derivatives, or with recombinant insulin.[498–504] Insulin is a polypeptide that is not active orally as it is broken down by enzymes in the gastrointestinal tract. Hence, it has to be administered parenterally. The major adverse effect of insulin is hypoglycaemia following overdose, and in large overdoses this may be severe and fatal.

There have been a number of incidences where insulin overdose has resulted in severe hypoglycaemia in humans. Although this can occur accidentally, it is frequently the result of a suicide attempt. As insulin is conveniently at hand to both diabetics and their physicians, it has been used by both groups to attempt suicide.[505–526] However, overdose is not always intentional and suicide attempts using insulin are not always by affected patients. Moreover, abuse of insulin, especially by adolescents, may play a part in some overdoses.[527–536]

Insulin has also been used in murders. In the first well-documented case, Elizabeth Barlow had been found dead in her bath at her home in Bradford, Yorkshire. She had felt ill earlier in the day, and had gone to bed. However, she felt warm and decided to take a bath. Her husband, Kenneth Barlow claimed that when he awoke, he had gone to the bathroom and found her submerged in the bath. He attempted to revive her but when this failed, he called a doctor. The doctor felt suspicious about the death as it was unusual for a healthy young woman to drown in the bath and because some of the details about the attempted resuscitation were flawed. Consequently, he called the police who found used syringes in the kitchen, but no injectable medicines.

A post-mortem examination revealed death was due to drowning. The pathologist took blood samples from various parts of the body and he also collected a urine sample. None of the common poisons were found in the samples but the analyst was convinced of poisoning and suspected insulin which would also explain two other findings – the excessive sweating which led her to take a bath and the dilated pupils reported before her death. The body was examined once again but in bright light and two injection sites where found, one on each buttock. The pathologist removed these with the surrounding tissue.

At the time, 1957, the structure of insulin had only been known for two years and there was no readily available method for determining insulin in tissues. The method for assaying insulin depended on a bioassay to quantify the dose that caused hypoglycaemic convulsions in mice. Extracts from the deceased woman's tissues were assayed in this manner, along with tissues from other bodies as a control. The analyst reported that he had examined three separate samples from Elizabeth Barlow's buttock samples and had found a total of 84 units of insulin. These amounts roughly equated to the therapeutic dose required to sustain two patients for a day. Elizabeth Barlow was not diabetic. Kenneth Barlow was arrested on suspicion of murder, and subsequently sentenced to life imprisonment at Leeds Assizes in December 1987. He was released in 1984 but still maintained his innocence.[537,538]

Of course, Elizabeth Barlow drowned. The amount of insulin injected was sufficient to produce an unconscious state but probably not to cause death. Consequently, it is believed that her husband drowned her because she failed to die as quickly as he expected. It has been pointed out that had he left her overnight, she may have died or suffered irreversible brain damage while at the same time her body may have absorbed most of the injected dose thus leaving no evidence for forensic investigators to find.[538]

Prior to this case and since, there have been a number of other murder cases where insulin did prove lethal, usually alone, but sometimes in combination with other drugs such as glipizide and glibeclamide.[539–550] At the time of writing in 2012 there is a current investigation into a series of deaths at Stepping Hill Hospital in Stockport, UK where saline drips had been adulterated with insulin.[551]

The suicides and homicides described appeared to have used medical insulin, that is, insulin intended for use in human diabetic patients. There appears to be no evidence that veterinary insulin has been used for nefarious purposes but as the various veterinary preparations are similar to or indeed identical to insulin used in human medical practice, the potential for abuse and misuse clearly exits.

13.12.2 Steroid Hormones

13.12.2.1 Natural and Synthetic Steroid Hormones

Steroid hormones are used in veterinary medicine for a variety of therapeutic purposes and some of the major ones are shown in Table 13.3. However, the steroid hormones also have another veterinary use, namely growth promotion that relies on their anabolic effects. A number of naturally occurring steroids have been used for growth promotion including 17β-oestradiol, testosterone and progesterone and in addition, a number of synthetic hormones have also been used including trenbolone acetate and melengestrol acetate (Figure 13.20).[554–557] Another growth promoter that has been widely used is zeranol. This is not a steroid (Figure 13.21) but a fungal metabolite isolated from *Fusarium* species.[558,559] At the correct doses, the oestrogens, progestins and androgens exert anabolic effects and it is this property which makes them useful as growth promoting agents or, more correctly, as agents that not only increase muscle mass but also improve carcass quality for example, by increasing marbling in meat. These drugs are normally given as implants behind the ear of cattle.

Some of these drugs are also used in human medicine, often for the treatment of reproductive conditions or for diseases of the reproductive tract and, of course, for contraceptive purposes.[560,561] However, the anabolic effects of these drugs can also be useful, especially in wasting diseases, in frailty of old age and

Table 13.3 Some Therapeutic Uses of Steroid Hormones.[160,552,553]

Hormone	*Major use*
17β-Oestradiol	Prevention of pregnancy in bitches, induction of oestrus
Testosterone	Reversal of feminisation in male dogs, suppression of oestrus, treatment of pseudopregnancy, treatment of some skin diseases (*e.g.* endocrine alopecia)
Allyltrenbolone (altrenogest)	Synchronisation of oestrus in pigs (for artificial insemination), suppression of oestrous in the horse
Nandrolone (19-nortestosterone)	Supportive therapy for chronic renal failure in cats and dogs
Progesterone	Luteal insufficiency to maintain pregnancy

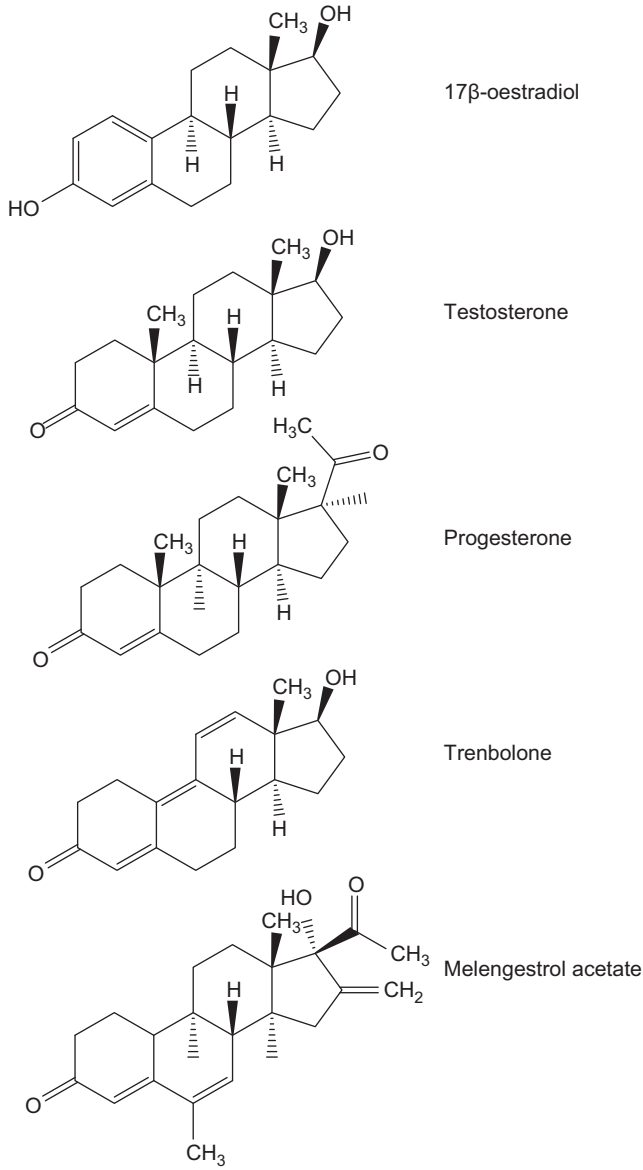


Figure 13.20 Chemical formulae of 17β-oestradiol, testosterone, progesterone, trenbolone acetate and melengestrol acetate.

in cancer cachexia where losses of body and muscle mass are not related to nutritional intake alone.[562–571]

Depending on the type of drug, these agents may have a variety of adverse effects. The nature of these effects depends on their pharmacology. For example, androgens may increase body hair in women and cause acneform

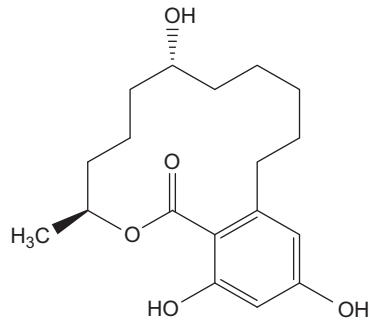


Figure 13.21 Chemical formula of zeranol.

eruptions, while oestrogens may cause feminisation in men and reductions in fertility.[572–576] Dependence may result.[577,578] Many of these drugs, including the veterinary versions, are freely available from internet sites, which only compounds the problems involved.[579]

In recent years, androgenic anabolic steroids have become substances subject to serious abuse. This may be for amateur body building and improvement of self-esteem, for illegal use by professional (and amateur) athletes or simply for abuse along with other illicit drugs.[580–587] Abuse of these substances, which generally involves higher doses and longer periods of use than with therapeutic treatment may induce any of the effects noted with clinical use but also convulsions, rhabdomyolysis, pulmonary embolism, pulmonary peliosis, necrotising myopathy, acute renal failure, adrenal changes and even sudden death.[588–594]

However, one of the major effects caused by use and abuse of anabolic androgenic steroids is ventricular myocardial dysfunction with decreased early and increased late diastolic filling. In fatal cases this may be accompanied with fibrosis and myocytolysis and together these form risk factors for ventricular arrhythmias and congestive heart failure.[595–601] These changes may not be reversible. Several years after ceasing to use anabolic steroids, strength athletes still had evidence of left ventricular hypertrophy when compared with similar athletes who had not used these drugs.[602]

Abuse of anabolic androgenic steroids may also lead to liver toxicity with intrahepatic cholestasis.[603–605] Their use has also been associated with the development of benign adenomas and adenocarcinoma of the liver,[606,607] and with other benign tumours.[608]

It is perhaps understandable that testosterone and oestradiol have been singled out as veterinary drugs with significant potential to adversely affect human health.[286]

13.12.2.2 Diethylstilboestrol

Diethylstilboestrol (DES) is an oestrogenic drug that is not a steroidal compound. In fact, it is a derivative of the aromatic hydrocarbon stilbene and it is

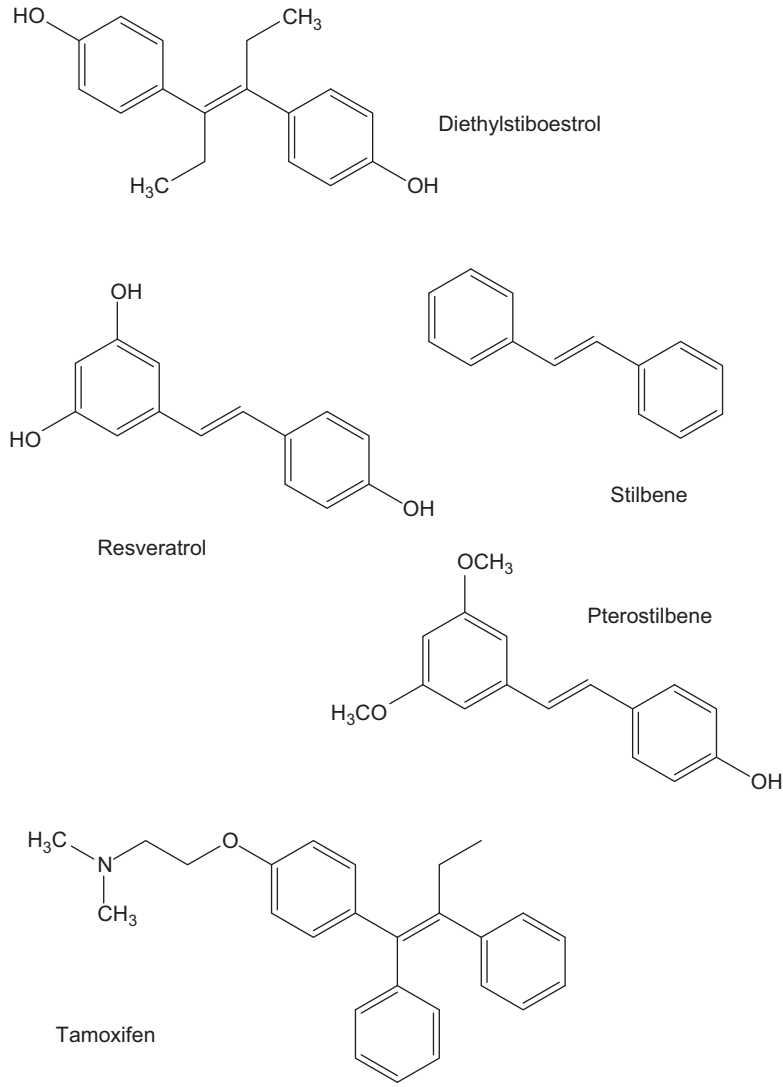


Figure 13.22 Chemical formulae of diethylstilboestrol, stilbene, resveratrol, pterostilbene and tamoxifen.

structurally related to the phytoalexins resveratrol and pterostilbene. Resveratrol is found in the skins of red grapes, other fruits and red wine, while pterostilbene is found in grapes and blueberries.[609] Resveratrol possesses oestrogenic activity and has been investigated for anticancer activity.[610–612] These compounds also have structural similarities with the anticancer drug tamoxifen (Figure 13.22).

DES currently has a role in the treatment of advanced metastatic prostate cancer.[613–615] Previously, it was used in the period 1940 to 1970 for the

maintenance of pregnancy and the prevention of various complications of pregnancy.[616–618] It has also been used as a growth promoter in cattle and, along with the related compound hexestrol, for the chemical castration (caponisation) of male poultry, and notably chickens.[619–621] This latter use produced chickens with higher fat content, especially abdominal and other muscle fat content.[622–624]

All of this was to change when it became apparent following the publication of several reports that a number of women who were exposed to DES *in utero* when their mothers were given the drug, developed clear-cell carcinoma of the vagina on reaching puberty or shortly afterwards.[625] Although tumours of this type were known previously, they were always in older women and recognising this, and following what appeared to be a cluster of cases, Herbst and his colleagues analysed the available data and recognised the association between development of these tumours and *in utero* exposure to DES.[626] Since that time, the adverse effects resulting from *in utero* exposure to DES have become well recognised.[627–638] The data on cancer risk in mothers given DES are less clear with some data suggesting no effects but other data suggesting an increased incidence of breast cancer.[639,640] Other adverse effects include genital tract abnormalities which may lower fertility in women, spontaneous abortion, preterm delivery, ectopic pregnancies and an increased risk of hypospadias in sons of women given DES during pregnancy.[641–644] A further major concern is that the effects of DES may be transgenerational and that adverse effects such as tumours or hypospadias may occur in future generations.[645,646] DES appeared to have no effects on secondary sex ratio in women exposed to DES *in utero*.[647]

13.12.2.3 Regulatory Aspects

DES was prescribed to over 2 million women in the 1990s,[647] and as noted above, was widely used as a growth promoter in poultry and cattle. These uses came to an abrupt end once the facts about the adverse effects of DES following *in utero* exposures became known. In 1980, high levels of DES were found in baby food made from veal from France and sold in Italy. It is thought that this resulted from the illegal use of injectable DES formulations leading to high concentrations of DES at the injection site in treated cattle.[648–650] These factors contributed firstly to the ban on the use of DES in animal production and then to the European ban on hormonal growth promoters and the uses of these drugs are now confined to non-food animals and some therapeutic uses as discussed earlier and shown in Table 13.3.[651,652]

As the growth promoting hormones had been banned in what is now the EU, there was little point in manufacturers applying for EU MRLs for these substances. However, the compounds were referred to JECFA for an evaluation for later consideration in the Codex Alimentarius system. What followed next is a rare example of how disagreements on toxicology and policy objectives can trigger a trade dispute. In the EU, the hormones were prohibited for growth promotion purposes under Council Directive 96/22/EC which replaced older legislation and included prohibitions on thyrostatic compounds and

β-agonists. These prohibitions were later refined by other amending legislation and notably by Directives 2003/74/EC and 2008/97/EEC.[653–655] However, JECFA evaluated a number of these drugs and in doing so, evaluated toxicology, pharmacology, residues and other appropriate data. In fact, JECFA evaluated trenbolone acetate, zeranol, 17β-oestradiol, testosterone, progesterone and melengestrol acetate.[656–660]

In these evaluations, JECFA has reviewed a vast quantity of reports on safety. As an example, when reviewing 17β-oestradiol, JECFA examined data related to metabolism, pharmacodynamics, acute toxicity, repeat dose toxicity, chronic toxicity and carcinogenicity, genotoxicity, reproductive toxicity, and observations in humans. When JECFA reviewed the natural hormones at its fifty-second meeting in Rome in 1999, it examined over 220 reports. At that meeting, JECFA calculated ADI values for 17β-oestradiol, progesterone and testosterone on the basis of hormonal parameters in menopausal women ($50\,ng\,kg^{-1}$ bw), changes in the uterus ($30\,\mu g\,kg^{-1}$ bw) and effects in eunuchs ($2\,\mu g\,kg^{-1}$ bw) respectively.[659] The synthetic hormones have also been favourably assessed by JECFA.[661]

In the USA, the FDA has based its assessments of the natural hormones on physiological effects, and the consideration that meat containing additional hormones arising from growth promoting uses is equal to 1% or less than the amounts produced daily by prepubertal children. In addition to the amounts of hormones naturally biosynthesised by animals, this is therefore insignificant. An ADI approach has been adopted for the synthetic analogues based on arguments that are generally similar to those of JECFA.[652,662] The end result of this is that the growth promoters are permitted in some countries such as the USA and Canada but prohibited in others such as the EU member states. As EU legislation also means that the ban extends to animal products such as meat derived from treated animals, a trade dispute arose between the EU and the USA, with the latter claiming that the EU's ban was based on political considerations and not on scientific arguments.

In the EU, the Lamming Committee, a group formed from members of the Scientific Committee for Food and the Scientific Committee on Animal Nutrition had reached a provisional conclusion that the use of the natural hormones was safe and posed no consumer risk.[663] Nevertheless, the EU proceeded to ban the growth promoting uses of these compounds and this, taken together with the favourable view of both the Lamming Committee and of JECFA has provided conclusive proof for some that the ban was politically motivated and not inspired by good science.[664] The resulting trade dispute was eventually referred to the World Trade Organisation (WTO). The EU and the USA agreed that this was a topic for consideration under the Agreement on Sanitary and Phytosanitary Measures made under the Sanitary and Phytosanitary Agreement (SPA), for discussions under the General Agreement on Tariffs and Trade (GATT). Under the SPA, WTO members must base their legislation on Codex standards and guidelines. JECFA MRLs, through the Codex Committee on Residues of Veterinary Drugs in Food eventually become Codex standards and the MRLs for some synthetic steroids, including those for

melengestrol acetate, trenbolone acetate and zeranol (as well as methods of residues of analysis), have been adopted by Codex Alimentarius based on JECFA recommendations.[665] Moreover, under the SPA, members are obliged to base their arguments on scientific and technical issues, and the USA argued that the EU ban was not made on scientific principles and nor was it based on sound evidence. In support of this, the USA cited the Lamming Committee report as well as reports by JECFA and Codex and hence, in its view, the ban was contrary to a number of Articles of the SPA agreement.[652]

A panel was formed under the terms of what has become known as the Uruguay Round to try to settle this dispute. Both the EU and the USA provided evidence and arguments. It has to be said that some of the EU arguments were flimsy and even whimsical. For example, the USA argued that meat from treated animals was like any other meat and that in the EU, meat from the USA received less favourable treatment than domestically produced meat. The EU argued that meat from treated animals was not like other meat and that all hormone treated meat was banned, not just meat from the USA. Furthermore, despite the wording of the SPS, the EU argued that Codex standards were in fact levels of protection and that in any case, Codex MRLs were not standards.[652]

The panel rejected the arguments put forward by the EU and found that as the EU's arguments were not based on international standards, then its prohibition was inconsistent with the SPS agreement. Eventually, the issue was referred to an appellate body.

To summarise, in 1999, a panel met under the auspices of the WTO to consider two complaints, one brought by the USA and the other by Canada to argue the case that an EU ban of imports of beef from cows treated with hormones (17β-oestradiol, progesterone, testosterone, trenbolone acetate, zeranol and melengestrol acetate) contravened the terms of the trade agreements mentioned earlier. The EU insisted the ban was essential for food safety while the USA and Canada claimed there was no harm to human health. The panel found that the EU violated a number of articles of the SPS agreement. The appellate panel agreed with the Panel's findings, especially that the EU measures reflected a higher level of protection than was justified by risk assessment, and that there was no rational relationship between the measure and the scientific evidence submitted on the safety of the hormones, although it reversed the decision on similarities or otherwise of treated and untreated meat. It concluded that there was a difference between the two.

The EU was unable to, and probably had no intention of, implementing the decision to reverse the ban by the date of 13 May 1999. Hence, the USA and Canada sought retaliatory rights against the EU of US$ 202 million and CDN$ 75 million a year. The arbitrators considered this and lowered the amounts to US$ 116 million and CDN$ 11.3 million.

In 2002, the Scientific Committee on Veterinary Measures Relating to Public Health in the EU issued a report which provided evidence from 17 studies of the various hormones. This looked at a number of issues including exposure, alteration of gene expression, oestrogenic potencies, and the genotoxicity of 17β-oestradiol. Needless to say, most of the conclusions were not supportive of

the use of the hormone growth promoters and the Committee used the new evidence to support its previous, rather negative conclusions.

In 2007, investigators from the USA and Denmark studied the semen quality of fertile American men and examined this against maternal beef consumption by their mothers during pregnancy. It was found that sperm concentration was inversely related to maternal beef meals per week. In the sons of high beef consumption mothers, sperm concentration was 24% lower and the proportion of men with sperm concentrations below 20×10^6 per mL was three times higher than in men who mothers had had a lower beef intake. The author's concluded that maternal beef consumption and "possibly xenobiotics in beef" may affect testicular development *in utero* and adversely affect reproductive potential.[668] An editorial in the same issue of the journal appears to support this view and suggests that JECFA reviews the issue of hormone toxicity again.[669] There may be some degree of truth in this speculation. However, a number of issues need to be considered. The men in this study had an average age of just over 31 years which means that the mothers of these men were pregnant in the mid-1970s, long before any safety evaluations by JECFA and before some of the compounds now in use had been introduced, although they could conceivably have been exposed to DES residues, as DES was withdrawn in the US in 1979. However, it also has to be recognised that cattle have naturally occurring levels of testosterone, progesterone and oestradiol and that the concentrations present depend on the physiological status of the animal *e.g.* calf *versus* cow, heifer *versus* pregnant or lactating cow, castrated male, and so on. However, even if the concentrations of endogenous hormones are the same in meat (and milk), it stands to reason that those who consume greater amounts of meat will be exposed to higher amounts of endogenous hormones than those who eat smaller amounts. This issue in particular needs to be addressed before concerns over residues are raised. This is emphasised by the fact that one of the authors (from the EU) has previously criticised JECFA and the US approach to assessing the safety of hormones and has suggested that JECFA evaluations be revised.[670] This paper was published in 1999 which coincidentally is the same year that the SPS Panel ruled on the USA/EU hormones trade dispute and since that time JECFA has obliged and revised its earlier views on the hormones. It also seems relevant that the high levels of illegal hormones and other growth promoters which some claim is a major problem might be a cause for greater concern.[671,672]

Perhaps a more justified concern is the fate of hormone growth promoters from intensive farming enterprises. These have the capacity to produce significant quantities of run-off, manure and slurry and yet the environmental fate of any exogenous hormones present is largely unknown (see also Chapter 16).[673–681]

13.13 Corticosteroids

The adrenal cortex produces two types of steroid hormones, the androgens and the corticosteroids. Natural and synthetic androgens have already been discussed. The corticosteroids can be divided into the glucocorticoids and the

mineralocorticoids depending on the receptor affinity and function. The physiological functions of the natural corticosteroids are widespread and range from roles in carbohydrate metabolism, lipid metabolism, electrolyte balance and anti-inflammatory responses, generally working in a concerted manner with other hormones. The major endogenous corticosteroids are hydrocortisone (cortisol), cortisone and aldosterone. In human medicine natural and synthetic corticosteroids are used for a variety of therapeutic purposes including (but not limited to) replacement therapy in adrenal insufficiency, for rheumatic disorders, in renal disease, the treatment of allergies and in the treatment of some malignancies.[682] Similarly, in veterinary medicine they have a range of uses, including the treatment of skin diseases, such as dermatitis, and local infections including otitis and mastitis in cattle.[683] The major glucocorticoids used in veterinary medicine, usually in combination with other drugs such as antimicrobials and antifungal drugs are hydrocortisone, prednisolone, betamethasone, dexamethasone and mometasone (note, betamethasone and dexamethasone are isomers of each other) and their chemical structures are shown in Figure 13.23). As an illustration, some formulations available in the UK and their therapeutic uses are shown in Table 13.4.

Although needlestick injuries may occur during the use of injectable products, the most likely route of human exposure to these substances is dermal during topical use, either through spillage of liquid products, for example when applying an otic product to the ear or on application of products that require dermal application. Infrequent application is unlikely to be a major occupational issue but more frequent administration may give rise to concerns as the corticosteroids and more specifically the glucocorticoids, are known to have adverse effects on the skin.

Topical application and systemic administration as well as administration by inhalation can lead to atrophy of the skin which may be severe and lead to skin thinning, skin tearing and other dermal effects which may result in increased morbidity and mortality.[684–690] The effects are also seen in animal models and hydrocortisone, dexamethasone, betamethasone and triamcinolone have induced dermal atrophy in the rat.[691]

The mechanism is not yet fully understood but it is thought to involve inhibition of collagen synthesis and hyaluronic acid synthesis. Inhibition of keratinocyte growth factors may also be involved.[692–696] Inhibition of hyaluronic acid synthesis, through suppression of hyaluronan synthase may also explain some of the adverse effects of glucocorticoids in bone.[697] Consequently, those who need to apply products containing corticosteroids to animal patients on a regular basis would be well advised to wear protective gloves to prevent the occurrence of dermal adverse effects.

Of course, the corticosteroids are known to produce a whole spectrum of effects when given at high therapeutic doses and for prolonged periods.[682,698]

13.14 Prostaglandins

The major prostaglandins used in veterinary medicine are dinoprost (prostaglandin $F_{2\alpha}$, Figure 13.24) and cloprostenol, which is a synthetic racemic

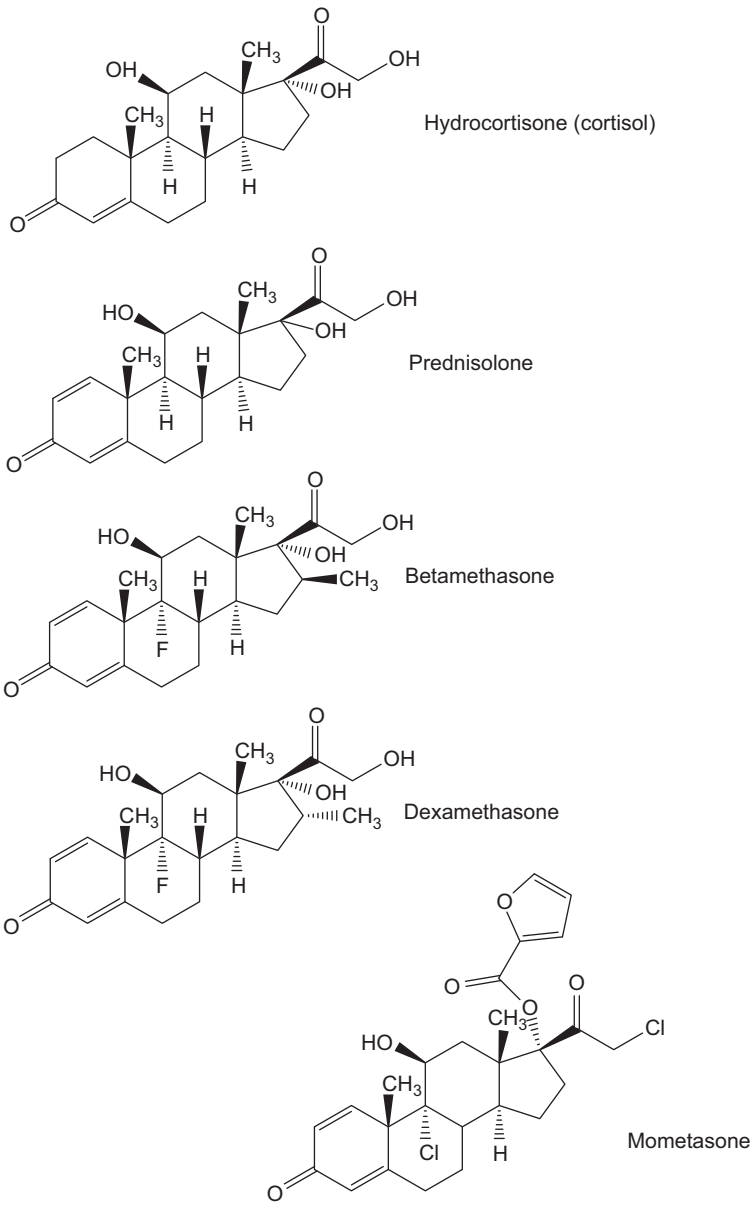


Figure 13.23 Chemical formula of hydrocortisone, prednisolone, betamethasone, dexamethasone and mometasone.

derivative of prostaglandin $F_{2\alpha}$. They are used for a number of purposes including regression of the corpus luteum in cattle to return the animals to oestrus, and for the induction of parturition in pregnant animals including cattle, pigs and horses. The prostaglandins have been identified as being a

Table 13.4 Some corticosteroid preparations available in the UK.[160]

Corticosteroid	*Other actives*	*Species*	*Route of administration/ main uses*
Betamethasone	Fusidic acid	Dogs	Topical/surface pyoderma, wet eczema
	Clotrimazole	Dogs	Otic/otitis externa
Dexamethasone	Marbofloxacin	Dogs	Otic/otitis externa
	—	Horses, cattle, pigs, dogs, cats	Intravenous or intramuscular in horses; intramuscular in other species/ inflammatory and allergic conditions; treatment of ketosis in cattle
Hydrocortisone	—	Dogs	Spray/inflammatory and pruritic skin conditions
	Miconazole nitrate, gentamicin	Dogs	Otic/otitis externa
Prednisolone	Framycetin, nystatin	Dogs, cats	Otic/otitis externa
	Cefapirin	Cattle	Intramammary/mastitis
	Amoxicillin, clavulanic acid	Cattle	Intramammary/mastitis
	Novobiocin, neomycin, procaine penicillin, dihydrostreptomycin	Cattle	Intramammary/mastitis
	Penethamate, framycetin, dihydrostreptomycin	Cattle	Intramammary/mastitis
	Cinchophen	Dogs	Oral/osteoarthritis
	—	Dogs, cats	Oral/anti-inflammatory, anti-allergic, autoimmune diseases, neoplastic disease
	Miconazole nitrate, polymixin B	Dogs, cats	Otic or suspension/otitis externa, skin disease
Mometasone	Orbifloxacin, posaconazole	Dogs	Otic/otitis externa

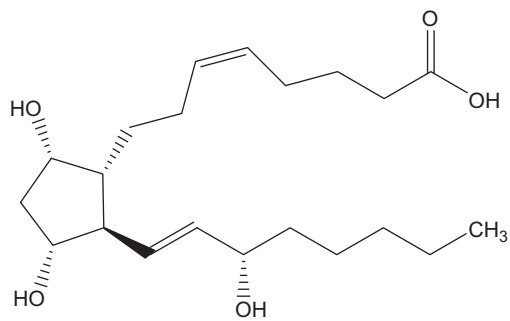


Figure 13.24 Chemical formula of prostaglandin $F_{2\alpha}$.

concern for human health when used in veterinary medicine because of their potency and the possibility of self-injection accidents.[286]

In human medicine, prostaglandin $F_{2\alpha}$ is used for the induction of therapeutic and elective abortions, and for the induction of labour, either alone or in conjunction with other drugs such as oxytocin.[699–707] Abortion with prostaglandins may involve relatively late abortion (second trimester to early third trimester). They are used for similar purposes in animal patients.[708–710] Entry of prostaglandin $F_{2\alpha}$ into the systemic circulation can cause life-threatening effects including intravascular collapse, hypertension and bronchoconstriction.[711]

Consequently, accidental self-injection with a prostaglandin product could have a serious outcome. This is particularly true when used by pregnant women and clearly, great care needs to be taken when working with these products, especially as prostaglandins are well absorbed through the skin. In the UK, the following phrase appears on the labels of these products: ''Direct contact with the skin or mucous membranes of the user should be avoided. Prostaglandins of the $F_{2\alpha}$ type may be absorbed through the skin and may cause bronchospasm or miscarriage. Care should be taken when handling the product to avoid self-injection or skin contact. Pregnant women, women of childbearing age, asthmatics and persons with other respiratory tract diseases should exercise caution when handling. Those persons should wear gloves during administration. Accidental spillage on the skin should be washed immediately with soap and water. In case of accidental injection, seek medical advice immediately and show the package insert or the label to the physician.'' This would appear to be sound advice. Oddly, the progesterone receptor antagonist aglepristone, which is used by injection to terminate pregnancy in cats and dogs,[712–719] is not subject to similar advice.

13.15 Somatotropins

Bovine somatotropin is used in cattle to enhance milk production. Porcine somatotropin is used in pigs as a growth promoter and carcass quality enhancer. They are authorised in a number of countries, but, not surprisingly, not in the EU. These agents are either endogenous hormones or recombinant analogues of endogenous polypeptide hormones. Their toxicity, or lack of it, has been reviewed in some depth by JECFA.[720–722] On ingestion, they are subject to normal digestive processes and are therefore not active orally.[723] JECFA reasoned that the only possible risk to human health was from elevated levels of insulin-like growth factor (IGF-1). However, treatment of animals with IGF-1 had no effects and JECFA considered that the somatotropins did not require the establishment of MRL values and that ADI values need not be specified.

An EU MRL application was made for another somatotropin, somatosalm. This drug is used to enable farmed Atlantic salmon to make the safe transition from freshwater to the marine environment. The applicant made the

application and the CVMP gave an opinion that no MRL was required on public health grounds, and the opinion was passed to the European Commission for adoption. The Commission did not take action to adopt the CVMP opinion but instead sought further advice from CVMP, seemingly amid fears that the product might be employable in other animal species as a growth promoter. As a result of the delays the applicant took the matter to the European Court of Justice (*Pharos S. A. vs. Commission of the European Communities*). Unfortunately, the Court of First Instance, a lower body, took a different view from the applicant. It considered that the Commission's delays while it sought further advice were reasonable, as was actually seeking that advice.[724] Eventually the favourable CVMP opinion on somatosalm was adopted.[725]

13.16 Conclusions

This chapter, along with other chapters in this book, demonstrates just what a wide variety of different drugs are used in animal medicine and how some may pose risks to consumers or to users, while others are devoid of significant user risk, consumer risk or both. With the correct regulatory framework and implementation of regulatory requirements, this means that veterinary drugs can be safely used in animals providing the correct precautions are taken, and that when used in food animals appropriate tolerances or MRLs are imposed and enforced. The substances which have been discussed in this chapter and which have EU MRL values for use in food animals are shown in Table 13.5. However, sometimes unseen consequences cannot be avoided. In the UK and in other countries, animals that are not considered fit for human consumption may be supplied to pet food manufacturers or to those who keep large numbers of animals. If the animals have been euthanized with toxic substances, then this can pose a risk to animal "consumers". In one case, a colony of otters died after being fed meat from a horse euthanized with barbiturate, while in others, dogs have slept for long periods after being fed barbiturate-contaminated meat.[726,727] Taken with the clenbuterol incidents described earlier in this chapter, this does help to underline the importance of the regulatory system and its enforcement, even if drugs intended for food animals are not considered for their safety to otters or to dogs.

As this is the final chapter in this book to discuss individual compounds or classes of drugs, it is perhaps a convenient point to briefly examine the substances which have been reviewed for their toxicological, pharmacological and microbiological properties, as described in Chapters 3 and 17, in the pursuit of EU MRLs. Over 700 substances have been evaluated and as can be seen from Figure 13.25, the majority of these were considered not to require MRL values.

These substances include many simple salts, herbal remedies and components of veterinary homeopathic formulations. Others include medical gases, drugs that are rapidly metabolised to materials which are not pharmacologically and toxicologically active and biologically inert materials such as some

Table 13.5 Substances discussed in Chapter 13 and their EU MRL status.

Etorphine	No MRL[a]
Butorphanol	No MRL required, equidae only, intravenous use only
Buprenorphine	No MRL
Fentanyl	No MRL
Alfaxalone/alphadalone	No MRL
Detomidine	No MRL required, cattle and horses
Medetomidine	No MRL
Romifidine	No MRL required, horses
Dexmedetomidine	No MRL
Xylazine	No MRL required, cattle and equidae
Atipamezole	No MRL
Chlorpromazine	Prohibited in food species
Acepromazine	No MRL
Azaperone	MRL established; pigs
Fluanisone	No MRL
Carazolol	MRLs established; pigs – excludes use in transport to slaughter to avoid injection site residues
Clenbuterol	MRLs established; solely for tocolysis in cattle and horses and respiratory ailments in horses
Ractopamine	No MRL – not authorised for use in EU
Zilpaterol	No MRL – not authorised for use in EU
Isoxsuprine	No MRL required; cattle and equidae
Carprofen	MRLs established; cattle including dairy cattle, equidae
Diclofenac	MRLs established; cattle including dairy cattle, equidae
Ketoprofen	No MRL required; pigs, cattle and equidae
Tolfenamic acid	MRLs established; cattle and pigs
Flunixin meglumine	MRLs established; cattle, pigs and horses
Phenylbutazone	No MRL
Meloxicam	MRLs established, cattle, pigs, equidae, rabbits, goats
Tepoxalin	No MRL
Cimicoxib	No MRL
Robenocoxib	No MRL
Mavacoxib	No MRL
Firocoxib	MRLs established; equidae
Atropine	No MRL required; all food producing species
Scopolamine	No MRL, but no MRL required for butylscopolamine bromide; all food producing species
Hyoscyamine	No MRL
Lidocaine	No MRL required; equidae, for regional anaesthesia only
Bupivacaine	No MRL
Mepivacaine	No MRL required; equidae for intra-articular and epidural use only
Tricaine mesylate	No MRL required; fin fish for water borne use only
Bromide, sodium and potassium salts	No MRL required; all food producing species
17-β oestradiol	No MRL required; cattle and horses, therapeutic purposes only
Testosterone	No MRL
Progesterone	No MRL required; for therapeutic and zootechnical use only
Allyltrenbolone (altrenogest)	No MRLs required; pigs and horses for zootechnical use only
Other steroid hormones	No MRLs have been established due to the EU prohibition for growth promoting purposes – refer to text

Table 13.5 (*Continued*)

Hydrocortisone	No MRL required; all food producing species, topical use only
Prednisolone	MRLs established, cattle
Betamethasone	MRLs established, cattle and pigs
Dexamethasone	MRLs established, cattle, pigs, goats and equidae
Mometasone	No MRL
Beclometasone dipropionate	No MRL required, equidae, inhalation use only
Dinoprost/dinoprostone	No MRL required, all mammalian food producing species
Cloprostenol and R-cloprostenol	No MRL required, bovine, pigs, goats, equidae
Bovine somatotropins	No MRLs have been established due to the EU prohibition for milk production enhancing purposes – refer to text
Somatosalm (fish somatotropin)	No MRL required

[a]No MRL means that MRL values or no MRL required status have not been determined. This may be because of insufficient data, data that suggests that the product may not be safe to use in food animals intended for human consumption or, more frequently, because the product was developed specifically for use in companion animals.

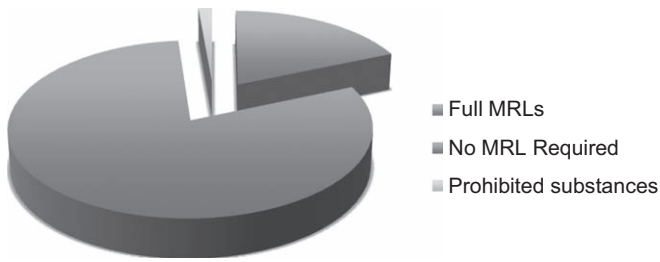


Figure 13.25 Substances evaluated for EU MRLs, 1992 to 2012 and their classifications.

polymers. A considerable number are approved food additives with E numbers. The remaining categories are those with numerical MRLs and those prohibited for use in food animals. The constituents of the group considered not requiring MRL values are shown in Figure 13.26.

For those drugs that have been awarded MRL values, the majority are antimicrobial agents followed closely by antiparasitics (Figure 13.27). This is not surprising as bacterial and parasitic diseases are among the most common conditions affecting farmed animals. The remaining drugs include the NSAIDs, but others, and notably the antifungal agents, are poorly represented.

All of this should provide reassurance that in the EU at least, drugs and other agents used in food producing animals are adequately assessed for their toxicological properties thus protecting consumer safety. As described in Chapter 3, similar measures apply in other countries.

Figure 13.26 Categories of substances not requiring MRLs.

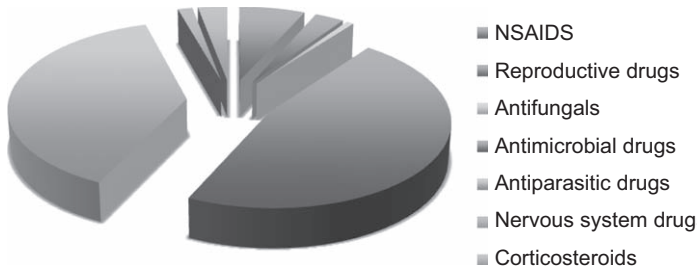


Figure 13.27 Categories of drugs with numerical MRL values.

It is perhaps disappointing therefore to note that in the period leading to the London Olympic Games in 2012, some Chinese athletes avoided the consumption of meat.[728] This is because of fears over residues of clenbuterol which is used illegally in China, and the consequences for drug surveillance during the games, thus highlighting the concerns of others.[276]

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CHAPTER 14

Human Safety of Veterinary Vaccines

14.1 Introduction

Vaccines are essential tools in preventing disease in humans and in animals. In veterinary medicine, there is a substantial range of vaccines available for disease prevention in companion animals and in farmed species. Table 14.1 shows just a few types of vaccines that are currently available, and the diseases they are intended to prevent. They may be supplied as solutions, suspensions or as lyophilisates with a suitable solvent system, usually water for injection (sterile) and reconstitution prior to use. They may be given by a number of routes but subcutaneous or intramuscular injections are the most frequently used. Although Table 14.1 suggests that each antigen is given separately, vaccines are frequently available as multivalent formulations containing a number of antigens. This is particularly true of vaccines intended for cats and dogs, and for oral coccidial vaccines for chickens, which usually contain several precocious eimerial strains.[1–10]

Human and animal vaccines are highly regulated products that are only authorised once the criteria of safety, quality and efficacy are satisfied. In the USA, vaccines are regulated by the US Department of Agriculture, while in the European Union, they are regulated as veterinary medicinal products by national regulatory authorities and by the European Medicines Agency.[11,12] Prior to authorisation, they must undergo stringent testing for safety, efficacy and quality, including tests for extraneous agents, for stability and reversion to virulence in addition to studies of single, repeat and overdose safety, effects on reproductive safety and immunologic function and interaction with other products that might be used at the same time in particular animals. In the EU, vaccines must also be subjected to a user risk assessment, in the same manner as pharmaceuticals.[13–24]

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By Kevin N. Woodward

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Table 14.1 Some typical animal vaccines.

Species	*Disease*	*Organism*	*Administration Route*
Chicken	Gumboro disease; avian infectious bursal disease	Virus	Subcutaneous; *in ovo*
	Coccidiosis; *Eimeria* strains	Protozoa; apicomplexan	Oral
	Newcastle disease	Virus	Ocular, ocular nasal
	Infectious bronchitis	Virus	Intramuscular
	Avian rhinotracheitis	Virus	Intramuscular
	Salmonellosis; *Salmonella typhimurium, S. enteritidis*	Bacteria	Intramuscular
	Chicken anaemia	Virus	Intramuscular/ subcutaneous
	Airsacculitis, tracheitis; *Mycoplasma gallisepticum*	Mycoplasma	Ocular nasal spray
	Marek's Disease (T-cell lymphoma, immunosuppression, limb paralysis)	Virus	Subcutaneous
Turkey	Erysipelas; *Erysipelothrix rhusiopathiae*	Bacteria	Subcutaneous
Pigeon	Pigeon paramyxo viral disease	Virus	Subcutaneous
	Newcastle disease	Virus	Subcutaneous
Rainbow trout	Enteric redmouth (*Yersinia ruckeri*)	Bacteria	Bath/oral
	Vibriosis (*Listonella (Vibrio) anguillarum*)	Bacteria	Bath/ intraperitoneal
	Furunculosis (*Aeromonas salmonicida*)	Bacteria	Intraperitoneal
Atlantic salmon	Furunculosis (*Aeromonas salmonicida*)	Bacteria	Intraperitoneal
	Salmon pancreatic disease	Virus	Intraperitoneal
Rabbit	Rabbit viral haemorrhagic disease	Virus	Subcutaneous
	Myxomatosis	Virus	Intradermal
Pig	Porcine proliferative enteritis, porcine circovirus	Virus	Intramuscular
	Porcine parvovirus	Virus	Intramuscular
	Mycoplasma hyopneumoniae	Mycoplasma	Intramuscular
	Glässer's disease, *Haemophilus parasuis*	Bacteria	Intramuscular
	Swine erysipelas; *Erysipelothrix rhusiopathiae*	Bacteria	Intramuscular
	Porcine reproductive and respiratory syndrome (PRRS); blue ear	Virus	Intramuscular/ intradermal
Sheep	Enzootic abortion (*Chlamydophila abortus*)	Bacterial[a]	Subcutaneous
	Louping-Ill	Virus	Subcutaneous
	Orf	Virus[b]	Skin scarification
	Toxoplasma gondii (embryonic death, abortion)	Protozoa[c]	Intramuscular

Table 14.1 (*Continued*)

Species	*Disease*	*Organism*	*Administration Route*
Cattle	Bovine herpes	Virus	Intramuscular
	Bovine respiratory syncytial disease	Virus	Subcutaneous
	Bovine diarrhoea	Virus	Intramuscular
	Lungworm (*Dictyocaulus viviparous*)	Nematode	Oral
	Ringworm (*Trichophyton verrucosum*)	Fungus	Intramuscular
	Leptospirosis (*Leptospira interrogans* serovar *hardjo*)	Bacteria (spirochete)	Subcutaneous
	Rotavirus	Virus	Intramuscular
	Coronavirus	Virus	Intramuscular
Cattle/sheep	Clostridia	Bacteria (toxoids)[d]	Subcutaneous
	Blue tongue	Virus	Subcutaneous
Horse	West Nile disease	Virus	Intramuscular
	Equine influenza	Virus	Intramuscular
	Lymph node abscesses (*Streptococcus equi*)	Bacteria	Submucosal
	Equine arteritis	Virus	Intramuscular
Cat	Rabies	Virus	Subcutaneous
	Feline rhinotracheitis herpes	Virus	Subcutaneous
	Calicivirus	Virus	Subcutaneous
	Feline panleucopenia	Virus	Subcutaneous
	Chlamydiosis (*Chlamydophila felis*)	Bacteria	Subcutaneous
	Feline bordetellosis (*Bordetella bronchiseptica*)	Bacteria	Intranasal
	Feline leukaemia	Virus	Subcutaneous
Dog	Rabies	Virus	Subcutaneous
	Canine distemper	Virus	Subcutaneous
	Parvovirus	Virus	Subcutaneous
	Infectious canine hepatitis	Virus	Subcutaneous
	Canine parainfluenza	Virus	Subcutaneous
	Leptospirosis (*Leptospira canicola; L. icterohemorrhagiae*)	Bacteria (spirochete)	Subcutaneous
	Kennel cough; acute tracheobronchitis (*Bordetella bronchiseptica*)	Bacteria	Intranasal

[a]Zoonotic disease, product should not be handled by pregnant women.
[b]Zoonotic disease, product may cause Orf in humans.
[c]Zoonotic disease, may cause toxoplasmosis in humans.
[d]Usually toxoids/cultures of one or more of *Clostridium perfringens*, *C. chauvoei*, *C. novyi*, *C. tetani*, *C. sordellii* and *C. haemolytica*.

In animal patients, the safety of vaccines is normally very high. However, adverse reactions may occur due to systemic reactions, allergic reactions, effects on the immune system, residual pathogenicity, inadequate inactivation, genetic recombination and contamination. Moreover, injection site reactions are

common in mammals, birds and fish.[16,25–34] Some of these effects may be caused or exacerbated by adjuvants used in vaccines which commonly include mineral oil and aluminium-based compounds such as aluminium hydroxide.[34–38] An elevated incidence of injection site sarcomas has been observed in cats. The aetiology of this condition is not well understood but it may be a result of chronic inflammation at the injection site.[39–45] Systemic effects include reduced milk yields in cattle, effects on pulmonary function and hyperthermia in several species, hepatocellular necrosis in dogs given a *Bordetella bronchiseptica*-canine parainfluenza virus vaccination, alopecia, and panniculitis in cats.[25,46–52] The mechanisms underlying many of these effects are poorly understood.

Several components of vaccines may give rise to allergic reactions in animals. These include cells, cellular debris, serum and other foreign proteins and preservatives including antibiotics and the antigens themselves and allergic reactions are relatively common.[16,25,53–55] In the UK, in the period 1985 to 1999, around 8% of suspected adverse reactions in cats and 20% in dogs were anaphylaxis and hypersensitivity reactions while in a retrospective cohort of 1.2 million dogs and 3.5 million doses of vaccine in the USA, 4678 adverse reactions occurred with the majority being allergic or possibly allergic reactions.[56,57]

Idiopathic arthritis or idiopathic immune-mediated arthritis is a relatively common disease of dogs and it has been suggested that it may be induced by vaccination.[58] Polyarthritis has frequently been associated with dog vaccination.[59,60] Autoimmune disease has been induced in beagles using a multivalent vaccine.[61]

Infective disease may be induced for several reasons. These include residual pathogenicity due to partial attenuation of pathogens used in vaccines or inadequate activation (as occurred in the infamous Cutter disaster and live poliovirus in humans), genetic recombination for examples where organisms with deleted genes for pathogenicity reacquire them, and reversion to virulence of an otherwise attenuated antigen.[16,25,49,62–70]

Cross contamination of vaccines with other pathogens has also resulted in mortality and morbidity in animals. These incidents have included pseudorabies virus contaminated with pestivirus, Marek's disease virus contaminated with reticuloendotheliosis virus, contamination of cell lines and vaccines with bovine diarrhoea virus, bluetongue in dogs arising from contaminated live canine vaccine and clostridial disease in ruminants – of 202 523 animals in affected herds, 41 767 were infected with *Clostridium sordellii* and 22 189 died, possibly as a result of a failure in a sterility test for detecting contaminants in a clostridial vaccine.[16,25,71–78]

Of course, these vaccines are designed and developed for use in animals and exposure of humans to these products is not expected to happen, at least under normal conditions of use. However, exposure to vaccines, or to components of vaccines may occur either through direct exposure or through exposure to residues of chemical components of vaccines intended for use in food animals. The direct exposure route has implications for zoonotic disease and physical injury, while the indirect route has implications for potential toxicity.

14.2 Zoonotic Diseases

A number of veterinary vaccines contain components that are pathogenic in humans and which could conceivably cause disease, although the vast majority are specific to the animals they infect. An example of a disease that infects both animals and humans is rabies, and rabies vaccines are commercially and widely available. However, the problem is overcome by inactivation of the virus to produce a product that is safe for both the vaccinated animal and for those who administer it. However, as Table 14.1 indicates, some products are available that may cause disease in humans if used improperly or should accidental injection or a needle stick injury occur. Clearly, the results of human contamination and infection are microbiological and not toxicological. Nevertheless, it is worth examining these briefly for the sake of completeness and to emphasise that user safety is not the sole domain of pharmaceutical products.

The types of veterinary vaccine products available differ from country to country. This not only reflects commercial considerations but also disease patterns, which vary depending on location. As with human medicine, animal diseases differ with climactic conditions and so different vaccines are required in equatorial conditions when compared with those needed in northern Europe. Table 14.1 is neither comprehensive nor is it representative of every climactic area. In fact, it is largely (but not entirely) based on the United Kingdom and the vaccine products commercially available there.[79] The table also shows that in the UK there only three enzootic disease vaccines available which are capable of causing disease in humans. Such vaccines are usually authorised, in spite of the obvious hazards and associated risks, either because the inactivated versions are not effective or if they are, they are not as efficacious as the live version.

Toxoplasmosis caused by *Toxoplasma gondii* is a disease of humans and other mammals that occurs on a worldwide basis. It results in acute effects characterised by swollen lymph nodes and influenza-like symptoms. It may also produce skin lesions in animals and humans, and has been associated with psychiatric disorders in the latter. Humans are usually infected due to consumption of infected meat or from exposure to cat faeces from infected animals. Immunocompromised individuals are known to be more susceptible to infection.[80–86] There is evidence that toxoplasmosis may be associated with an increased incidence of brain tumours in humans.[87,88] Congenital toxoplasmosis may occur. The vaccine for sheep available in the UK contains live tachyzoites of *T. gondii* and is intended to protect infected animals by reducing the effects on the infection, and specifically to reduce early embryonic death, infertility and abortion.[79] The operator warnings in the product literature preclude use by pregnant women or women of child-bearing age. They also warn against use by immunocompromised individuals including those with HIV infection, people undergoing cancer chemotherapy and those taking immunosuppressive drugs. Users are advised to wear gloves, to avoid self-injection and to seek medical advice if self-injection occurs. A statement recognises that pyrimethamine treatment is currently recognised as being effective although there is no recommendation to use it. Presumably this is left to the judgment of the

physician. Hence, the risk communication and risk management consist of sound advice and sensible recommendations for the use of this product.

Sheep also suffer from enzootic abortion caused by *Chlamydophila abortus* infection (Table 14.1). Vaccination is effective in controlling this disease.[89] This and related bacterial species can infect humans and cause abortion in pregnant women.[90–93] Like the *Toxoplasma* vaccine, this product requires careful handling particularly by pregnant women and women of child bearing age. The advice to users is similar, especially with regard to immunocompromised individuals and the need to seek medical attention. The product literature for this vaccine notes that treatment with tetracyclines is effective therapy for infections due to *Chlamydophila abortus*.

Orf (contagious ecthyma, contagious pustular dermatitis, sore mouth, scabby mouth) is a contagious disease of sheep and goats caused by a parapox virus and it one of the commonest diseases of these animals in some parts of the world.[94–98] It is another zoonotic disease that can be contracted from sheep and goats, and especially through bottle-feeding of lambs, contact with animal products, contact through religious activities and direct contact through other means with infected animals.[99–124] In humans, it is normally a mild disease affecting the skin and eyes; it produces small nodular lesions of the skin. These may enlarge but they tend to resolve over the course of a few weeks.[111,125–129] Unlike the diseases discussed above, it appears to have no adverse consequences on pregnancy outcome.[127,130] However, it may be more severe in immunocompromised patients.[131,132] In some agricultural communities, a significant proportion of the population may have been infected. In England, up to 15% of farmers have reported suffering from orf and in Wales around 29% reported the disease.[133,134] The disease may occasionally be difficult to diagnose and other conditions can be similar in appearance. For example, a case of sealpox appeared superficially similar to orf.[135] Human infection can now be diagnosed by the polymerase chain reaction.[136] Despite the fact that the disease is very infectious and the vaccines contain live viruses, there are no well-documented cases of disease arising from vaccine use.

In contrast to orf, brucellosis infection has developed in humans following self-injection with live brucella vaccine.[137–142] In the USA, the Centers for Disease Control and Prevention (CDC) received reports of 26 cases through passive surveillance and 21 of these had suffered needle stick injuries with contaminated needles used for animal vaccination, 4 had received conjunctival spray exposure and one individual had contamination through an open wound. There were no cases of brucellosis in these subjects. There is currently insufficient data to determine if the strain involved can cause systemic brucellosis in humans.[143] Self-injection with *Mycobacterium paratuberculosis* bacterin (Johne's disease bacterin) produced only minor local reactions.[144]

14.3 Physical Injury – High-pressure Injection Injuries

Vaccines intended for use in individual animals including cats, dogs or rabbits, and those intended for use in relatively small numbers of animals such as cattle

and horses are normally given using a conventional syringe and needle. However, vaccines intended for administration to very large numbers of animals, and notably to poultry, are usually given by automatic equipment from a reservoir of vaccine, using high pressure delivery systems. On a global scale, this practice has resulted in serious injuries to the hands and digits of those involved. In the United Kingdom, injuries have arisen while administering vaccines through high-pressure equipment to pigs, poultry and other animals.[145–149] The amounts injected are usually precalibrated and appear relatively small. With poultry the volume is around 0.5 mL and for larger animals this may be 2 mL or higher, and these larger volumes may lead to severe tissue damage following self-injection.[150]

These types of accident are not confined to agriculture or indeed to vaccines. They have been recognised for many years and may involve oil, grease, paint, water, industrial solvents and even sand with injection into the palmar area of the hand or into a digit.[151–174] Occasionally, other body areas may be involved including the penis and scrotum.[175–178] They normally occur in an industrial setting such as paint spray workshops, service stations and other automotive undertakings, as well as in other areas of industry.

Superficial examination might suggest that these injuries are relatively benign and their significance has been overlooked in the past, leading to delays in treatment.[179–188] In reality, these are medical emergencies. Internally, there is frequently extensive damage to tissue that is caused by a combination of pressure, kinetic energy of entry and the chemical and biological properties of the material injected.[182,189–191] Injected material may penetrate tendon sheaths and fascial planes.[190] Tissue damage may include haemorrhage, vascular pressure and occlusion of digital and other blood vessels, oedema, local ischaemia, necrosis and inflammation and foreign body granulomatous change.[192–197]

If untreated, secondary infection may develop and gangrene can follow. Depending on the material injected, systemic toxicity is possible.[145,190,194,198] These injuries have been described as ‘devastating’ and they may require amputation of a digit, part of the hand or the entire hand if not treated promptly.[148,149,169,184,186,190,199–202] They are more severe when the substances injected are paint and solvents when compared with the effects of oils and water.[145,165,203]

Treatment of these injuries must be prompt and should include excision of the penetration point, irrigation to remove foreign material, debridement, synovectomy, decompression, removal of necrotic tissue and, where necessary, amputation. Antibiotic prophylaxis and anti-inflammatory drugs should be used as appropriate.[145,148,179,182,183,188,195,197,199–210]

Veterinary vaccines are frequently complex formulations. In addition to one or more antigens, they also contain a solvent or solvent system. To achieve maximum efficacy, these products usually contain an adjuvant. The nature of these adjuvants can vary widely but the most common ones are aluminium compounds, mineral oil, saponins and nanoparticles, and although their activities are poorly understood they may have immunomodulatory effects or

assist with antigen presentation.[211–216] They may cause inflammation following self-injection accidents.[214]

The oil-based vaccines appear to be the major problem group after self-injection. Following the injection of poultry vaccines where the dose delivered is usually small, the patient can frequently be treated with anti-inflammatory drugs and corticosteroids. However, self-injection of a 2 mL volume of a porcine vaccine has resulted in amputation of a digit and self-injection of 1 mL of a bovine vaccine into the thigh caused extensive tissue damage and long-lasting disability, and so these larger doses require the type of intervention described previously.[150,217] Injection of 1 mL of a bovine vaccine into a little finger base resulted in ischaemia and eventual amputation.[218] Self-injection with a vaccine containing Freund's complete adjuvant (Gudair) for the control of Johne's disease caused by *Mycobacterium paratuberculosis* resulted in several cases of hand injury which required surgical intervention.[144,218–220] Self-injection of a vaccine for *Salmonella enteriditis* resulted in necrosis of the digits in four cases.[221] A vaccine intended for use in aquaculture resulted in anaphylaxis after self injection.[222] Perhaps of greater concern is the observation that some oils used as adjuvants in human and veterinary vaccines, and some of their constituent hydrocarbons, can induce autoimmune disorders.[223–226]

The UK regulatory agency for veterinary medicinal products, the Veterinary Medicines Directorate (VMD) produced advice in 2003 to highlight the problem of self-injection and broader advice was published in the *British Medical Journal* and elsewhere to raise awareness of this accident, and how it should be treated.[227–230]

The VMD publishes its pharmacovigilance reporting results for animal, human and environmental adverse effects annually in the *Veterinary Record*. In 2007, five cases of self-injection were reported to the VMD and the treatment of these ranged from irrigation to skin grafts.[231] In 2008 and 2009 accidents involving vaccine administration were largely restricted to needle stick injuries.[232,233] However, in 2010, 36 reports involved vaccines and of these, 31 (86%) were simple needle stick injuries. The remaining 5 involved accidental self-injection and resulted in hospital out-patient treatments.[234]

14.4 Human Consumer Safety of Vaccine Excipients

Globally, the scientific requirements for safety, quality and efficacy testing of veterinary medicinal products apply equally to veterinary vaccines as they do to pharmaceutical products. Of course, by the very nature of vaccines, the testing requirements differ from those of pharmaceuticals and there is less of an emphasis on toxicity testing, and more of an emphasis on biological testing. As already described in this chapter, the major components of vaccines and indeed other biological products are antigens and other proteinaceous materials along with a suitable solvent or suspension system, usually water, and none of these offer a risk to consumers of food of animal origin. However, the majority of veterinary (and human) vaccines also contain other substances. The adjuvants have already been described earlier. However, other materials are also used as

stabilizers, preservatives, suspending agents, pH adjusting agents, vitamins and components of the solvent system including co-solvents with water as well as residual chemicals left over from the deactivation process, for example formaldehyde and glutaraldehyde. Some of these substances have the capacity for toxicity, and consumers must be given adequate protection from chemicals used in vaccines intended for use in food species. Table 14.2 shows examples of some of these substances. It is meant to be illustrative and not exhaustive.

These substances are assessed for toxicity and their potential to impact on consumer safety (as well as animal safety) before they are permitted in vaccines intended for food animal use as there is a recognition that some of them may be toxic.[235] The most controversial of these is thiomersal (thimerosal, Figure 14.1) an antiseptic and antifungal preservative and an organomercury compound. Its safety in human vaccines has long been the subject of debate, especially over its alleged association with autism and neurotoxic effects.[236–249] The current consensus appears to be that any risks associated with the use of thiomersal in human vaccines are outweighed by the undisputed benefits of preventing serious diseases.[239,243,244,248] In the European Union, excipients used in vaccines intended for use in food animals are subject to the same legislation on

Table 14.2 Examples of substances frequently used as components of veterinary vaccines and other biological products (including those used in animals intended for food production).

Aluminium hydroxide	Isopropanol
Aluminium salts	Lactic acid and lactates
Amino acids	Lecithin
Ammonium chloride	Montanide
n-Butanol	Neomycin
Benzoic acid	Nitrogenous bases
Benzyl alcohol	Orgotein
Betaine	Orotic acid
Butylated hydroxyanisole (BHA)	Permitted colours
Butylated hydroxtolune (BHT)	Poloxamers
Carnitine	Poloxalene
Cetrimide	Polyethylene glycols
Chlorocresol	Polymixin B
Diethanolamine	Polysorbates
Dimethyl sulphoxide	Propylene glycol
EDTA[a]	Salts of mono- & diglycerides
Ethanol	Sorbitol, mannitol, xylitol
Ethyl lactate	Sorbic, lactic, citric acid
Folic acid	Thiomersal
Formaldehyde	Thymol
Gentamicin	Titanium dioxide
Gluconates	Tocopherols
Glutaraldehyde	Urea
Glycerol formal	Vitamins
Iron salts	

[a]Ethylenediaminetetraacetic acid.

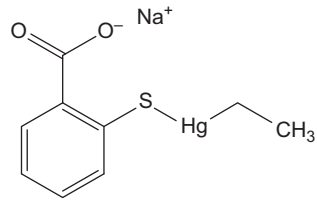

Figure 14.1 Chemical formula of thiomersal.

maximum residue limits (MRLs) as are pharmaceuticals and excipients used in pharmaceuticals. Hence they are assessed for human safety and either gain full MRLs (*e.g.* the antibiotics used both as chemotherapeutants and as preservatives) or they are considered not to require MRLs on public health grounds (see Chapter 3). Substances shown in Table 14.2 have been assessed in this way. Thiomersal was not considered to pose a risk to human health from its use in veterinary vaccines as animals are not sent to slaughter immediately after vaccination and hence residues will deplete. This use contributes negligible amounts to the overall exposure to mercury and so the risk to the consumer is negligible. The maximum concentration of thiomersal permitted in vaccines intended for food animal use is 0.02%.[250]

14.5 Conclusions

Veterinary vaccines are essential tools in protecting animal health and welfare and public health. They protect companion animals and those animals that are farmed for human consumption against a variety of infectious diseases. Veterinary vaccines are only authorised if they meet the usual criteria of safety, quality and efficacy and their chemical components are selected to ensure consumer safety. Careful use of veterinary vaccines, following advice and warnings mandated by regulatory authorities that appear in product literature and on product labels should ensure protection against needle stick and self-injection accidents. Immediate medical attention must be sought for the latter. Only a small number of veterinary vaccines contain live, zoonotic organisms but where exposures occur, medical attention is required.

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CHAPTER 15

Adverse Drug Reactions in Humans – Data from Veterinary Pharmacovigilance Schemes

15.1 Introduction

As already discussed elsewhere in this work, it is the users of veterinary drugs who are primarily exposed to these products during their use, as opposed for example, to their residues in food of animal origin. These include, but are not limited to, veterinarians, veterinary nurses, farmers, fish farmers and the owners of companion animals.[1–9] Exposure to drugs on treated animals may also occur, for example following topical treatments including spot-on formulations, sprays, pour-on products and after dipping of cattle or sheep. As a result of occupational exposures or exposure through treated animals, adverse drug reactions may occur in humans, and in many countries, including the EU member states and the USA, these reactions are subject to reporting under veterinary pharmacovigilance requirements.[10–14] It is therefore useful to examine the information collected by these schemes to determine the extent to which toxicity in humans plays a role in adverse reactions in humans. Unfortunately, these data are not always readily available. However, both the UK and US authorities make this information available to a limited extent, and this will be examined in this chapter.

15.2 The Suspected Adverse Reactions Reporting Scheme – United Kingdom

The Veterinary Medicines Directorate (VMD) operates the Suspected Adverse Reaction Surveillance Scheme (SARRS) in the UK. The SARRS covers all

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aspects of adverse reaction reporting for veterinary medicinal products including adverse drug reactions in exposed humans and in doing so, the VMD implements all aspects and requirements of European legislation pertaining to veterinary drugs and pharmacovigilance. Reporting to VMD is usually done using a "yellow form" and there is a specific form for suspected adverse reactions in humans. This form requires details relating to the product and the suspected adverse reaction. Details of adverse reactions reported to the VMD are published annually in the *Veterinary Record*.

The numbers of adverse reactions in humans reported to the VMD is shown in Figure 15.1 for the period 1985 to 2010. The increase in reactions in the early 1990s is probably due to a number of factors including the greater publicity given to the scheme and to its applicability to human adverse reactions. In addition, at about the same time, there was an increased interest in a specific topic, namely suspected adverse reactions to organophosphorus-containing sheep dips, and the numbers of reports for these rose dramatically. This will be discussed later in this chapter.

The majority of human suspected adverse reactions are reported by marketing authorisation holders. For example, in the period 1985 to 2001, 57% of reports were submitted by marketing authorisation holders. Of the remainder, 16% were submitted by farmers, 6% by veterinarians, 6% by the general public, 5% by physicians and pharmacists and 4% by the National Poisons Information Service (NPIS). The remaining 6% originated from a number of sources, including from officials of the Health and Safety Executive, the UK government agency responsible for occupational safety and health.

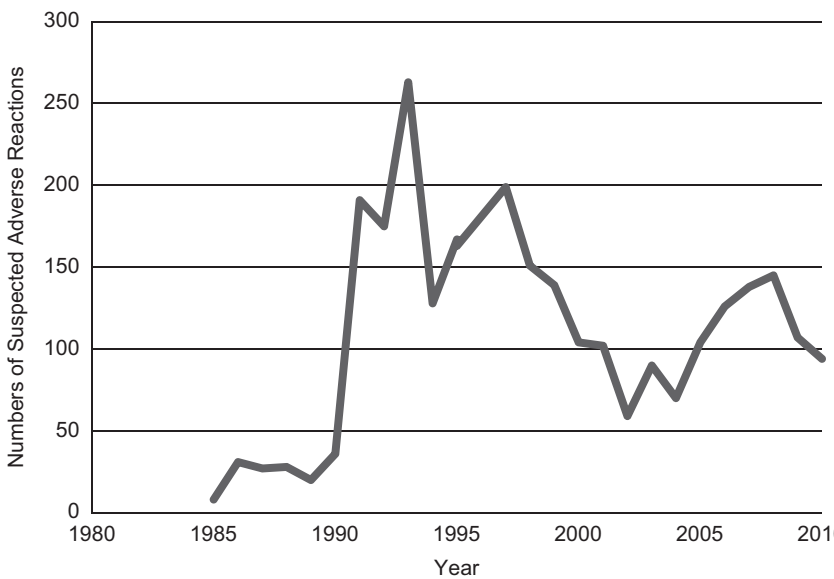

Figure 15.1 Adverse reactions in humans 1985–2010.

Table 15.1 Human suspected adverse reactions 2002 to 2010.

Product Class	*2002*	*2003*	*2004*	*2005*	*2006*	*2007*	*2008*	*2009*	*2010*
Total number of human reactions	59	90	70	104	126	138	145	107	94
Ectoparasiticides and endectocides	26	46	26	45	62	67	64	45	46
Organophosphorus sheep dips	2	3	0	2	—[a]	—[a]	—[a]	—[a]	—[a]
Vaccines	18	22	19	29	29	29	48	34	18
Other veterinary medicines	15	22	35	29	29	42	33	28	30
Needle stick injuries	—[b]	19	24	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]
Serious adverse reactions	13	17	8	11	12	6	7	8	2
Deaths	0	0	0	1	0	0	1[c]	0	0

[a]Individual values not available.
[b]The majority of adverse reactions involving vaccines and other injectable products were minor needle stick injuries.
[c]Patient hospitalised but death not due to adverse reaction.

In the period 1985 to 2001, the majority (75%) of suspected adverse reactions reported followed exposure to ectoparasiticides. The majority of the remainder were accounted for by vaccines (15%) and other products (anaesthetics, antimicrobials, anthelmintic agents, hormones, antiseptics; total 7%). After 2000, the numbers of reports for suspected adverse reactions to organophosphorus sheep dips declined. The reasons for this are complex and included the fact that many of the previous reports were historical in nature but had been reported in the period 1990 to 1995 as a result of greater publicity given to the SARRS in general and to sheep dips in particular. Furthermore, alternative products including cypermethrin-based dips and endectocides became more widely available. Figure 15.2 illustrates the numbers of suspected adverse reactions reported for organophosphorus-containing ectoparasiticides for the period 1985 to 2005. From 2002 to 2010, the numbers of suspected adverse reactions in humans were dominated by a combination of ectoparasiticides and endectocides, along with vaccines.[15–23] These figures are shown in Table 15.1.

A number of more specific issues also arose. These included adverse reactions to products containing imidacloprid, sprays containing dichlorvos and, as already mentioned, the organophosphorus sheep dips.

15.2.1 Dog and Cat Products Containing Imidacloprid

A number of adverse reactions in exposed humans were reported for this product. A proportion of the reactions reported (18%) were respiratory but others involved skin and eye effects.[9] It was thought that this might be due to the solvent in the formulation, benzyl alcohol. However, the respiratory symptoms were thought unlikely to be due to benzyl alcohol as the substance is of low volatility. Nevertheless, it was still considered plausible that benzyl

alcohol might be the culprit constituent, and that it might also produce signs of respiratory distress in individuals with asthma, and the signs reported were consistent with those of a respiratory irritant. However, there is an alternative explanation; that at least some of the adverse reactions are due to allergy to cats. Nevertheless, concern over its effects on skin remained. The UK's independent advisory committee, the Veterinary Products Committee (VPC) recommended that the warning on the label at the time (''People with known skin sensitivity may be particularly sensitive to this product'') be amended to take into account the solvent (''This product contains benzyl alcohol which may cause some transient irritation to the skin. Avoid skin contact'').

15.2.2 Sprays Containing Dichlorvos

Between 1989 and 1999 there were 33 reports arising from the use of these products, and these involved skin rashes or propellant burns. In addition, a number of reports concerned 'generalised' reactions to these products. The VMD and its advisors considered the available data, including medical reports and questionnaires sent to and returned by the affected patients or their medical advisers. The conclusion was eventually reached that there was no evidence of cholinergic effects attributable to dichlorvos but one adverse reaction report made reference to vomiting while three others referred to diarrhoea. Despite there being no specific mechanism evident, it was concluded that the effects might be product related but no regulatory action was taken.

Where heavy exposures occur, dichlorvos results in significant morbidity and mortality. These exposures normally involve accidents which arise during pesticide use rather than with the employment of small aerosols of veterinary medicinal product, or they may arise from suicide attempts.[24–28] Dichlorvos is also genotoxic *in vitro* although the evidence for *in vivo* effects is not overwhelming, while animal carcinogenicity data and data from epidemiological studies show no strong evidence of carcinogenic effects.[29,30] Regardless, the precautionary approach is to take suitable measures when using dichlorvos-containing products, particularly when supplied in aerosol, and therefore in respirable form. The majority of these products have now been discontinued in favour of safer alternatives.

15.2.3 Organophosphorus Sheep Dips

The OP dips (and sprays) have long been used for the treatment and attempted eradication of sheep scab. This disease has major animal welfare implications for the sheep and major economic implications for the farmer. The disease can result in loss of the fleece and severe lesions on the animal that are then open to secondary infection by bacteria and parasites. The disease is caused by the sheep scab mite *Psoroptes ovis*. Organophosphorus-based dips have long been used for the treatment and attempted eradication of the disease.[31,32] In this process, animals are submerged in the dip bath fluid so that the entire body surface, including the head, is submerged. They then exit the bath and in

modern dip systems are retained in the dipping area to allow the excess dip fluid to drain back into the bath.

It is known that human exposure to organophosphorus compounds can result in a variety of acute toxic effects. These arise primarily as a result of the inhibition of acetylcholinesterase. Signs of acute toxicity are due to effects on the central nervous system (anxiety, ataxia, hypotension), to muscarinic effects (wheezing, cough, rhinitis) and to nicotinic effects (muscle weakness, mydriasis and tachycardia). Other acute effects include chest tightness, abdominal cramps, confusion and convulsions.[33–36] With some organophosphorus compounds, a specific syndrome may develop. This is delayed peripheral neuropathy or OP-induced delayed neuropathy (OPIDN).[37–42] (For a more detailed discussion on the toxicity of organophosphorus compounds see Chapter 10.)

Acute effects have certainly been observed after sheep dipping.[43] The ability of organophosphorus compounds to induce chronic effects after exposure to low concentrations is more controversial.[44–46] Some workers have found subtle adverse effects after such exposures, while others have found no effects.[45–47] It has proved difficult to define the routes of exposure during sheep-dipping. The vapour pressure values of the majority of organophosphorus compounds are low, and airborne concentrations of diazinon during dipping have also been shown to be low. In fact these were below the limits of detection of the analytical assay used (<0.1 mg m^{-3}).[48] Splashing may occur when sheep enter and exit the dip bath, but the available evidence suggests that most splashing occurs when the concentrate is being diluted prior to use rather than with diluted dip bath itself.[48–50] No significant differences in plasma or erythrocyte cholinesterase were detected in workers employed in dipping sheep and exposed to organophosphorus compounds regardless of whether they wore protective clothing.[48,51] From this information at least, it is difficult to identify critical routes of exposure to organophosphorus compounds during sheep dipping and to quantify any exposure that might occur.

As already described, over the period 1985 to 2000 there was an increase in the numbers of suspected adverse reactions to veterinary medicinal products reported to the VMD and this is reflected in Figure 15.1. Much of this increase was accounted for in the early stages by increases in the numbers of suspected adverse reactions to organophosphorus-containing dips (Figure 15.2). These products are usually supplied as emulsion concentrates and they are added to dip bath water to make up the formulation and to recharge it as the active ingredient is removed by the treated sheep during the dipping process and diluted by topping up the bath with water to replenish the content and to maintain an adequate depth. In the period 1985 to 2001 the VMD received a total of 1967 reports relating to possible adverse reactions.[52–64]

A special group was established to review adverse reactions to veterinary medicinal products in humans, the Appraisal Panel for Human Suspected Adverse Reactions.[65] By 2000, the Appraisal Panel had considered a large number of adverse reactions in humans, including many relating to exposure to organophosphorus-containing dips. As a result, the Appraisal Panel realised that there was a similarity between the reported signs and symptoms in

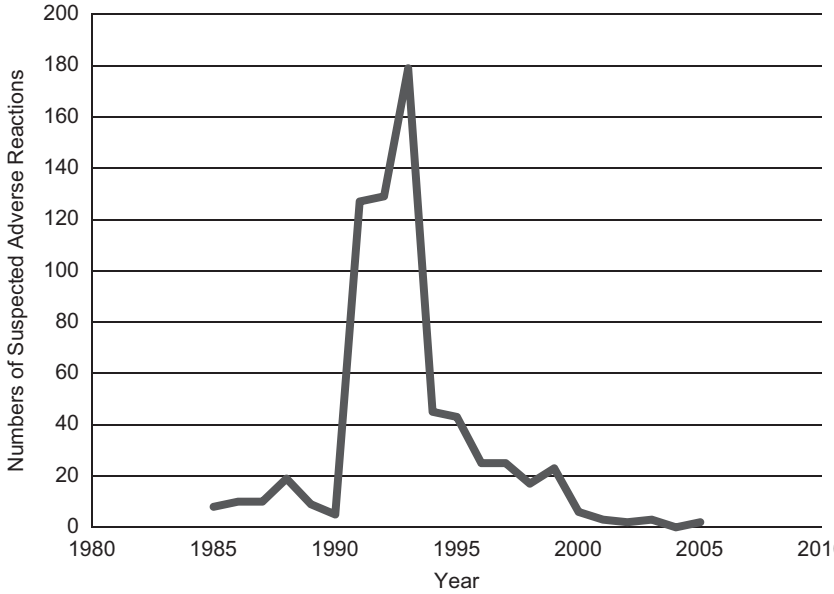

Figure 15.2 Suspected adverse reactions to organophosphorus sheep dips.

individuals potentially exposed to sheep dips and chronic fatigue syndrome (CFS), a condition associated with psychological, biological and social factors.[66–70] Thus, the major symptoms reported included:

- Myalgia
- Arthralgia
- Attention disturbances
- Irritability
- Depression
- Chronic headache
- Chronic fatigue

Other signs reported included:

- Confusion
- Sore throat
- Sleep-disorders
- Muscle weakness
- Pyrexia
- Memory impairment

The overall condition was frequently referred to as ''dipper's flu''. As a result of the similarity between these effects and CFS, the Appraisal Panel decided to

consult an expert on CFS. This expert went on to comment that headaches were common in those with CFS but they were also common in the general population although the rates of headaches in CFS patients were higher. On this basis of this advice the Appraisal Panel concluded that there were no diagnostic features sufficiently adequate to distinguish the reported effects in sheep dippers from those with CFS and that more adverse drug reaction data or the results of epidemiological studies were required to determine if sheep dipping, organophosphorus compounds and ill health were inter-related.[71,72]

It should be noted that similar signs have been reported in individuals working with large animal pour-on products containing organophosphorus compounds. With these products, headache was again the most common symptom reported along with other CFS-type problems. However, these signs were not reported for non-organophosphorus-containing products such as those formulated with synthetic pyrethroids. Here, the main sign reported was paraesthesia, which is a known effect with these substances.[73–80] As the organophosphorus-containing pour-on products were no longer available on the UK market, the Appraisal Panel made no recommendations regarding their use.

The associations between possible exposure to organophosphorus compounds and ill health, and notably CFS-like effects, have been studied in sheep farmers. Many of the subjects examined reported chronic fatigue as a major problem and higher scores for this effect were associated with higher exposures. Only weak evidence of a chronic effect and an association with cumulative exposures was found.[50,81]

Eventually, the entire issue of possible adverse reactions to sheep dips was referred to the UK's independent Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT), a committee that provides advice to the Food Standards Agency, to the Department of Health and to other government departments. The COT first reviewed this in 1998 and it finally reported in 2000.[82] It focussed largely on neurotoxicity and epidemiology, and it examined a number of studies including those relating to exposure of those involved in sheep dipping.[83–85] It also had access to the reports of suspected adverse reactions submitted to the VMD.

The COT concluded that the evidence did not support the induction of adverse neuropsychological effects as a result of prolonged exposure to organophosphorus compounds and that the balance of evidence did not suggest that exposures to organophosphorus-containing sheep dips could result in peripheral neuropathy. It considered the data to have a number of limitations because of differences between control and exposed populations, because of biases due to the association between willingness to participate in the studies and health problems and because of the small study sample sizes. Difficulties also arose due to the inclusion of patients with a past history of acute organophosphorus poisoning and the inclusion of patients who at the time were potentially exposed to organophosphorus compounds or who had been recently exposed.

The COT also recognised that there were major gaps in knowledge relating to the effects of these substances and relating to patterns of exposure.

Specifically, it recognised that there was no information relating to the possibility that they could cause "disabling neurological or neuropsychiatric disease in a small sub-group of exposed persons." It went on to make recommendations for further research:

- To determine what the common patterns of exposure to sheep dips and their components actually were and what constituted the clinical presentation and clinical course.
- How common was sheep dipper's flu?
- Does low-level exposure to organophosphorus compounds cause disabling neurological or psychiatric disease in a small subgroup of individuals?
- Do people with chronic disabling disease differ metabolically from the general population?
- What mechanism, other than effects on acetylcholinesterase, play a causative role in the development of ill health in sheep dippers?

The results of some of the work commissioned as a result of the COT's recommendations became available in the mid-2000s and the COT reviewed these in 2007. Unfortunately, much of the data generated were inconclusive and some of the projects were still in progress. One of the major issues identified was that "dipper's flu" did not appear to be a specific syndrome. There was some evidence of neurological illness in persons who had used organophosphorus compounds but unfortunately, these people had been exposed to other products, including pesticides. There was also evidence of metabolic differences in those with chronic disabling illnesses, but these did not correlate with enhanced susceptibility to organophosphorus compound toxicity. Research showed that psychological mechanisms may be involved in those suffering neurological symptoms after exposure to sheep dips or to pesticides.[86,87] Consequently, it may be difficult to differentiate between any effects that sheep dips may induce and the effects induced by other chemicals, as well as those arising from genetic and phenotypic effects.

Human paraoxonase protein, paraoxonase 1 (PON 1) detoxifies some organophosphorus compounds including diazoxon, the active metabolite of diazinon, and the PON1 Q1192R polymorphism affects paraoxonase activity. Individuals with low PON 1 activity may be susceptible to the toxic effects of some organophosphorus compounds.[88–90] There is some evidence for PON1 deficiency in sheep farmers who have reported adverse reactions following exposures to sheep dip. Farmers reporting chronic ill health have a higher proportion of PON1 polymorphisms related to a lower capacity to hydrolyse and detoxify diazoxon.[91,92] The role of polymorphisms in other genes, if any, is unclear.[93] Whether these findings fully address the dipper's flu issue is debatable. The degree of exposure to organophosphorus compounds is generally very low and the chronic nature of the condition remains to be explained.

The labelling for organophosphorus sheep dips in the UK has been strengthened. These are now required to carry a skull and crossbones symbol and the words TOXIC IF SWALLOWED. There is also advice on suitable

protective clothing, equipment and methods of safely preparing the dip bath. Methods have been developed for safely transferring the concentrate to the dip bath to prevent inadvertent exposure to the product before dilution with water. One of these included the provision of the concentrate in a water-soluble sachet that is added to the dip bath thus preventing any need to handle the product itself. A training scheme for the use of sheep dips has been introduced and this is accompanied by the award of a Certificate of Competence.[94] The Health and Safety Executive has also published a booklet providing comprehensive advice on the safe dipping of sheep. This provides specific advice on protective clothing and the design of sheep dips so that run-off is contained and the handling of treated sheep minimised.[95]

It is undisputed that the active ingredient in sheep dips, diazinon (and previously also chlorfenvinphos) can induce neurotoxicological effects including under some circumstances neuropathies. For example, cognitive effects and developmental neurotoxicity have been reported in rats.[96,97] However, many of the effects reported in those potentially exposed to organophosphorus-containing sheep dips were ill-defined, such as the influenza-like effects mentioned earlier, sore throats and general malaise. Although these effects may have been caused by exposure to sheep dips, they could also have been due to a range of other factors, and one of the functions of the Appraisal Panel was to address these, and to advise the VPC accordingly.

The Appraisal Panel has reviewed all of the reports of suspected adverse reactions to sheep dips submitted to the VMD. The VPC, through the advice of the Appraisal Panel, has made several recommendations regarding the safe use of sheep dips and in 1999 suspended the marketing authorisations for these products pending the redesign of containers and delivery systems to reduce contact with the concentrate, as described above. The suspensions on these marketing authorisations were not lifted until late 2000. Even then, this was regarded as an interim measure pending further improvements to reduce operator exposure to the undiluted product.[98–103] Furthermore, the issue of chronic illness following low exposure to organophosphorus dips, as alluded to earlier, has yet to be resolved. There is agreement that long-term effects can result from acute organophosphorus poisoning.[47,104–107] Some researchers believe that long-term effects can result from low-level exposure to organophosphorus compounds without frank acute effects occurring.[45,46,105,108] However, others consider that from the results of controlled studies, from information arising from accidental exposures and from animal studies, these chronic effects do not exist.[37,107] Hence, preventing late neurological effects means preventing acute organophosphorus poisoning.[104]

In 2001, a telephone survey was launched of individuals who had reported that they had experienced adverse effects following exposure to sheep dips. Those interviewed were nominated by support groups for those said to be affected by exposure to sheep dips and 524 eligible participants were identified. Of these, 367 individuals were identified with neurological effects. These had been screened for contributory diseases such as diabetes and medications with known neurological side-effects. The cumulative exposure to sheep dips varied

but potential exposures were not considered to be unusually high. All had been included because of claimed long-term health effects due to organophosphorus dip exposure. However, there was inadequate evidence to determine what effects, if any, exposure had contributed to the adverse heath effects.[109]

Data from 646 reports of suspected adverse reactions to organophosphorus sheep dips were subjected to a detailed analysis. It was noted that 232 respondents had failed to provide information on their exposure histories and many of these reports involved nervous disorders (447), general disorders (389), psychiatric disorders (203), musculoskeletal disorders (192) and ocular effects (50). The majority of symptoms reported were headaches. Others included paraesthesia, fatigue, influenza-like symptoms, lethargy, depression, amnesia, arthralgias, myalgias and dyspnoea. However, there were no obvious patterns of morbidity in these reports although nausea and dizziness were associated, as were memory loss and depression. Short- and long-term exposures were associated with psychiatric disorders and musculoskeletal effects, notably myalgia. These associations were weakened by the confounding effects of age at onset, fear of reporting and missing exposure data. There were no obvious explanations for any of the effects reported.[110]

The Health and Safety Executive conducted a risk assessment. It concluded that for acute toxicity at least, the wearing of personal protective equipment was an important factor when handling sheep dips and sheep dip concentrates.[111]

The true reasons that lie behind the sheep dip story in the UK may never be fully known. Sheep dipping is an intensive activity that involves physical labour and long working days, often in adverse weather conditions (heat or cold). Some argue that wearing heavy protective clothing (waterproof boots, rubber aprons, gloves, face-shield) is impractical for such work and is more likely to result in heat related illnesses rather than in any degree of practical protection. The products that are now available have been designed to minimise exposure of sheep farmers and farm workers to sheep dip concentrates. However, many of these organophosphorus dips have now disappeared. The synthetic pyrethroid dips, which had themselves had sufficient problems including adverse environmental effects and potential human toxicity, have also been withdrawn.[112–114] The dips have now been largely replaced by injectable products containing ivermectin, moxidectin or doramectin, by pour-on products containing dicyclanil, cypermethrin, alpha-cypermethrin, deltamethrin or cyromazine and by oral products containing ivermectin or moxidectin.[115]

15.3 Adverse Reaction Reporting in the USA

In the United States, adverse reaction reporting for veterinary pharmaceuticals is conducted under a programme operated by the Center for Veterinary Medicine (CVM), which is part of the US Food and Drug Administration (FDA). The FDA provides cumulative veterinary adverse drug experience reports (currently 1987 to 30 April 2012) on its website (http://www.fda.gov/AnimalVeterinary/default.htm). These reports cover adverse drug reaction

reports in animals and in humans. These reports are not always easy to comprehend. As the advice provided on the website makes clear, there is no certainty that the drug actually caused the reported adverse event. In other words, there is no causality assessment implied in the report, and causality assessment is essential in linking exposure to a drug with an adverse outcome.[116–120] These reports cannot be used to calculate incidence or drug risk as there is no way of knowing how many animals were treated or how many humans were exposed. The number of reports solely indicates the numbers of adverse drug experiences received by CVM for a particular drug. Moreover, some phrases may appear more than once in the same report. There is also no information provided on how the drugs were used (or abused, misused) or if they were subject to off-label use.

With these limitations in mind, it is useful to examine the adverse drug experience reports in humans for the period 1987 to 2012. These are shown in Table 15.2. It is evident from this table that injection site injuries are relatively

Table 15.2 Human adverse drug experiences for veterinary drugs, 1987 to 2012.

Drug	*Route*	*Signs*	*Number of times Reported*
Acepromazine	Oral	Stupor	1
		Unconsciousness	1
Albendazole	N/A	Dyspnoea	1
		Rash	1
	Oral	Haematochezia	1
	Topical	Application site inflammation	1
		Headache	1
	Unknown	Death	1
Altrenogest	N/A	Taste abnormality	1
	Ophthalmic	Congestion	1
		Headache	1
		Irritation	1
		Eyelid pain	1
	Oral	Diarrhoea	2
		Abdominal pain	2
		Headache	1
		Nausea	1
	Parenteral	Hair abnormality	1
		Mammary hyperplasia	1
		Weight increase	1
	Topical	Abnormal menses	25
		Headache	8
		Abdominal pain	7
		Rash	5
		Reproductive disorder	5
		Oestrous behaviour	4
		Oestrous cycle disorder	4
		Anoestrous	3

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Altrenogest *(cont.)*		Behavioural disorder	3
		Nausea	3
		Pruritus	3
		Vaginal bleeding	2
		Dyspnoea	2
		Reduced fertility	2
		Pain	2
		Muscle pain	2
		Vomiting	2
		Abortion	1
		Vulva discharge	1
		Dizziness	1
		Dysuria	1
		Fever	1
		Hot flash	1
		Raised liver enzymes	1
		Joint pain	1
		Ovarian pain	1
	Topical	Pruritus, feet/digits	1
		Mammary swelling	1
		Low testosterone	1
		Uterine contractions	1
		Vision disorder	1
		Weight loss	
	Unknown	Abnormal menses	2
		Nausea	2
	Unknown	Abdominal pain	2
		Behaviour disorder	1
		Dizziness	1
		Abnormal oestrous cycle	1
		Headache	1
		Insomnia	1
		Chest pain	1
		Rash	1
		Weight increase	1
	Various	Taste abnormality	1
		Vomiting	1
Amitraz	Inhalation	Headache	3
		Paraesthesia	1
	Ophthalmic	Eye irritation	2
		Congestion, eyelid	1
		Pain, eyelid	1
	Oral	Unconsciousness	2
		Apnoea	2
		Convulsions	1
		Dyspnoea	1
		Sedation	1
		Raised liver enzymes	1
		Stupor	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
	Parenteral	Ecchymoses	1
		Injection site swelling	1
	Topical	Rash	23
		Pruritus	8
		Congestion, skin	4
		Depression/lethargy	4
		Urticaria	4
		Dizziness	3
		Eye irritation	3
		Dyspnoea	3
		Ill	2
		Nausea	2
		Papules	2
		Sedation	2
		Somnolence	2
		Swellings	2
		Vesicles/bullae	2
		Alopecia	1
		Conjunctivitis	1
		Convulsions	1
		Diarrhoea	1
		Eye discharge	1
		Dysphagia	1
		Epiphora	1
		Hair abnormality	1
		Hypoethesia	1
	Topical	Faecal incontinence	1
		Skin inflammation	1
		Skin irritation	1
		Raised liver enzymes	1
		Pain	1
		Eyelid pain	1
		Head/face pain	1
		Joint pain	1
		Pallor	1
		Pruritus, eyes	1
		Pustules	1
		Skin scales	1
		Stiffness	1
		Stupor	1
		Limb swelling	1
		Syncope	1
		Trembling	1
		Vomiting	1
		Weakness	1
	Unknown	Congestion, skin	3
		Headache	3
		Nausea	3
		Ill	2

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Amitraz *(cont.)*		Reaction, unspecified	2
		Weakness	2
		Dizziness	1
		Fever	1
		Abnormal gastrointestinal	1
		Skin inflammation	1
		Back pain	1
		Pruritus	1
		Tongue swelling	1
		Taste abnormality	1
		Urticaria	1
		Vomiting	1
Amoxicillin, Clavulanate	Oral	Flatulence	1
		Mouth/lips irritation	1
		Abdominal pain	1
		Pruritus	1
		Swelling, mouth/lips	1
	Topical	Urticaria	4
		Anaphylaxis/ anaphylactoid	3
		Dyspnoea	1
		Oedema, face/head	1
		Pruritus	1
		Rash	1
		Swelling, tongue	1
Amoxicillin	Oral	Diarrhoea	2
		Abdominal pain	1
		Vomiting	1
	Topical	Headache	1
		Swelling, head/face	1
		Urticaria	1
Ampicillin	Inhalation	Irritation, pharynx	1
Amprolium	Inhalation	Irritation, pharynx	1
	Oral	Abdominal pain	2
	Topical	Skin irritation	1
		Taste abnormality	1
	Unknown	Headache	1
		Nausea	1
		Pain	1
		Muscle pain	1
Atipamezole	N/A	Injection site swelling	1
	Ophthalmic	Eye irritation	2
		Mydriasis	1
		Eye pain	1
		Vision disorder	1
	Parenteral	Injection site pain	1
		Injection site swelling	1
Bacitracin	Inhalation	Coughing	1
		Irritation	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Betamethasone, clotrimazole, gentamicin	Topical	Skin inflammation	1
		Skin, vesicles, bullae	1
Betamethasone, gentamicin	Ophthalmic	Eye irritation	1
		Eye pain	1
	Topical	Erythema, application site	1
		Urticaria	1
Butorphanol	Oral	Dizziness	1
		Ataxia	1
		Headache	1
		Nausea	1
		Abdominal pain	1
		Sedation	1
		Vomiting	1
	Parenteral	Injection site phlebitis	1
Carprofen	Ophthalmic	Eye irritation	47
		Eyelid pain	21
		Congestion, eyes/lid	17
		Vision disorder	4
		Congestion	2
		Swelling, eyes/lids	2
		Abrasions, corneas	1
		Bleeding, eyes	1
		Conjunctivitis	1
		Discharge, eyes	1
		Oedema, eyes	1
		Epiphora	1
		Eye infection	1
		Photosensitisation	1
		Pruritus, eyes	1
		Ulcers, corneas	1
	Oral	Nausea	15
		Vomiting	10
		Diarrhoea	7
		Depression/lethargy	5
		Dizziness	4
		Hypersalivation	3
		Abdominal pain	3
		Hyperesthesia	2
		Raised liver enzymes	2
		Xerostomia	2
		Acidosis	1
		Aggression	1
		Raised alkaline phosphatase	1
		Vaginal bleeding	1
		Elevated blood urea nitrogen	1
		Convulsions	1
		Diarrhoea	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Carprofen *(cont.)*		Fever	1
		Froth, mouth/lips	1
		Headache	1
		Irritation pharynx	1
		Kidney failure	1
		Liver failure	1
		Myositis	1
		Nervousness	1
		Chest pain	1
		Head/face pain	1
		Papules	1
		Paraesthesia, mouth/lip	1
		Rash	1
		Sedation	1
		Somnolence	1
		Stupor	1
		Tachycardia	1
		Weakness	1
	Parenteral	Injection site pain	3
		Injection site inflammation	2
		Hypoesthesia	1
		Injection site oedema	1
		Injection site swelling	1
	Topical	Congestion, skin	2
		Pruritus	2
		Rash	2
		Unconsciousness	2
		Anaphylaxis/ anaphylactoid	1
		Dizziness	1
		Hyperesthesia	1
		Skin inflammation	1
		Nausea	1
		Sores	1
		Swelling, eyelids	1
		Swelling, head/face	1
		Swelling, mouth/lips	1
		Urticaria	1
	Unknown	Eye irritation	2
		Eyelid pain	2
		Chest pain	1
		Vision disorder	1
Cefovecin	Ophthalmic	Conjunctivitis	1
		Eye irritation	1
	Topical	Anaphylaxis/ anaphylactoid	2
		Ear disorder	1
		Oedema, multiple sites	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
		Skin inflammation	1
		Eye irritation	1
		Irritation, pharynx	1
		Nose abnormality	1
		Urticaria	1
	Unknown	Eye irritation	1
		Keratoconjunctivitis	1
	Various	Trembling	1
Cefpodoxime	Topical	Rash	2
Ceftiofur	Missing	Bleeding, injection site	1
		Injection site pain	1
		Injection site inflammation	1
	Parenteral	Injection site pain	34
		Injection site swelling	28
		Injection site inflammation	15
		Hypoesthesia	5
		Injection site abnormality	4
		Dizziness	3
		Nausea	3
		Skin congestion	2
		Ill	2
		Bleeding, injection site	2
		Ecchymoses	1
		Diarrhoea	1
		Fever	1
		Hypothermia	2
		Skin irritation	1
		Chest pain	1
		Limb pain	1
		Paraesthesia	1
		Rash	1
		Hand stiffness	1
		Swelling	1
		Swelling, tongue	1
		Weakness	1
	Topical	Rash	3
		Ill	2
		Congestion, eyes	1
		Congestion, skin	1
		Fever	1
		Eye irritation	1
		Skin irritation	1
		Pruritus, eye	1
		Skin, slough	1
		Administration site swelling	1
		Swelling, eyelids	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Ceftiofur *(cont.)*	Unknown	Injection site pain	13
		Injection site swelling	11
		Injection site inflammation	6
		Accidental exposure	2
		Congestion, skin	2
		Nausea	2
		Abdominal pain	2
		Swelling	2
		Depression/lethargy	1
		Dizziness	1
		Fever	1
		Hyperesthesia	1
		Ill	1
		Skin inflammation	1
		Injection site oedema	1
		Injection site stiffness	1
		Liver disorder	1
		Chest pain	1
		Paraesthesia	1
		Stiffness, hands	1
		Syncope	1
		Urticaria	1
		Vomiting	1
Chloramphenicol	Oral	Endocrine disorder	1
Chlorhexidine	Topical	Skin congestion	1
		Skin inflammation	1
		Skin lesions	1
		Wheezing	1
Chlortetracycline	Topical	Skin abnormality	1
Clenbuterol	Oral	Apprehension	1
		Breathing abnormality	1
		ECG abnormality	1
		Atrial fibrillation	1
		Tachycardia	1
Clindamycin	Oral	Vomiting	1
	Parenteral	Injection site pain	1
	Topical	Partial deafness	1
		Nausea	1
Clomipramine	Oral	Depression/lethargy	127
		Nausea	63
		Dizziness	34
		Vomiting	31
		Xerostomia	17
		Diarrhoea	12
		Headache	10
		Nervousness	8
		Tachycardia	6
		Apprehension	5
		Weakness	4

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
		Hyperesthesia	3
		Insomnia	3
		Abdominal pain	3
		Shaking	3
		Taste abnormality	3
		Anorexia	2
		Confusion	2
		Hot flush	2
		Mydriasis	2
		Paraesthesia	2
		Elevated blood pressure	2
		Somnolence	2
		Vision disorder	2
		Arrhythmia	1
		Ataxia	1
		Diarrhoea	1
		Dissociation	1
		Fever	1
		Gagging	1
		Abnormal gastrointestinal	1
		Hypoesthesia	1
		Hyposalivation	1
		Paresis	1
		Paraesthesia	1
		Sedation	1
		Sleep abnormality	1
		Sweating	1
		Tongue abnormality	1
		Trembling	1
		Uterine contractions	1
		Voice disorder	1
Cloprostenol	Parenteral	Injection site pain	1
		Injection site bleeding	1
	Topical	Respiratory disorder	3
		Abdominal pain	2
		Abortion	1
		Anaemia	1
		Behavioural disorder	1
		Lack of fertility	1
		Abnormal menses	1
		Nervousness	1
		Tachycardia	1
Clorsulon, ivermectin	Parenteral	Injection site pain	5
		Headache	2
		Injection site	2
		Nausea	2
		Congestion, skin	1
		Hot flash/flush	1
		Injection site bleeding	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Clorsulon, ivermectin *(cont.)*		Injection site inflammation	1
		Pain	1
		Back pain	1
		Paraesthesia	1
		Limb swelling	1
	Topical	Hypoesthesia	1
	Unknown	Fever	1
		Eye pain	1
		Vision disorder	1
Clotrimazole, gentamicin, mometasone	Ophthalmic	Eye irritation	1
	Topical	Partial deafness	1
		Urticaria	1
Cyclosporin	Ophthalmic	Eye irritation	1
	Oral	Nausea	6
		Confusion	3
		Diarrhoea	1
		Dizziness	1
		Dysphagia	1
		Irritation, oesophagus	1
		Abdominal pain	1
	Topical	Nasal congestion	1
		Depression/lethargy	1
		Dizziness	1
		Skin inflammation	1
		Nausea	1
		Pain	1
		Muscle pain	1
		Paraesthesia, mouth/lips	1
		Pruritus	1
		Taste abnormality	1
		Vomiting	1
	Unknown	Urticaria	1
Danofloxacin mesylate	Oral	Taste abnormality	1
	Parenteral	Injection site swelling	4
		Injection site pain	3
		Ecchymoses	1
		Congestion, skin	1
		Injection site inflammation	1
		Paraesthesia	1
	Topical	Anorexia	1
		Depression/lethargy	1
		Diarrhoea	1
		Discomfort	1
		Fever	1
		Pain	1
		Weakness	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Deracoxib	Oral	Depression/lethargy	3
		Nausea	2
		Dizziness	2
		Abdominal pain	1
		Limb pain	1
	Topical	Skin abscess	1
		Arrhythmia	1
		Slough skin	1
Desoxycorticosterone	Ophthalmic	Eye irritation	1
Detomidine	Ophthalmic	Eye irritation	1
	Oral	Depression/lethargy	2
		Tachycardia	1
	Parenteral	Bradycardia	2
		Depression/lethargy	2
		Somnolence	2
		Injection site pain	1
		Skin irritation	1
		Shock	1
	Unknown	Behaviour disorder	1
		Bradycardia	1
		Breathing abnormality	1
		Confusion	1
		ECG abnormality	1
		Hallucination	1
		Hydrocephalus	1
		Low blood pressure	1
		Unconsciousness	1
Dexamethasone, neomycin, thiabendazole	Ophthalmic	Eye irritation	83
		Pain, eyes	53
		Congestion, eyes/lids	16
		Vision disorder	8
		Swelling, eyelids	5
		Eye discharge	4
		Headache	2
		Pruritus, eye	2
		Corneal abrasions	1
		Eye oedema	1
		Keratoconjunctivitis	1
		Corneal ulceration	1
	Oral	Death	1
		Vomiting	1
	Topical	Pain, application site	1
		Pruritus, application site	1
		Skin congestion	1
		Paraesthesia	1
		Ears, pruritus	1
		Swelling tongue	1
	Unknown	Rash	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Dexamethasone	Ophthalmic	Conjunctivitis	1
		Eye irritation	1
		Eye/eyelid pain	1
		Photosensitisation	1
		Swelling, eyes/lids	1
	Oral	Behaviour disorder	1
Dexmedetomidine	Ophthalmic	Headache	2
		Epiphora	1
		Eye irritation	1
		Eye/lid pain	1
	Oral	Dizziness	1
		Tongue swelling	1
	Parenteral	Dizziness	6
		Hypoesthesia	3
		Hyperesthesia	2
		CNS disorder	1
		Depression/lethargy	1
		Ear disorder	1
		Headache	1
		Injection site abnormality	1
		Injection site oedema	1
		Miosis	1
		Nausea	1
		Pallor	1
		High blood pressure	1
		Sweating	1
		Taste abnormality	1
	Unknown	Hypoesthesia	1
		High blood pressure	1
		Sedation	1
		Swelling, feet/digits	1
		Tachycardia	1
Diclofenac	Ophthalmic	Congestion, eye/lid	2
		Headache	2
		Eye irritation	2
		Depression/lethargy	1
		Diarrhoea	1
		Dizziness	1
		Oestrus cycle abnormality	1
		Gastrointestinal abnormality	1
		Haematochezia	1
		Irritation pharynx	1
		Pain, eyes/lid	1
		Bone marrow lesion	1
		Swelling, pharynx	1
Difloxacin	Oral	Nausea	1
		Abdominal pain	1
		Vomiting	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Fluocinolone, dimethyl sulphoxide	Ophthalmic	Eye irritation	4
		Pain, eyes/lids	3
		Headache	1
	Topical	Skin exfoliation	1
Dinoprost tromethamine	Inhalation	Ill	1
		Pain	1
		Chest pain	1
	Intrauterine	Choking	1
		CNS disorder	1
		Coughing	1
		Dysphagia	1
	N/A	Anorexia	1
		Birth defects	1
		Congestion, skin	1
		Dysphagia	1
		Nausea	1
		Neurological disorder	1
		Abdominal pain	1
		Recumbency	1
	Oral	Irritation, pharynx	2
		Nausea	2
		Abortion	1
		Anorexia	1
		Atrophy, muscles	1
		Congestion, lungs	1
		Coughing	1
		Diarrhoea	1
		Dyspnoea	1
		Abdominal pain	1
		Swelling, eyes/lids	1
		Swelling, head/face	1
		Taste abnormality	1
		Vomiting	1
	Parenteral	Abdominal pain	4
		Injection site swelling	2
		Abnormal menses	2
		Depression/lethargy	1
		Diarrhoea	1
		Headache	1
		Ecchymosis	1
		Injection site oedema	1
		Injection site inflammation	1
		Injection site pain	1
		Skin irritation	1
		Uterine contractions	1
	Topical	Abdominal pain	7
		Vaginal bleeding	2
		Abnormal menses	2

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Dinoprost, tromethamine *(cont.)*		Nausea	2
		Pruritus	2
		Swelling, vagina/vulva	2
		Breathing abnormality	1
		Depression/lethargy	1
		Diarrhoea	1
		Dyspnoea	1
		Headache	1
		Skin irritation	1
		Macules	1
		Testicular pain	1
		Low blood pressure	1
		Reproductive disorder	1
		Sweating	1
		Vaginitis	1
		Vomiting	1
	Unknown	Froth, mouth/lips	2
		Injection site pain	2
		Premature labour	2
		Tachycardia	2
		Injection site bleeding	1
		Injection site inflammation	1
		Injection site pruritus	1
		Liver disorder	1
		Abdominal pain	1
		Joint pain	1
		Weight increase	1
Dirlotapide	Ophthalmic	Conjunctivitis	1
		Eye irritation	1
	Oral	Diarrhoea	1
		Nausea	1
		Stool abnormality	1
		Vomiting	1
	Unknown	Hyperactivity	1
Doramectin	Inhalation	Headache	1
		Irritation, pharynx	1
		Nausea	1
		Muscle pain	1
	N/A	Injection site pain	1
	Ophthalmic	Partial blindness	1
		Congestion, eyes/lids	1
		Eye irritation	1
		Pain, eyes/lids	1
	Oral	Nausea	2
		Abdominal pain	2
		Diarrhoea	1
		Dizziness	1
		Muscle pain	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
		High blood pressure	1
		Vomiting	1
		Weakness	1
	Parenteral	Injection site swelling	12
		Injection site pain	7
		Injection site inflammation	5
		Ecchymoses	3
		Depression/lethargy	3
		Dizziness	3
		Headache	3
		Haematoma	2
		Abdominal pain	2
		Swelling	2
		Blood, urine	1
		Convulsions	1
		Diarrhoea	1
		Dyspnoea	1
		Dysuria	1
		Fever	1
		Hypoesthesia	1
		Injection site abnormality	1
		Injection site bleeding	1
		Lameness	1
		Nausea	1
		Pain	1
		Back pain	1
		Limb pain	1
		Stiffness	1
		Stiffness, hands	1
		Stranguria	1
		Vision disorder	1
		Vomiting	1
	Topical	Nausea	7
		Diarrhoea	5
		Dizziness	5
		Abdominal pain	4
		Pruritus	4
		Skin congestion	3
		Headache	3
		Eye irritation	3
		Skin irritation	3
		Rash	3
		Vomiting	3
		Depression/lethargy	2
		Dyspnoea	2
		Hypoesthesia	2
		Ill	2
		Shaking	2
		Swelling, feet/digits	2

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Doramectin *(cont.)*		Swelling, head/face	2
		Urticaria	2
		Application site lesions	1
		Ataxia	1
		Coughing	1
		Nasal discharge	1
		Gastrointestinal abnormality	1
		Hyperesthesia	1
		Skin inflammation	1
		Insomnia	1
		Irritation, pharynx	1
		Myositis	1
		Neurological disorder	1
		Pain, Head/face	1
		Pain, muscles	1
		Paraesthesia	1
		Pruritus, eyes	1
		Stiffness	1
		Swelling	1
		Swelling, joints	1
		Swelling, limbs	1
		Trembling	1
		Vesicles/bullae, skin	1
		Volvulus	1
		Severe vomiting	1
		Weakness	1
	Unknown	Injection site inflammation	4
		Injection site pain	3
		Injection site bleeding	2
		Injection site swelling	2
		Injection site erythema	1
		Ecchymoses	1
		Temporary blindness	1
		Skin congestion	1
		Depression/lethargy	1
		Dizziness	1
		Hypoesthesia	1
		Nausea	1
		Pain	1
		Chest pain	1
		Muscle pain	1
		Pruritus	1
		Swelling, limbs	1
		Vesicle/bullae mouth	1
		Vesicle/bullae skin	1
	Various	Congestion eyes/lids	1
		Hypothermia	1
		Shaking	1
		Vomiting	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Droperidol, fentanyl	N/A	Death (suicide)	1
	Parenteral	Hypoesthesia	1
Embutramide, mebezonium, tetracaine	Ophthalmic	Amblyopia	1
		Conjunctivitis	1
		Dizziness	1
		Hypoesthesia	1
		Eye irritation	1
		Eye pain	1
		Paraesthesia	1
		Swelling, eyes	1
	Oral	Abdominal pain	1
		Unconsciousness	1
	Parenteral	Hypoesthesia, limbs	2
		Injection site pain	1
		Injection site swelling	1
		Pain, feet/digits	1
		Sedation	1
	Topical	Dyspnoea	1
		Chest pain	1
	Unknown	Hypoesthesia	1
Emodepside, praziquantel	Ophthalmic	Eye irritation	1
	Oral	Taste abnormality	1
	Topical	Anaphylaxis/ anaphylactoid	1
		Congestion	1
		Congestion, skin	1
		Diarrhoea	1
		Discomfort	1
		Dyspnoea	1
		Oedema, head/face	1
		Hypoesthesia	1
		Ill	1
		Nausea	1
		Abdominal pain	1
		Chest pain	1
		Pruritus	1
		Swelling, head/face	1
		Swelling, pharynx	1
		Urticaria	1
		Vomiting	1
	Unknown	Dizziness	2
		Eye irritation	2
		Nausea	2
		Somnolence	2
		Anaphylaxis/ anaphylactoid	1
		Congestion/eyelids	1
		Depression/lethargy	1
		Discomfort	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Emodepside, praziquantel *(cont.)*		Dry skin	1
		Hyperesthesia	1
		Nail disorder	1
		Pain, eyes/lids	1
		Pain, feet	1
		Rash	1
		Swelling, eyes/lids	1
		Swelling, tongue	1
		Vomiting	1
		Weakness	1
Enrofloxacin	Inhalation	Nausea	1
	N/A	Eye irritation	1
		Pain, eyes/lids	1
	Ophthalmic	Eye irritation	4
		Pain, eyes/lids	3
		Congestion, eyes/lids	1
		Conjunctivitis	1
		Mydriasis	1
		Pruritus	1
		Swelling, eyes	1
		Vision disorder	1
	Oral	Insomnia	3
		Rash	2
		Diarrhoea	1
		Dizziness	1
		Fever	1
		Headache	1
		Irritation, pharynx	1
		Nausea	1
		Chest pain	1
		Taste abnormality	1
	Parenteral	Injection site pain	29
		Injection site swelling	21
		Injection site inflammation	17
		Injection site bleeding	15
		Congestion, skin	4
		Hypoesthesia	3
		Pain	3
		Injection site oedema	2
		Cellulitis	1
		Convulsions	1
		Ecchymosis	1
		Skin inflammation	1
		Injection site pruritus	1
		Injection site stiffness	1
		Skin irritation	1
		Pain, feet/digits	1
		Pain, joints	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
		Pallor	1
		Paraesthesia	1
		Rash	1
		Shaking	1
		Sweating	1
		Swelling, feet/digits	1
		Taste abnormality	1
		Vision disorder	1
		Vomiting	1
		Weakness	1
Enrofloxacin/Silver sulfadiazine	Ophthalmic	Conjunctivitis	1
		Pruritus, eye	1
	Topical	Erythema, application site	1
		Epiphora	1
		Irritation	1
		Irritation, pharynx	1
		Irritation, skin	1
Enrofloxacin	Topical	Depression	2
		Hyperesthesia	2
		Application site erythema	1
		Fever	1
		Headache	1
		Hypoesthesia	1
		Injection site pain	1
		Pain	1
		Back pain	1
		Paraesthesia, mouth/lips	1
		Rash	1
		Slough, skin	1
		Swelling, head/face	1
		Swelling, tongue	1
		Vesicles/bullae	1
		Weakness	1
	Unknown	Injection site pain	5
		Injection site bleeding	3
		Headache	1
		Injection site abnormality	1
		Nausea	1
Eprinomectin	Parenteral	Injection site bleeding	1
		Injection site oedema	1
	Topical	Diarrhoea	4
		Headache	3
		Abdominal pain	3
		Congestion, skin	2
		Hypoesthesia	2
		Nausea	2
		Rash	2
		Convulsions	1
		Diarrhoea, mild	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Eprinomectin *(cont.)*		Abdominal distension	1
		Dizziness	1
		Oedema, head/face	1
		Oedema, neck	1
		Fever	1
		Flatulence	1
		Skin irritation	1
		Lesions, mouth/lips	1
		Abnormal menses	1
		Obstruction, larynx	1
		Joint pain	1
		Muscle pain	1
		Pruritus	1
		Sores	1
		Syncope	1
		Trembling	1
		Unconsciousness	1
		Urticaria	1
		Vomiting	1
Oestradiol, progesterone	Parenteral	Injection site pain	1
		Injection site swelling	1
Oestradiol	Topical	Alopecia	1
		Mammary swelling	1
Famphur	Inhalation	Coughing	1
		Dyspnoea	1
		Headache	1
		Inflammation	1
		Irritation	1
		Irritation, eye/lid	1
		Nausea	1
		Abdominal pain	1
		Aspiration pneumonia	1
		Swelling/ eyes/lids	1
		Swelling, pharynx	1
	Missing	Abortion	1
	Ophthalmic	Irritation, eyes/lids	4
		Pain, eyes/lids	2
		Conjunctivitis	1
		Epiphora	1
		Irritation, pharynx	1
		Paraesthesia, eyes	1
		Photophobia	1
		Swelling, eyes/lids	1
		Swelling, pharynx	1
	Parenteral	Diarrhoea	1
		Dizziness	1
		Abdominal pain	1
		Sweating	1
		Vomiting	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
	Suppository	Epiphora	1
	Topical	Irritation, eyes/lids	13
		Nausea	8
		Vomiting	6
		Fever	4
		Headache	4
		Rash	4
		Congestion, skin	3
		Inflammation, skin	3
		Sweating	3
		Skin disorder	2
		Confusion	1
		Congestion, eyelid	1
		Conjunctivitis	1
		Depression/lethargy	1
		Diarrhoea	1
		Dizziness	1
		Oedema, face	1
		Oedema, limbs	1
		Hypoesthesia	1
		Insomnia	1
		Skin irritation	1
		Neurological disorder	1
		Pain	1
		Pain, head/face	1
		Pain, limbs	1
		Joint pain	1
		Muscle pain	1
		High blood pressure	1
		Swelling, eyes/lids	1
		Vesicles/bullae, skin	1
		Weakness	1
	Unknown	Coughing	1
		Swelling, head/face	1
		Vomiting	1
Febantel, praziquantel, pyrantel	Oral	Abdominal pain	1
	Topical	Swelling, head/face	1
		Urticaria	1
Fenbendazole	Inhalation	Irritation, pharynx	2
		Coughing	1
		Nose, inflammation	1
		Sneezing	1
		Taste abnormality	1
	Not applicable	Hyperactivity	1
		Irritation, eyes/lids	1
		Skin, abnormal	1
	Ophthalmic	Irritation, eyes/lids	1
	Oral	Diarrhoea	5
		Abdominal pain	3

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Fenbendazole *(cont.)*		Vomiting	3
		Nausea	2
		Respiratory disorder	1
	Topical	Skin disorder	2
		Gastritis	1
		Hypoesthesia	1
		Skin lesions	1
		Abdominal pain	1
		Paraesthesia, mouth/lip	1
		Rash	1
		Abnormal skin	1
		Swelling	1
		Twitch	1
		Skin ulcers	1
		Urticaria	1
		Weakness	1
	Unknown	Nausea	2
		Diarrhoea	1
		Dizziness	1
		Fever	1
		Abdominal pain	1
		Vomiting	1
Fenthion	Inhalation	Dizziness	1
		Gastroenteritis	1
	Topical	Dizziness	1
		Hypoesthesia	1
		Rash	1
	Unknown	Hypoesthesia, feet/digits	1
Firocoxib	Oral	Dizziness	3
		Nausea	3
		Abdominal pain	3
		Depression/lethargy	2
		Gastrointestinal, abnormal	2
		Bloody diarrhoea	1
		Severe diarrhoea	1
		Dysuria	1
		Fever	1
		Gagging	1
		Gastritis	1
		Irritation, oesophagus	1
		Melena	1
		Joint pain	1
		Low blood pressure	1
		Elevated liver enzymes	1
		Ulcers, stomach	1
		Vasculitis	1
		Vomiting	1
		Vomiting, severe	1
		Weakness	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
	Unknown	Pruritus	1
		Urticaria	1
Florfenicol, flunixin	Parenteral	Abnormality, application site	1
		Swelling, application site	1
		Skin irritation	1
		Nausea	1
		Paraesthesia	1
	Unknown	Injection site pain	2
		Injection site swelling	2
Florfenicol	Missing	Injection site pain	2
		Injection site inflammation	1
		Injection site swelling	1
		Limb pain	1
	N/A	Injection site pain	2
		Arthritis	1
		Cellulitis	1
		Hypoesthesia	1
		Injection site swelling	1
		Pain	1
		Pruritus, eyes	1
	Ophthalmic	Irritation, eyelids	1
		Vision disorder	1
	Oral	Hot flush	1
		Joint pain	1
	Parenteral Topical	Injection site pain	14
		Injection site swelling	7
		Injection site inflammation	4
		Injection site oedema	4
		Injury	4
		Injection site bleeding	3
		Bleeding	2
		Hypoesthesia	2
		Injection site stiffness	2
		Swelling	2
		Congestion, skin	1
		Diarrhoea	1
		Injection site induration	1
		Injection site mass	1
		Abdominal pain	1
		Pain, feet/digits	1
		Joint pain	1
		Limb pain	1
		Muscle pain	1
		Paraesthesia	1
		Stiffness, hands	1
		Swelling, joints	1
		Dizziness	2

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Florfenicol *(cont.)*		Oedema, head/face	1
		Injection site pain	1
		Rash	1
		Taste abnormality	1
	Unknown	Injection site swelling	3
		Ecchymoses	1
		Injection site pain	1
		Taste abnormality	1
	Various	Application site erythema	1
		Application site pain	1
		Congestion, skin	1
Flunixin	Ophthalmic	Pain, eyes/lids	4
		Irritation, eyes/lids	1
		Vision disorder	1
		Congestion, eyes/lids	1
		Headache	1
		Irritation, skin	1
		Nausea	1
	Oral	Vomiting	1
		Vomiting, bloody	1
	Parenteral	Injection site swelling	1
		Congestion, skin	1
		Dizziness	1
		Injection site pain	1
		Sweating	1
		Swelling feet, digits	1
		Weakness	1
	Topical	Ataxia	1
		Hyperesthesia	1
		Irritation, skin	1
		Rash	1
	Unknown	Confusion	2
		Elevated liver enzymes	2
		Gastrointestinal bleeding	1
		Abnormal chemistry	1
		Depression/lethargy	1
		Encephalopathy	1
		Injection site pain	1
Fluoxetine	Oral	Headache	1
Furazolidone	Topical	Inflammation	1
Glycosaminoglycan polysulphate	Parenteral	Injection site bleeding	2
		Injection site inflammation	1
		Injection site pain	1
	Unknown	Joint pain	2
		Joint swelling	2
		Anaphylaxis/ anaphylactoid	1
		Abnormal chemistry	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
		Death	1
		Liver failure	1
		Low platelet count	1
		Urticaria	1
Gonadorelin	Ophthalmic	Irritation, eyes/lids	2
	Parenteral	Injection site pain	2
		Ill	1
		Injection site swelling	1
		Joint pain	1
		Weakness	1
	Topical	Behaviour disorder	1
		Depression/lethargy	1
		Dyspnoea	1
		Irritation, eyes/lids	1
		Abnormal menses	1
		Nausea	1
		Abdominal pain	1
		Chest pain	1
		Urticaria	1
	Unknown	Injection site bleeding	1
Imidacloprid, moxidectin	Inhalation	Nausea	1
		Wheezing	1
		Dyspnoea	1
		Vomiting	1
		Headache	1
	Missing	Nausea	2
		Abdominal pain	2
	Ophthalmic	Irritation, eyes/lids	19
		Conjunctivitis	3
		Pain, eyes/lids	2
		Congestion, eyes	1
		Epiphora	1
		Eye disorder	1
		Pruritus, eyes	1
		Vesicles/bullae	1
	Oral	Diarrhoea	2
		Hypoesthesia, mouth/lips	2
		Nausea	2
		Paraesthesia, mouth/lips	2
		Tongue abnormality	2
		Borborygmus	1
		Congestion, skin	1
		Hyperesthesia	1
		Irritation, mouth/lips	1
		Vomiting	1
	Topical	Nausea	12
		Rash	10
		Urticaria	9
		Anaphylaxis/ anaphylactoid	8

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Imidacloprid, moxidectin *(cont.)*		Headache	7
		Pruritus	7
		Depression/lethargy	5
		Dizziness	5
		Hypoesthesia	5
		Taste abnormality	5
		Vomiting	5
		Congestion, skin	5
		Irritation, eyes/lids	4
		Paraesthesia, mouth/lip	4
		Application site erythema	3
		Irritation, pharynx	3
		Tongue abnormality	3
		Diarrhoea	2
		Hyperesthesia	2
		Hypoesthesia	2
		Ill	2
		Irritation	2
		Pain	2
		Paraesthesia	2
		Swelling, eyes/lids	2
		Application site pain	1
		Application site pruritus	1
		Apprehension	1
		Breathing abnormality	1
		Congestion, lungs	1
		Convulsions	1
		Dehydration	1
		Discharge, nose	1
		Discomfort	1
		Discomfort, mouth/lips	1
		Dyspnoea	1
		Ear disorder	1
		Oedema, limbs	1
		Epistaxis	1
		Hypersalivation	1
		Irritation, mouth/lips	1
		Nail disorder	1
		Nose abnormality	1
		Odour	1
		Abdominal pain	1
		Pain, feet/digits	1
		Pain, head/face	1
		Paraesthesia	1
		Pharyngitis	1
		Pruritus, eyes	1
		Pruritus, feet/digits	1
		Skin abnormality	1
		Spasm	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
		Swelling	1
		Swelling, ears	1
		Swelling, mouth/lips	1
		Swelling, pharynx	1
	Unknown	Congestion, skin	8
		Pruritus	7
		Urticaria	5
		Irritation, skin	4
		Dizziness	3
		Nausea	3
		Anaphylaxis/ anaphylactoid	2
		Congestion	2
		Discharge, nose	2
		Paraesthesia, mouth/lip	2
		Application site abnormality	1
		Coughing	1
		Diarrhoea	1
		Hypoesthesia, mouth/lips	1
		Inflammation skin	1
		Irritation pharynx	1
		Lesions, mouth/lips	1
		Nail disorder	1
		Nervousness	1
		Odour	1
		Pain	1
		Pain, mouth/lips	1
		Paraesthesia	1
		Swelling, mouth/lips	1
		Tongue, abnormality	1
		Twitch	1
		Vision disorder	1
		Xerostoma	1
	Various	Anaphylaxis/ anaphylactoid	2
		Hypoesthesia, mouth/lips	2
		Paraesthesia, mouth/lip	2
		Taste abnormality	2
		Tongue abnormality	2
		Congestion, eyes/lid	1
		Congestion, sinus	1
		Conjunctivitis	1
		Ear disorder	1
		Enlargement, lymph node	1
		Hyperesthesia	1
		Irritation, eyes/lid	1
		Irritation, mouth/lips	1
		Nausea	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Imidacloprid, moxidectin *(cont.)*		Pain	1
		Pain, eyes/lids	1
		Paraesthesia	2
		Stomatitis	1
		Stool abnormality	1
		Sweating	1
		Swelling, eyes/lids	1
		Swelling, head/face	1
		Swelling, mouth/lips	1
Imidocarb	Ophthalmic	Congestions, eyes/lids	1
	Parenteral	Injection site swelling	2
		Injection site pain	1
		Nausea	1
Insulin	Parenteral	Injection site pain	5
		Congestion, skin	2
		Injection site abnormality	2
		Depression/lethargy	1
		Hypoesthesia	1
		Incision site, ecchymosis	1
		Injection site bleeding	1
		Injection site pruritus	1
		Injection site swelling	1
		Pain, feet, digits	1
	Various	Rash	1
Isofluredone, neomycin	Ophthalmic	Swelling, eyes/lids	1
		Vision disorder	1
Isoflupredone, neomycin, tetracaine	Topical	Congestion, skin	1
		Depression/lethargy	1
		Headache	1
		Inflammation skin	1
		Swelling, head/face	1
Isoflupredone	Topical	Paraesthesia	1
Isoflurane	Inhalation	Headache	1
		Death	1
		Dizziness	1
		Ear disorders	1
		Vision disorders	1
	Topical	Irritation, skin	1
		Skin disorder	1
Ivermectin	N/A	Dizziness	2
		Syncope	2
		Headache	1
	Ophthalmic	Irritation	18
		Pain, eyes/lids	7
		Congestion, eyes/lids	4
		Pruritus, eyes/lids	2
		Swelling, eyes/lids	2
		Discharge, eyes/lids	1
		Vision disorder	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
	Oral	Vomiting	4
		Dizziness	3
		Depression/lethargy	2
		Nausea	2
		Abdominal pain	2
		Xerostoma	2
		Arrhythmia	1
		Ataxia	1
		Bradycardia	1
		Diabetes mellitus	1
		Diarrhoea	1
		Diarrhoea, mild	1
		Diplopia	1
		Dyspnoea	1
		Enlargement, lymph node	1
		Glossitis	1
		Hypopnoea	1
		Ill	1
		Irritation, mouth/lip	1
		Kidney failure	1
		Liver failure	1
		Chest pain	1
		Pneumonia	1
		Low blood pressure	1
		Sedation	1
		Somnolence	1
		Stool abnormality	1
		Stupor	1
		Urticaria	1
		Vision disorder	1
		Severe vomiting	1
	Parenteral	Injection site pain	6
		Injection site bleeding	5
		Injection site inflammation	4
		Injection site swelling	3
		Hypoesthesia	2
		Ecchymoses	1
		CAT scan abnormality	1
		Congestion, skin	1
		Death	1
		Depression/lethargy	1
		Dizziness	1
		Effusion	1
		Hypomotililty	1
		Injection site abnormality	1
		Injection site stiffness	1
		Nausea	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Ivermectin *(cont.)*		Gastrointestinal perforation	1
		Rash	1
		Sepsis	1
		Shock	1
		Suicide attempt	1
		Joint swelling	1
Ivermectin, praziquantel	Ophthalmic	Irritation, eyes/lids	2
		Pain, eyes/lids	1
	Oral	Vomiting	4
		Nausea	3
		Tongue abnormality	3
		Diarrhoea	2
		Hypoesthesia	2
		Abdominal pain	2
		Breathing abnormality	1
		Confusion	1
		Gagging	1
		Hallucination	1
		Hypoesthesia, mouth/lips	1
		Hypopnoea	1
		Paraesthesia	1
		Paraesthesia, mouth/lips	1
		Vision disorder	1
Ivermectin, praziquantel, pyrantel	Oral	Diarrhoea	2
		Nausea	2
		Abnormal odour, urine	1
		Vomiting	1
Ivermectin, praziquantel	Topical	Headache	2
		Congestion, skin	1
		Depression/lethargy	1
		Diarrhoea	1
		Dizziness	1
		Epiphora	1
		Gastrointestinal abnormality	1
		Hyperesthesia	1
		Skin irritation	1
		Nausea	1
		Abdominal pain	1
		Limb pain	1
		Papules	1
		Paraesthesia	1
		Tachycardia	1
		Taste abnormality	1
		Vision disorder	1
		Vomiting	1
Ivermectin, pyrantel	Oral	Dizziness	4
		Diarrhoea	3

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
		Abdominal pain	3
		Ill	2
		Circulatory disorder	1
		Depression/lethargy	1
		High blood pressure	1
		Stool abnormality	1
		Vomiting	1
		Vomiting, bloody	1
	Topical	Congestion, skin	1
		Pruritus	1
		Swelling, head/face	1
	Unknown	Diarrhoea	1
		Abdominal pain	1
		Vomiting	1
Ivermectin	Topical	Nausea	12
		Headache	7
		Depression/lethargy	5
		Diarrhoea	4
		Abdominal pain	4
		Taste abnormality	4
		Vomiting	4
		Anorexia	3
		Dizziness	3
		Rash	3
		Congestion, skin	2
		Hypoesthesia	2
		Irritation, eyelid	2
		Somnolence	2
		Stupor	2
		Sweating	2
		Vision disorder	2
		Abrasion, cornea	1
		Ataxia	1
		Breathing abnormality	1
		Confusion	1
		Congestion, sinus	1
		Conjunctivitis	1
		Coughing	1
		Diarrhoea, mild	1
		Diplopia	1
		Discomfort	1
		Respiratory distress	1
		Dry skin	1
		Dysphagia	1
		Dyspnoea	1
		Enlargement, lymph node	1
		Eruptions	1
		Eye disorder	1
		Fever	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Ivermectin *(cont.)*		Hyperesthesia	1
		Hypoesthesia, mouth/lips	1
		Ill	1
		Skin inflammation	1
		Irritation, pharynx	1
		Kidney failure	1
		Mydriasis	1
		Oliguria	1
		Chest pain	1
		Pain, eyes/lids	1
		Pain, testicle	1
		Slough, skin	1
		Stiffness, hands	1
		Swelling, mouth/lips	1
		Swelling, pharynx	1
		Trembling	1
		Vesicles/bullae, skin	1
	Unknown	Infection, eyes	2
		Anorexia	1
		Confusion	1
		Congestion, eyes/lids	1
		Abnormal defecation	1
		Diarrhoea	1
		Dizziness	1
		Dysuria	1
		Epiphora	1
		Haematochezia	1
		Irritation, eyes/lids	1
		Nausea	1
		Pain	1
		Pain, eyes/eyelids	1
		Joint pain	1
		Prostration	1
		Pruritus, eyes	1
		Abnormal urination	1
		Vomiting	1
	Various	Hypoesthesia	1
		Irritation, eyes/lid	1
Ketamine	Ophthalmic	Congestion, eyes/lid	1
		Dizziness	1
		Irritation, eyes/lid	1
		Nausea	1
		Pain, eyes/lids	1
	Oral	Dissociation	1
		Hallucination	1
	Parenteral	Urticaria	1
	Unknown	Hallucination	1
	Various	Irritation, eyes/lids	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Levamisole	N/A	Rash	1
	Parenteral	Injection site swelling	1
		Injection site abnormality	1
		Injection site pain	1
	Topical	Diarrhoea	2
		Abdominal pain	2
		Arrhythmia	1
		Constipation	1
		Depression/lethargy	1
		Headache	1
		Hypoesthesia	1
		Ill	1
		Skin irritation	1
		Nausea	1
		Neuritis	1
		Back pain	1
		Joint pain	1
		Limb pain	1
		Muscle pain	1
		Paraesthesia	1
		Rash	1
		Taste abnormality	1
		Vision disorder	1
		Vomiting	1
	Unknown	Injection site pain	1
		Injection site swelling	1
Levothyroxine	Oral	Nausea	1
		Pain	1
		Abdominal pain	1
		Vomiting	1
Lincomycin	Unknown	Injection site infection	1
		Injection site pain	1
Lufenuron	Ophthalmic	Irritation, eyes/lids	1
		Pain, eyes/lids	1
	Oral	Nausea	1
	Parenteral	Injection site pain	2
		Diarrhoea	1
		Ecchymosis	1
		Injection site mass	1
		Injection site swelling	1
		Nausea	1
Maropitant	Inhalation	Irritation, pharynx	1
		Pruritus	1
	Ophthalmic	Irritation, eyes/lids	4
		Pain, eyes/lids	2
		Swelling, eyes/lids	2
		Haematoma	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Maropitant *(cont.)*	Oral	Dizziness	3
		Anaphylaxis/ anaphylactoid	1
		Confusion	1
		Depression/lethargy	1
		Headache	1
		Nausea	1
		Chest pain	1
		Pruritus	1
		Urticaria	1
	Parenteral	Injection site pain	1
		Hypoesthesia	1
		Injection site swelling	1
		Haematoma	1
		Congestion, skin	1
		Injection site abnormality	1
		Hyperesthesia	1
		Injection site inflammation	1
		Nausea	1
		Paraesthesia	1
	Topical	Application site pain	1
		Paraesthesia	2
		Rash	1
	Unknown	Injection site swelling	2
		Haematoma	1
		Headache	1
		Injection site inflammation	1
		Injection site pain	1
Mebendazole	Oral	Depression/lethargy	1
		Gastritis	1
Medetomidine	Ophthalmic	Hypoesthesia, eyes/lid	1
	Oral	Dizziness	1
	Parenteral	Hypoesthesia	2
		Anaphylaxis/ anaphylactoid	1
		Coughing	1
		Injection site oedema	1
		Injection site swelling	1
		Irritation, pharynx	1
		Pruritus	1
		Swelling, pharynx	1
	Unknown	Bradycardia	2
		Ataxia	1
		Depression/lethargy	1
		Headache	1
		Hypoesthesia	1
		Injection site abnormality	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
		Miosis	1
		Pain, eyes/lids	1
		Low blood pressure	1
		Abnormal reflexes	1
		Suicide attempt	1
		Unconsciousness	1
	Various	Hypoesthesia	1
		Irritation, skin	1
		Nausea	1
		Pain, eyes/lids	1
Melarsomine	Ophthalmic	Irritation, eyes/lids	13
		Congestion, eyes/lids	2
		Pain, eyes/lids	2
	Oral	Paraesthesia, mouth/lip	1
	Parenteral	Injection site inflammation	4
		Injection site pain	4
		Injection site swelling	4
		Congestion, skin	2
		Injection site bleeding	2
		Oedema, corneas	1
		Eye disorder	1
		Inflammation, skin	1
		Injection site pruritus	1
		Irritation, eyes/lids	1
		Paraesthesia	1
		Ulcers, corneal	1
	Topical	Congestion, skin	1
		Discomfort	1
		Dizziness	1
		Hypoesthesia	1
		Irritation, skin	1
		Pain, eyes/lids	1
		Vesicles/bullae	1
	Unknown	Injection site bleeding	1
		Injection site pain	1
	Various	Irritation, eyes/lids	1
Meloxicam	Intranasal	Nose, inflammation	1
	Ophthalmic	Irritation, eyes/lids	35
		Pain, eyes/lids	10
		Congestion, eyes/lids	8
		Vision disorder	6
		Conjunctivitis	1
		Epiphora	1
		Eye disorder	1
		Inflammation	1
		Swelling, eyes/lids	1
	Oral	Congestion, lungs	1
		Hypoesthesia	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Meloxicam *(cont.)*		Irritation, pharynx	1
		Irritation, skin	1
		Abdominal pain	1
		Pruritus	1
		Swelling, tongue	1
		Vision disorder	1
	Topical	Pruritus	4
		Congestion, skin	3
		Blepharospasm	1
		Dizziness	1
		Impaired healing	1
		Hypoesthesia	1
		Ill	1
		Inflammation, skin	1
		Irritation, eyes/lids	1
		Irritation, skin	1
		Paraesthesia	1
		Photophobia	1
		Rash	1
		Elevated liver enzymes	1
		Slough, skin	1
		Spasm	1
		Swelling	1
		Swelling, mouth/lips	1
		Taste abnormality	1
		Vesicles/bullae	1
	Various	Irritation, eye	2
		Headache	1
		Nausea	1
		Abdominal pain	1
		Paraesthesia	1
		Tongue abnormality	1
Mepivacaine	Ophthalmic	Pain, eyes/lids	1
Mercaptobenzothiazole	Missing	Application site pain	1
	Ophthalmic	Irritation, eyes/lids	7
		Congestion, eyes/lids	2
		Pain, eyes/lids	2
		Swelling, eyes/lids	1
		Vision disorder	1
	Oral	Vomiting	5
		Irritation, pharynx	2
		Anorexia	1
		Behaviour disorder	1
		Flatulence	1
		Hypersalivation	1
		Abdominal pain	1
		Taste abnormality	1
	Topical	Congestion, skin	2
		Pruritus	2

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
		Rash	2
		Headache	1
		Ill	1
		Inflammation, skin	1
		Irritation, mouth/lip	1
		Pain, head/face	1
		Paraesthesia	1
		Skin abnormality	1
		Sweating	1
		Vocalisation	1
	Unknown	Irritation, eyes/lids	2
		Rash	2
		Congestion, eyes/lids	1
		Coughing	1
		Gagging	1
		Odour	1
		Pruritus	1
		Taste abnormality	1
	Various	Gagging	1
Methoxyflurane	Inhalation	Birth defects	1
		Discharge, nose	1
		Dizziness	1
		Headache	1
		Pain, head/face	1
		Paraesthesia	1
		Urticaria	1
Miconazole	Ophthalmic	Irritation, eyes/lids	1
		Pain, eyes/lids	1
Miconazole, polymixin B, prednisone	Ophthalmic	Irritation, eyes/lids	1
Milbemycin, lufenuron	Oral	Nausea	11
		Vomiting	6
		Headache	3
		Abdominal pain	3
		Dizziness	2
		Taste abnormality	2
		Apprehension	1
		Congestion, skin	1
		Abnormal defecation	1
		Diarrhoea	1
		Mild diarrhoea	1
		Discharge, nose	1
		Eructation	1
		Hot flush/flash	1
		Hot flush	1
		Hypoesthesia, limbs	1
		Paraesthesia	1
		Pruritus	1
		Sweating	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Milbemycin, lufenuron *(cont.)*		Swelling, neck	1
		Transudate	1
		Xerostomia	1
	Parenteral	Irritation, skin	1
	Topical	Congestion, skin	2
		Pruritus	2
		Inflammation, skin	1
		Lesions, skin	1
		Rash	1
		Skin, crusts	1
		Swelling, limbs	1
	Unknown	Depression/lethargy	1
		Dermatitis, moist	1
		Discharge, eyes	1
		Ill	1
		Pain, eyes/lids	1
		Pain, joints	1
		Pruritus, eyes	1
		Swelling, eyes/lids	1
		Urticaria	1
Milbemycin	Oral	Nausea	34
		Diarrhoea	12
		Headache	7
		Vomiting	7
		Abdominal pain	6
		Dizziness	6
		Congestion, skin	3
		Rash	3
		Congestion, eyes/lids	2
		Depression/lethargy	2
		Fever	2
		Ill	2
		Nervousness	2
		Somnolence	2
		Discomfort	1
		Distention, abdomen	1
		Ear disorder	1
		Flatulence	1
		Gastritis	1
		Gastrointestinal abnormality	1
		Hot flash	1
		Hot flash/flush	1
		Hot flush	1
		Infection	1
		Inflammation, skin	1
		Insomnia	1
		Irritation, pharynx	1
		Chest pain	1
		Pain, eyes/lids	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
		Pain, limbs	1
		High blood pressure	1
		Pruritus, eyes	1
		Stomach reflux	1
		Sedation	1
		Tachycardia	1
		Taste abnormality	1
		Vocalisation	1
		Weakness	1
	Topical	Paraesthesia, mouth/lip	1
		Rash	1
		Taste abnormality	1
		Urticaria	1
	Unknown	Pruritus	1
		Rash	1
Monensin	Inhalation	Arrest, heart	1
		Diarrhoea	1
		Oedema, lungs/trachea	1
		Headache	1
		Hypoesthesia	1
		Irritation, oesophagus	1
		Paraesthesia	1
		Lungs, lesions	1
		Tongue abnormality	1
		Weakness	1
	Oral	Bleeding, lungs/trachea	1
	Topical	Urticaria	2
		Congestion, skin	1
		Discharge, eyes/lids	1
		Healing impaired	1
		Pruritus	1
		Rash	1
		Swelling, eyes/lids	1
Monensin, tylosin	Topical	High blood pressure	1
Moxidectin	Inhalation	Irritation, eyes/lid	1
		Irritation, mouth/lip	1
	Ophthalmic	Irritation, eyes/lid	2
		Congestion, eyes/lid	1
		Pain, eyes/lid	1
		Skin disorder	1
		Swelling, eyes/lids	1
	Parenteral	Injection site inflammation	2
		Ecchymosis	1
		Injection site pain	1
		Injection site stiffness	1
		Injection site swelling	1
		Pain	1
		Swelling	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Moxidectin, praziquantel	Ophthalmic	Irritation, eyes/lids	1
		Pain, eyes/lids	1
Moxidectin	Topical	Diarrhoea	5
		Headache	5
		Inflammation, skin	4
		Nausea	4
		Abdominal pain	4
		Anorexia	3
		Fever	3
		Hypoesthesia	3
		Pain, face/head	3
		Diarrhoea, mild	2
		Hyperesthesia	2
		Lesions, skin	2
		Pain, muscles	2
		Paraesthesia	2
		Rash	2
		Swelling, feet/digits	2
		Vomiting	2
		Abnormal colour urine	1
		Application site erythema	1
		Application site pain	1
		Application site pruritus	1
		Application site swelling	1
		Congestion, eyes/lids	1
		Congestion, skin	1
		Depression/lethargy	1
		Diarrhoea, bloody	1
		Exfoliation, skin	1
		Insomnia	1
		Irritation, eyes/lids	1
		Nail disorder	1
		Pain, joints	1
		Paraesthesia	1
		Pruritus	1
		Swelling	1
		Swelling, eyes/lids	1
		Swelling, head/face	1
		Swelling, tongue	1
		Taste abnormality	1
		Urticaria	1
	Unknown	Anaemia	1
		Dizziness	1
		Injection site inflammation	1
		Injection site pain	1
		Injection site swelling	1
		Kidney failure	1
		Abdominal pain	1
	Various	Headache	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Narasin	Unknown	Congestion, lungs	1
		Irritation, pharynx	1
		Rhinitis	1
n-Butyl chloride	Ophthalmic	Pain	1
Nitazoxanide	Topical	Skin abnormality	3
		Depression/lethargy	2
		Nail disorder	2
		Anorexia	1
		Application site abnormality	1
		Dizziness	1
		Hyperpigmentation, skin	1
		Skin disorder	1
		Somnolence	1
Nitenpyram	Ophthalmic	Eye disorder	1
		Irritation, eyes	1
		Pruritus, eyes	1
	Oral	Nausea	8
		Diarrhoea	5
		Headache	5
		Vomiting	5
		Apprehension	5
		Depression/lethargy	3
		Hot flush	3
		Abdominal pain	2
		Pruritus	2
		Somnolence	2
		Taste abnormality	2
		Anaphylaxis/ anaphylactoid	1
		Congestion, skin	1
		Dehydration	1
		Dizziness	1
		Glossitis	1
		Ill	1
		Rash	1
		Abnormal stool	1
		Swelling, eyes/lids	1
		Xerostomia	1
	Topical	Rash	4
		Nausea	2
		Anaphylaxis/ anaphylactoid	1
		Congestion, skin	1
		Dizziness	1
		Hypoesthesia	1
		Irritation, pharynx	1
		Pruritus	1
		Swelling	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Nitenpyram *(cont.)*		Swelling, eyes/lids	1
		Swelling, head/face	1
		Swelling, mouth/lips	1
		Taste abnormality	1
		Urticaria	1
		Vesicles/bullae, skin	1
		Vomiting	1
	Unknown	Congestion, skin	1
		Pruritus	1
Omeprazole	Oral	Vomiting	1
Orbifloxacin	Oral	Dizziness	1
		Nausea	1
Ormetoprim, sulfadimethoxine	Oral	Diarrhoea	1
		Nausea	1
		Stranguria	1
		Vomiting	1
Oxfendazole	Topical	Pruritus	1
		Rash	1
		Vesicles/bullae, skin	1
Oxytetracycline	Oral	Abnormal colour urine	1
		Congestion, skin	1
		Gastritis	1
		Headache	1
		Hepatitis	1
		Chest pain	1
		High blood pressure	1
	Parenteral	Injection site swelling	6
		Injection site bleeding	3
		Fever	2
		Injection site inflammation	2
		Injection site mass	2
		Dizziness	1
		Injection site abnormality	1
		Injection site pain	1
		Septicaemia	1
Oxytetracycline, polymixin B	Ophthalmic	Irritation, eyes/lids	2
		Diarrhoea	1
		Oedema, head/face	1
		Vomiting	1
Oxytetracycline	Topical	Congestion, skin	1
		Headache	1
		Irritation, skin	1
		Paraesthesia	1
		High blood pressure	1
	Unknown	Eye disorder	1
		Injection site pain	1
		Liver disorder	1
		Elevated liver enzymes	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
		Pain, limbs	1
		Paraesthesia	1
Penicillin G benzathine, penicillin G procaine	Parenteral	Injection site infection	1
		Injection site pain	1
	Topical	Skin disorder	1
Penicillin G procaine	Unknown	Fever	1
		Neurological disorder	1
		Rash	1
Pentobarbital	Ophthalmic	Irritation, eyes/lids	1
		Pain, eyes/lids	1
Pentobarbital, phenytoin	Intranasal	Dizziness	1
	Ophthalmic	Irritation, eyes/lids	26
		Pain, eyes/lids	7
		Congestion, eyes/lids	4
		Dizziness	3
		Irritation, eyes/lids	3
		Vision disorder	3
		Conjunctivitis	2
		Oedema, cornea	2
		Ulcers, cornea	2
		Abrasion, cornea	1
		Eye disorder	1
		Hyperpnoea	1
		Hyperesthesia	1
		Nausea	1
		Pruritus, eyes	1
		Tachycardia	1
	Oral	Bradycardia	1
		Confusion	1
		Headache	1
		Irritation, eyes/lids	1
		Taste abnormality	1
	Parenteral	Injection site pain	7
		Hypoesthesia	5
		Injection site swelling	3
		Injection site abnormality	2
		Injection site bleeding	2
		Injection site inflammation	2
		Vesicles/bullae, skin	2
		Death (suicide)	1
		Lesions, skin	1
		Suicide attempt	1
		Unconsciousness	1
	Topical	Taste abnormality	2
		Temporary blindness	1
		Conjunctivitis	1
		Depression/lethargy	1
		Dizziness	1
		Eye disorder	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Pentobarbital, phenytoin *(cont.)*		Headache	1
		Hypoesthesia	1
		Hypoesthesia, feet/digits	1
		Irritation, eyes/lids	1
		Pain, eyes/lids	1
		Pain, feet/digits	1
		Photophobia	1
		Pruritus	1
		Rash	1
		Swelling, feet/digits	1
	Unknown	Headache	1
		Hyperactivity	1
		Ill	1
		Injection site pain	1
		Irritation, eyes/lids	1
		Nausea	1
		Pneumonia, aspiration	1
		Rash	1
		Suicide attempt	1
		Unconsciousness	1
		Urticaria	1
	Various	Irritation, eyes/lids	3
		Headache	1
		Swelling, eyes/lids	1
Pimobendan	Oral	Dizziness	1
		Nausea	1
		Low blood pressure	1
		Xerostomia	1
Poloxalene	Oral	Diarrhoea	1
		Eructation	1
		Irritation, skin	1
		Nausea	1
		Pain, limbs	1
		Spasm	1
		Vomiting	1
Ponazuril	Topical	Dizziness	1
		Rash	1
Praziquantel	Ophthalmic	Irritation, eyes/lids	4
		Congestion, eyes/lids	1
		Pain, eyes/lids	1
	Oral	Insomnia	1
		Abdominal pain	1
		Vomiting	1
	Parenteral	Hypoesthesia	1
		Injection site swelling	1
		Irritation, eyes/lids	1
Praziquantel, pyrantel	Unknown	Diarrhoea	1
		Nausea	1
		Abdominal pain	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Prednisolone, trimeprazine	Oral	Depression/lethargy	2
		Somnolence	2
		Gastrointestinal abnormality	1
		Xerostomia	1
Primidone	Oral	Drug interaction	1
		Somnolence	1
Progesterone	Oral	Vomiting	1
	Topical	Abnormal menses	2
		Fever	1
		Insomnia	1
	Unknown	CNS disorder	1
		Depression/lethargy	1
		Vesicles/bullae/skin	1
Propofol	Parenteral	Injection site bleeding	1
		Injection site swelling	1
	Various	Hypoesthesia	1
Pyrantel	Oral	Depression/lethargy	3
		Arrhythmia	1
		Diarrhoea	1
		Hypersalivation	1
		Nausea	1
		Abdominal pain	1
		Vocalisation	1
	Topical	Rash	2
		Congestion, skin	1
		Dyspnoea	1
		Inflammation, skin	1
		Irritation, skin	1
		Lesions, skin	1
	Unknown	Rash	1
		Vomiting	1
Ractopamine	Inhalation	Epistaxis	2
		Nausea	2
		Arrhythmia	1
		Discharge, nose	1
		Fever	1
		Headache	1
		Hypoesthesia	1
		Pruritus, eyes	1
		Sinusitis	1
		Sneezing	1
		Tachycardia	1
		Weakness	1
	Oral	Arrhythmia	1
		Myositis	1
		Muscle pain	1
		Unconsciousness	1
	Topical	Application site pain	1
		Application site swelling	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Ractopamine *(cont.)*		Circulatory disorder	1
		Dizziness	1
		Eruptions	1
		Limb pains	1
	Unknown	Chest pain	2
		Anaphylaxis/ anaphylactoid	1
		Anaemia	1
		Heart disorder	1
		Pain	1
		Pain, limbs	1
		Platelet abnormalities	1
		Respiratory disorder	1
		Tachycardia	1
	Various	Tachycardia	3
		Arrhythmia	1
		Coughing	1
		Discharge, mouth/lips	1
		Dizziness	1
		ECG abnormality	1
		Respiratory disorder	1
		Spasm	1
Romifidine	Parenteral	Bradycardia	1
		Somnolence	1
Roxarsone	Various	Death	1
		Immune disorder	1
		Neoplasm	1
Selamectin	Inhalation	Headache	4
		Nausea	4
		Taste abnormality	4
		Irritation, pharynx	4
		Anaphylaxis/ anaphylactoid	2
		Coughing	2
		Depression/lethargy	2
		Discharge, eyes/lids	2
		Distress, respiratory	2
		Dizziness	2
		Dyspnoea	2
		Hypoesthesia	2
		Irritation, eyes/lids	2
		Respiratory disorder	2
		Swelling, eyes/lids	2
		Swelling, pharynx	2
		Wheezing	2
		Confusion	1
		Congestion, nose	1
		Congestion, sinus	1
		Discharge, nose	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
		Epiphora	1
		Gagging	1
		Hypoesthesia, feet/digits	1
		Irritation	1
		Nose, abnormality	1
		Odour	1
		Pain	1
		Pain, eyes/lids	1
		Pain, head/face	1
		Pain, joints	1
		Palpitations	1
		Paraesthesia	1
		Polypnoea	1
		Pruritus, eyes	1
		Rhinitis	1
		Sinusitis	1
		Swelling, joints	1
		Swelling, mouth/lips	1
		Trembling	1
	Intranasal	Enlargement, lymph node	1
		Pain, neck	1
		Respiratory disorder	1
	Missing	Rash	2
		Urticaria	2
		Anaphylaxis/ anaphylactoid	1
		Application site pain	1
		Headache	1
		Inflammation, skin	1
		Pain, back	1
		Swelling	1
	N/A	Rash	3
		Irritation, skin	2
		Pruritus	2
		Skin, bleeding	1
		Congestion, skin	1
		Headache	1
		Lesions, skin	1
		Pain, mouth/lips	1
		Paraesthesia, mouth/lip	1
		Swelling, head/face	1
		Swelling, mouth/lips	1
		Urticaria	1
	Ophthalmic	Irritation, eyes/lids	20
		Pain, eyes/lids	10
		Congestion, eyes/lids	6
		Swelling, eyes/lids	4
		Conjunctivitis	3
		Eye disorder	3

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Selamectin *(cont.)*		Discharge, eyes/lids	2
		Vision disorder	2
		Abrasion, cornea	1
		Anorexia	1
		Application site pain	1
		Temporary blindness	1
		Confusion	1
		Congestion, skin	1
		Fever	1
		Irritation, pharynx	1
		Pain, joints	1
		Pain, mouth/lips	1
		Pruritus	1
		Pruritus, eyes	1
		Skin irritation, eyes/lids	1
		Sleep abnormality	1
		Swelling, head/face	1
		Swelling, mouth/lips	1
		Syncope	1
		Twitch	1
		Vesicle/bullae, skin	1
		Weakness	1
	Oral	Nausea	7
		Taste abnormality	7
		Diarrhoea	4
		Tongue abnormality	3
		Dizziness	2
		Irritation, mouth/lips	2
		Urticaria	2
		Vomiting	2
		Breathing abnormality	1
		Coughing	1
		Discomfort, mouth/lips	1
		Fever	1
		Glossitis	1
		Hyperesthesia	1
		Hypoesthesia	1
		Hypopnoea	1
		Irritation, mouth/lips	1
		Irritation, pharynx	1
		Odour	1
		Pain, mouth/lips	1
		Pain, muscles	1
		Paraesthesia	2
		Paraesthesia, mouth/lips	1
		Pruritus	1
		Rash	1
		Shaking	1
		Somnolence	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
		Swelling, eyes/lids	1
		Swelling, head/face	1
		Swelling, mouth/lips	1
		Vesicles/bullae, skin	1
		Wheezing	1
	Parenteral	Injection site swelling	2
		Application site pain	1
		Application site pruritus	1
		Ecchymosis	1
		Headache	1
		Injection site inflammation	1
		Injection site pain	1
		Abdominal pain	1
		Urticaria	1
	Topical	Rash	148
		Pruritus	115
		Urticaria	106
		Irritation, skin	55
		Congestion, skin	44
		Headache	28
		Taste abnormality	27
		Hypoesthesia	26
		Nausea	26
		Dizziness	25
		Hyperesthesia	24
		Anaphylaxis/ anaphylactoid	22
		Diarrhoea	22
		Inflammation, skin	22
		Vomiting	22
		Irritation, eyes/lids	19
		Swelling, eyes/lids	18
		Swelling, mouth/lips	18
		Pain	15
		Paraesthesia	25
		Application site pain	13
		Dyspnoea	13
		Vesicles/bullae, skin	13
		Skin abnormality	12
		Irritation, pharynx	10
		Swelling	10
		Application site erythema	9
		Lesions, skin	9
		Pruritus, eyes	9
		Application site inflammation	8
		Abdominal pain	8
		Swelling, head/face	8

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Selamectin *(cont.)*		Congestion, eyes/lids	7
		Depression/lethargy	7
		Apprehension	6
		Coughing	6
		Discomfort, mouth/lips	6
		Epiphora	6
		Skin, dry	6
		Swelling, pharynx	6
		Vision disorder	6
		Weakness	6
		Application site swelling	5
		Fever	5
		Irritation, mouth/lips	5
		Pain, eyes/lids	5
		Skin disorder	5
		Swelling, feet/digits	5
		Swelling, tongue	5
		Alopecia	4
		Discharge, nose	4
		Leukoderma	4
		Pain, feet/digits	4
		Pain, joints	4
		Sweating	4
		Diplopia	3
		Dry skin	3
		Lesions, mouth/lips	3
		Nail disorder	3
		Nose abnormality	3
		Pain, limbs	3
		Pain, mouth/lips	3
		Paraesthesia, mouth/lips	3
		Respiratory disorder	3
		Scales, skin	3
		Somnolence	3
		Trembling	3
		Application site lesions	2
		Application site, dry skin	3
		Temporary blindness	2
		Congestion	2
		Conjunctivitis	2
		Discomfort	2
		Ear disorder	2
		Oedema	2
		Gagging	2
		Glossitis	2
		Ill	2
		Inflammation	2
		Nervousness	2
		Neurological disorder	2
		Odour	2

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
		Chest pain	2
		Pain, muscles	2
		Palpitations	2
		Skin crusts	2
		Swelling, ears	2
		Ulcers, skin	2
		Xerostomia	2
		Skin abscess	1
		Arrhythmia	1
		Ataxia	1
		Behaviour disorder	1
		Petechiae	1
		Bleeding, vagina	1
		Choking	1
		Congestion, face	1
		Congestion, pharynx	1
		Congestion, sinus	1
		Constipation	1
		Convulsions	1
		Diarrhoea, severe	1
		Discharge, eyes/lids	1
		Distension, abdomen	1
		Respiratory distress	1
		Dry mouth syndrome	1
		Dysphagia	1
		Oedema, eyes/lids	1
		Oedema, head/face	1
		Oedema, tongue	1
		Enlargement, lymph node	1
		Epistaxis	1
		Eruptions	1
		Erythema multiforme	1
		Exfoliation, skin	1
		Eye disorder	1
		Flatulence	1
		Gastritis	1
		Gastrointestinal abnormality	1
		Hallucination	1
		Heart disorder	1
		Hot flash/flush	1
		Hot flush	1
		Hyperkeratosis	1
		Hyperpigmentation, skin	1
		Hyperpnoea	1
		Hypersalivation	1
		Hypoesthesia, feet/digits	1
		Hyposalivation	1
		Infection	1
		Infection, eyes	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Selamectin *(cont.)*		Injection site abnormality	1
		Iris, inflammation	1
		Neuropathy	1
		Nystagmus, horizontal	1
		Pain, head/face	1
		Paresis	1
		High blood pressure	1
		Pruritus, feet/digits	1
		Elevated liver enzymes	1
		Sinusitis	1
		Sneezing	1
		Sores	1
		Swelling, joints	1
		Swelling, limbs	1
		Syncope	1
		Trembling face	1
		Ulcers, mouth/lips	1
		Urine abnormality	1
		Uterine contractions	1
		Vesicles/bullae, skin	1
	Unknown	Rash	48
		Urticaria	44
		Pruritus	42
		Congestion, skin	14
		Irritation, eyes/lids	13
		Swelling, eyes/lids	14
		Dyspnoea	9
		Irritation, skin	9
		Vomiting	7
		Headache	6
		Inflammation, skin	6
		Irritation, pharynx	6
		Nausea	6
		Pain	6
		Pruritus, eyes	6
		Diarrhoea	5
		Sneezing	5
		Taste abnormality	5
		Anaphylaxis/ anaphylactoid	4
		Confusion	4
		Coughing	4
		Dizziness	4
		Hypoesthesia	4
		Pain, eyes/lids	4
		Vesicles/bullae, skin	4
		Vision disorder	4
		Alopecia	3
		Congestion, eyes/lids	3
		Depression/lethargy	3

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
		Discharge, nose	3
		Abdominal pain	3
		Pain, limbs	3
		Dry skin	3
		Ataxia	2
		Enlargement, lymph node	2
		Fever	2
		Hypersalivation	2
		Irritation, mouth/lips	2
		Joint disorder	2
		Lesions, mouth/lips	2
		Lesions, skin	2
		Pain, head/face	2
		Pain, mouth/lips	2
		Paraesthesia	2
		Sinusitis	2
		Voice, abnormal sound	2
		Swelling, head/face	2
		Tongue, abnormality	2
		Voice disorder	2
		Aplastic anaemia	1
		Application site pain	1
		Application site swelling	1
		Apprehension	1
		Bleeding, eyes	1
		Bleeding, vagina	1
		Temporary blindness	1
		CNS disorder	1
		Congestion	1
		Congestion, lungs	1
		Conjunctivitis	1
		Discharge, eyes	1
		Discomfort	1
		Discomfort, mouth/lips	1
		Dysphagia	1
		Oedema, head/face	1
		Epiphora	1
		Exfoliation, skin	1
		Gastrointestinal abnormality	1
		Abnormal hair	1
		Hyperesthesia	1
		Hypoesthesia, mouth/lips	1
		Hypomotility	1
		Hypothermia	1
		Skin infection	1
		Nervousness	1
		Neurological disorder	1
		Chest pain	1
		Pains, joints	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Selamectin *(cont.)*		Paraesthesia	1
		Paraesthesia, mouth/lips	1
		High blood pressure	1
		Low blood pressure	2
		Scales, skin	1
		Skin disorder	1
		Slough, skin	1
		Sores	1
		Spasm	1
		Stiffness	1
		Stomatitis	1
		Stupor	1
		Swelling	1
		Swelling, feet/digits	1
		Swelling, limbs	1
		Swelling, mouth/lips	1
		Swelling, pharynx	1
		Swelling, tongue	1
		Tachycardia	
		Wheezing	1
		Tachycardia	1
	Various	Taste abnormality	3
		Headache	2
		Congestion, tongue	1
		Skin exfoliation	1
		Gagging	1
		Hyperesthesia	1
		Irritation, eyes/lids	1
		Irritation, mouth/lips	1
		Paraesthesia, mouth/lips	1
		Pruritus	1
		Abnormal reflexes	1
		Vision disorder	1
		Vomiting	1
Selegiline	Oral	Nausea	1
		High blood pressure	1
		Apprehension	1
		Diarrhoea	1
		Dizziness	1
		Eye disorder	1
		Headache	1
		Hypoesthesia	1
		Nervousness	1
		Abdominal pain	1
		Tachycardia	1
		Taste abnormality	1
Selenium, vitamin D	Missing	Injection site pain	1
	Ophthalmic	Congestion, eyes/lids	1
		Irritation, eyes/lids	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
	Parenteral	Death	1
		Pain, feet/digits	1
Semduramicin	Topical	Pruritus	1
		Rash	1
		Vesicles/bullae, skin	1
Sevoflurane	Inhalation	Dizziness	2
		Nausea	2
		Congestion, skin	1
		Hair abnormality	1
		Headache	1
		Lesions, skin	1
		Slough, skin	1
Sometribove	N/A	Injection site pain	9
		Injection site swelling	8
		Injection site bleeding	1
		Injection site inflammation	1
		Pain	1
		Rash	1
		Stiffness, hands	1
	Oral	Arthritis	1
		Eruptions	1
		Flatulence	1
		Joint disorder	1
		Odour, mouth	1
		Abdominal pain	1
		Muscle pain	1
		Pruritus	1
	Parenteral	Injection site pain	29
		Injection site swelling	25
		Injection site inflammation	10
		Injection site bleeding	4
		Injection site oedema	3
		Anaphylaxis/ anaphylactoid	2
		Purpura	2
		Congestion, skin	2
		Nausea	2
		Abdominal pain	2
		Rash	2
		Urticaria	2
		Congestion, eyes/lids	1
		Dysphagia	1
		Dyspnoea	1
		Injection site sepsis	1
		Injection site abscess	1
		Injection site necrosis	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Sometribove *(cont.)*		Injection site slough	1
		Injection site stiffness	1
		Irritation	1
		Pain	1
		Paraesthesia	1
		Pruritus	1
		Swelling, head/face	1
		Swelling, limbs	1
		Swelling, pharynx	1
		Tachycardia	1
	Topical	Skin lesions	1
	Unknown	Injection site swelling	3
		Injection site inflammation	2
		Swelling	1
Spinosad	Inhalation	Headache	1
		Irritation, pharynx	1
		Nausea	1
		Chest pain	1
Spinosad, milbemycin	Oral	Apprehension	1
		Diarrhoea	1
		Irritation, pharynx	1
		Nausea	1
		Abnormal voice	1
		Vision disorder	1
		Vomiting	1
	Topical	Hyperesthesia	1
		Congestion, skin	1
		Irritation, mouth/lips	1
		Taste abnormality	1
		Urticaria	1
	Unknown	Rash	1
Spinosad	Missing	Nausea	1
	Ophthalmic	Congestion, eyes/lids	1
		Irritation, eyes/lids	1
		Pain, eyes/lids	1
		Swelling, eyes/lids	1
	Topical	Rash	7
		Congestion	4
		Urticaria	4
		Hypoesthesia	3
		Inflammation, skin	3
		Pruritus	3
		Pain, eyes/lids	2
		Conjunctivitis	1
		Oedema	1
		Oedema, head/face	1
		Hyperesthesia	1
		Nausea	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
		Neurological disorder	1
		Chest pain	1
		Pain, mouth/lips	1
		Paraesthesia	1
		Swelling	1
		Swelling, ears	1
		Tongue abnormality	1
	Unknown	Rash	2
		Congestion, nose	1
		Coughing	1
		Dyspnoea	1
		Irritation, pharynx	1
		Pain, eyes/lids	1
		Pruritus	1
		Swelling, eyes/lids	1
		Swelling, mouth/lips	1
Stanozolol	Parenteral	Blood, abnormality	1
		Cardiomegaly	1
		Kidney failure	1
		Liver failure	1
	Unknown	Injection site pain	
Sulfadiazine, trimethoprim	Oral	Depression/lethargy	2
		Irritation, pharynx	2
		Exfoliation, skin	1
		Nose abnormality	1
		Paraesthesia	1
		Paraesthesia, mouth/lip	1
		Rash	1
		Unconsciousness	1
		Vomiting	1
Sulfadimethoxine	Oral	Flatulence	1
		Leg disorder	1
		Abdominal pain	1
		Shaking	1
Sulfurated lime solution	Topical	Dyspnoea	1
Tiletamine, zolazepam	Ophthalmic	Congestion, eyes/lids	1
		Irritation, eyes/lids	1
		Pain, eyes/lids	1
	Parenteral	Injection site swelling	2
		Convulsions	1
		Delirium	1
		Depression/lethargy	1
		Hypoesthesia, feet/digits	1
		Injection site abnormality	1
		Nausea	1
		Paraesthesia	1
		Twitch	1
		Unconsciousness	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Tiletamine, zolazepam *(cont.)*	Unknown	Death	2
		Human exposure, injection	1
		Hypoesthesia	1
		Nausea	1
Tilmicosin	Inhalation	Discomfort	1
		Irritation, pharynx	1
		Taste abnormality	1
	Missing	Elevated liver enzymes	1
		Suicide attempt	1
	Ophthalmic	Irritation, eyes/lids	19
		Pain, eyes/lids	19
		Application site pain	6
		Congestion, eyes/lids	4
		High blood pressure	4
		Taste abnormality	4
		Vision disorder	4
		Application site erythema	2
		Apprehension	2
		Headache	2
		Nausea	2
		Swelling, eyes/lids	2
		Abrasion, corneas	1
		Application site inflammation	1
		Conjunctivitis	1
		Depression/lethargy	1
		Dizziness	1
		Hypothermia	1
		Chest pain	1
		Muscle pain	1
		Photophobia	1
		Pruritus, eyes	1
		Shaking	1
		Spasm	1
		Tachycardia	1
		Trembling	1
	Oral	Taste abnormality	88
		Chest pain	23
		Nausea	22
		Headache	21
		Dizziness	19
		Vomiting	15
		Hypoesthesia	13
		Tachycardia	13
		Apprehension	11
		Irritation, pharynx	9
		Fever	8
		Hypoesthesia, mouth/lips	8

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
		Depression/lethargy	7
		Irritation, mouth/lips	7
		High blood pressure	7
		Bradycardia	6
		Diarrhoea	6
		Dyspnoea	6
		Tongue abnormality	6
		Ill	5
		Abdominal pain	5
		Shaking	5
		Sweating	5
		Nervousness	4
		Swelling, tongue	4
		Arrhythmia	3
		Discomfort, mouth/lips	3
		Pain, mouth/lips	3
		Pain, muscles	3
		Swelling, pharynx	3
		Trembling	3
		Application site pain	2
		ECG abnormality	2
		Gagging	2
		Hypersalivation	2
		Rash	2
		Somnolence	2
		Vision disorder	2
		Xerostomia	2
		Anaphylaxis	1
		Anorexia	1
		Application site inflammation	1
		Ataxia	1
		Bronchitis	1
		Confusion	1
		Congestion, skin	1
		Coughing	1
		Death (suicide)	1
		Dehydration	1
		Dementia	1
		Dry mouth syndrome	1
		Dysphagia	1
		Enlargement, salivary gland	1
		Hot flush	1
		Hyperactivity	1
		Hypoesthesia, feet/digits	1
		Hypopnoea	1
		Neurological disorder	1
		Pain, back	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Tilmicosin *(cont.)*		Pain, limb	1
		Pain, neck	1
		Paraesthesia	1
		Paraesthesia, mouth/lip	1
		Pneumonia	1
		Polydipsia	1
		Low blood pressure	1
		Pruritus	1
		Spasm	1
		Stomatitis	1
		Swelling, mouth/lips	1
		Syncope	1
		Ulcers, tongue	1
		Urticaria	1
		Vocalisation	1
		Weakness	1
	Parenteral	Injection site pain	202
		Injection site bleeding	113
		Injection site swelling	103
		Injection site inflammation	77
		Dizziness	81
		Nausea	49
		Tachycardia	46
		High blood pressure	42
		Apprehension	31
		Hypoesthesia	31
		Chest pain	23
		Taste abnormality	22
		Injection site abnormality	21
		Weakness	18
		Depression/lethargy	16
		Injection site stiffness	15
		Pain, limbs	15
		Arrhythmia	13
		Congestion, skin	13
		Nervousness	13
		Sweating	13
		Dyspnoea	12
		Fever	11
		Vomiting	11
		Hypoesthesia, limbs	10
		Pain	9
		Shaking	9
		Abdominal pain	8
		Xerostomia	8
		Ill	7
		Hyperpnoea	6

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
		Injection site oedema	6
		Injection site infection	6
		Pain, joints	6
		Low blood pressure	6
		Syncope	6
		Diarrhoea	5
		ECG abnormality	5
		Hypoesthesia, feet/digits	5
		Stiffness, hands	5
		Stiffness, limbs	5
		Swelling, limbs	5
		Unconsciousness	5
		Bradycardia	4
		Collapse	4
		Death (suicide)	4
		Pain, muscles	4
		Somnolence	4
		Urticaria	4
		Vision disorder	4
		Bleeding	3
		Haematoma	3
		Cyanosis	3
		Death	3
		Hyperactivity	3
		Irritation, pharynx	3
		Pallor	3
		Paresis, fore limbs	3
		Paresis, lower limbs	3
		Spasm	3
		Trembling	3
		Application site pain	2
		Arrest, heart	2
		Cellulitis	2
		Convulsions	2
		Heart disorder	2
		Hot flash	2
		Hot flush/flash	2
		Hyperesthesia	2
		Hypertonia	2
		Insomnia	2
		Pain, back	2
		Pain, neck	2
		Palpitations	2
		Paraesthesia	2
		Stupor	2
		Abortion	1
		Anaphylaxis/ anaphylactoid	1
		Anorexia	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Tilmicosin *(cont.)*		Apnoea	1
		Arthritis	1
		Ataxia	1
		Atrophy, muscles	1
		Behaviour disorder	1
		Ecchymoses	1
		Bloat	1
		Bronchitis	1
		Low blood calcium	1
		Cardiomegaly	1
		Abnormal chemistry	1
		Confusion	1
		Congestion	1
		Congestion, eyes/lids	1
		Congestion, nose	1
		Dehydration	1
		Dementia	1
		Discomfort	1
		Ear disorder	1
		Abnormal ECG	1
		Oedema, limbs	1
		Oedema, lungs/trachea	1
		Enlargement, lymph node	1
		Eructation	1
		Fibrillation, ventricular	1
		Low blood glucose	1
		Hypoesthesia, mouth/lips	1
		Hypomotility	1
		Hypopnoea	1
		Hypothermia	1
		Injection site effusion	1
		Injection site mass	1
		Injection site phlebitis	1
		Injection site pruritus	1
		Injection site slough	1
		Irritation, eyes/lids	1
		Irritation, skin	1
		Low blood potassium	1
		Lameness	1
		Elevated liver enzymes	1
		Melaena	1
		Neurological disorder	1
		Odour, mouth	1
		Pain, eyes/lids	1
		Pain, feet/digits	1
		Pain, lower limbs	1
		Prostration	1
		Pruritus	1
		Pulse thread	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
		Rash	1
		Recumbency	1
		Respiratory disorder	1
		Staggering	1
		Stiffness	1
		Stiffness, upper limbs	1
		Swelling, feet/digits	1
		Swelling, head/face	1
		Swelling, joints	1
		Swelling, tongue	1
		Thrashing/paddling	1
		Tongue abnormality	1
		Vomiting, projectile	1
		Vomiting, severe	1
	Topical	Ill	11
		Dyspnoea	10
		Fever	10
		Hypoesthesia	9
		Vomiting	9
		Depression/lethargy	9
		Diarrhoea	8
		Congestion, skin	7
		Inflammation, skin	7
		Rash	7
		Application site inflammation	6
		Application site abnormality	6
		Hyperesthesia	5
		Nervousness	5
		Abdominal pain	5
		Sweating	5
		Application site swelling	5
		Irritation, skin	3
		Pain	3
		Pain, limbs	3
		Pallor	3
		Paraesthesia	3
		Low blood pressure	3
		Pruritus	3
		Spasm	3
		Swelling, head/face	3
		Trembling	3
		Vesicles/bullae, skin	3
		Application site lesion	2
		Ataxia	2
		Heart disorder	2
		Hot flash/flush	2
		Hypoesthesia, limbs	2

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Tilmicosin *(cont.)*		Injection site bleeding	2
		Injection site inflammation	2
		Irritation pharynx	2
		Pain, feet/digits	2
		Pain, muscles	2
		Paraesthesia	2
		Paraesthesia, mouth/lip	2
		High blood pressure	2
		Shaking	2
		Skin abnormality	2
		Skin disorder	2
		Somnolence	2
		Stiffness, neck	2
		Unconsciousness	2
		Anaphylaxis/ anaphylactoid	1
		Anorexia	1
		Application site pruritus	1
		Arrhythmia	1
		Balance disorder	1
		Behaviour disorder	1
		Haematoma	1
		Blood, urine	1
		Confusion	1
		Congestion	1
		Distension, abdomen	1
		Respiratory distress	1
		Dry skin	1
		Dysphagia	1
		Eructation	1
		Eruptions	1
		Exfoliation, skin	1
		Gastritis	1
		Hot flash	1
		Hot flush	1
		Hyperpnoea	1
		Hypersalivation	1
		Hypopnoea	1
		Hypothermia	1
		Infection, skin	1
		Injection site pain	1
		Injection site swelling	1
		Irritation	1
		Irritation, eyes/lids	1
		Jerking	1
		Neurological disorder	1
		Pain, back	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
		Paraesthesia, nose	1
		Polydipsia	1
		Polypnoea	1
		Shock	1
		Skin, scabs	1
		Staggering	1
		Stool abnormality	1
		Swelling	1
		Swelling, feet/digits	1
		Swelling/limbs	1
		Swelling, mouth/lips	1
		Swelling, tongue	1
		Syncope	
	Unknown	Injection site pain	13
		Injection site bleeding	8
		Injection site swelling	8
		Dizziness	6
		Injection site inflammation	6
		Nausea	6
		Tachycardia	5
		Headache	4
		Vomiting	4
		Apprehension	3
		Death (suicide)	3
		Hypoesthesia	3
		Chest pain	3
		High blood pressure	3
		Apnoea	2
		Arrest, heart	2
		Death	2
		Swelling, head/face	2
		Swelling, limbs	2
		Taste abnormality	2
		Unconsciousness	2
		Acidosis	1
		Aggression	1
		Anaphylaxis/ anaphylactoid	1
		Application site erythema	1
		Arrhythmia	1
		Behaviour disorder	1
		Breathing abnormality	1
		Confusion	1
		Congestion	1
		Congestion, skin	1
		Convulsions	1
		Dementia	1
		Depression/lethargy	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Tilmicosin *(cont.)*		Destructiveness	1
		Dissociation	1
		Fever	1
		Fibrillation, atrial	1
		Ill	1
		Injection site abnormality	1
		Injection site oedema	1
		Injection site mass	1
		Injection site pruritus	1
		Injection site slough	1
		Nervousness	1
		Low blood pressure	1
		Pruritus	1
		Pupils, areflexia	1
		Rash	1
		Sneezing	1
		Swelling	1
		Swelling, mouth/lips	1
		Vision disorder	1
		Xerostomia	1
	Various	Taste abnormality	64
		Nausea	20
		Headache	16
		Depression/lethargy	13
		Hypoesthesia, mouth/lips	12
		Tachycardia	12
		Dizziness	11
		Chest pain	11
		Pain, eyes/lids	11
		Hypoesthesia	10
		Irritation, eyes/lids	9
		Application site pain	8
		Vomiting	7
		Weakness	6
		Dyspnoea	5
		Fever	5
		Application site erythema	4
		Apprehension	4
		Congestion, eyes/lids	4
		Injection site pain	4
		Nervousness	4
		High blood pressure	4
		Tongue abnormality	4
		Congestion, skin	3
		Discomfort, mouth/lips	3
		Abdominal pain	3
		Application site inflammation	2
		Diarrhoea	2

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
		Eye disorder	2
		Hypopnoea	2
		Injection site inflammation	2
		Pain	2
		Paraesthesia, mouth/lip	2
		Somnolence	2
		Stomatitis	2
		Sweating	2
		Urticaria	2
		Anorexia	1
		Application site lesions	1
		Ataxia	1
		Haematoma	1
		Breathing abnormality	1
		Cardiomegaly	1
		Confusion	1
		Congestion	1
		Congestion, sinus	1
		Discharge, nose	1
		Oedema, lungs/trachea	1
		Epiphora	1
		Epistaxis	1
		Gagging	1
		Glossitis	1
		Heart disorder	1
		Hyperesthesia	1
		Hypoesthesia, eyes/lids	1
		Hypoesthesia, feet/digits	1
		Hypoesthesia, limbs	1
		Hypothermia	1
		Ill	1
		Injection site bleeding	1
		Irritation, mouth/lips	1
		Irritation, pharynx	1
		Irritation, skin	1
		Leg disorder	1
		Lesions, mouth/lips	1
		Locomotion disorder	1
		Odour	1
		Otitis	1
		Pain, lower limbs	1
		Pain, joints	1
		Pain, muscles	1
		Pallor	1
		Palpitations	1
		Photophobia	1
		Mouth/lips lesion	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Tilmicosin *(cont.)*		Radiographs abnormal	1
		Rash	1
		Skin irritation, eyes/lids	1
		Spasm	1
		Staggering	1
		Stiffness limbs	1
		Suicide attempt	1
		Swelling, head/face	1
		Swelling, mouth/lips	1
		Trembling	1
		Twitch	1
		Ulcers, mouth/lips	1
		Vesicles/bullae, skin	1
		Wheezing	1
		Xerostomia	1
Toceranib	Topical	Paraesthesia	1
		Skin abnormality	1
Trenbolone	Parenteral	Myositis	1
Triamcinolone	Inhalation	Irritation, pharynx	1
	Ophthalmic	Irritation, eyes/lids	1
	Oral	Hypersalivation	1
		Taste abnormality	1
	Topical	Taste abnormality	2
		Application site pruritus	1
		Congestion, skin	1
		Diarrhoea	1
		Dizziness	1
		Inflammation, skin	1
		Nausea	1
		Chest pain	1
		Pruritus	1
		Rash	1
		Skin, dry	1
	Unknown	Dyspnoea	1
		Mydriasis	1
		High blood pressure	1
		Vision disorder	1
Trilostane	Oral	Dizziness	1
Tulathromycin	Inhalation	Nausea	1
	Missing	Injection site pain	1
		Injection site inflammation	1
		Injection site swelling	1
	N/A	Injection site pain	1
	Ophthalmic	Irritation, eyes/lids	4
		Pain, eyes/lids	2
		Vision disorder	2
		Congestion, eyes/lids	1
		Headache	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
	Oral	Depression/lethargy	1
		Dyspnoea	1
		Headache	1
		Pain	1
		Pain, mouth/lips	1
		Taste abnormality	1
	Parenteral	Injection site pain	34
		Injection site swelling	12
		Injection site inflammation	11
		Injection site bleeding	4
		Hypoesthesia	3
		Dizziness	2
		Ecchymosis	1
		Fever	1
		Injection site oedema	1
		Injection site stiffness	1
		Joint disorder	1
		Paraesthesia	1
		Vomiting	1
		Weakness	1
	Topical	Rash	3
		Application site pain	2
		Application site erythema	1
		Application site swelling	1
		Fibrillation, atrial	1
		Heart disorder	1
		Hypoesthesia	1
		Irritation, eyes/lids	1
		Nausea	1
		Pain	1
		Abdominal pain	1
		Skin abnormality	1
		Tachycardia	1
	Unknown	Injection site pain	21
		Injection site swelling	10
		Injection site inflammation	8
		Dizziness	3
		Injection site abnormality	2
		Trembling	2
		Ecchymosis	1
		Congestion, skin	1
		Oedema, limbs	1
		Injection site bleeding	1
		Injection site pruritus	1
		Lesions, skin	1
		Nausea	1
		Pain, limbs	1
		Paraesthesia	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Tulathromycin *(cont.)*		Pruritus	1
		Taste abnormality	1
		Vomiting	1
Tylosin	Inhalation	Coughing	2
		Headache	2
		Taste abnormality	2
		Apprehension	1
		Bronchitis	1
		Congestion, lungs	1
		Congestion, nose	1
		Irritation, pharynx	1
		Nausea	1
		Pain, mouth/lips	1
		Sinusitis	1
		Vomiting	1
	Missing	Inflammation, skin	1
		Pruritus	1
	Ophthalmic	Irritation, eyes/lids	6
		Congestion, eyes/lids	2
		Pain, eyes/lids	2
		Abrasions, corneas	1
		Dizziness	1
		Oedema, eyes/lids	1
		Nausea	1
		Pruritus	1
		Rash	1
		Swelling, eyes/lids	1
		Taste abnormality	1
	Oral	Taste abnormality	10
		Hyperesthesia	2
		Coughing	1
		Diarrhoea	1
		Dizziness	1
		Fever	1
		Headache	1
		Hot flush	1
		Hypoesthesia	1
		Nausea	1
		Abdominal pain	1
		Pain, eyes/eyelids	1
		Pneumonia	1
		High blood pressure	1
		Pruritus	1
		Pruritus, eyes	1
		Swelling, mouth/lips	1
	Parenteral	Injection site pain	24
		Injection site bleeding	9
		Injection site inflammation	9

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
		Injection site swelling	7
		Injection site oedema	5
		Headache	2
		Taste abnormality	2
		Ecchymosis	1
		Congestion	1
		Congestion, skin	1
		Dyspnoea	1
		Oedema	1
		Erection, abnormal	1
		Hypoesthesia	1
		Ill	1
		Injection site abnormality	1
		Injection site infection	1
		Irritation, pharynx	1
		Paraesthesia	1
		Phlebitis	1
		Vision disorder	1
	Topical	Rash	10
		Congestion, skin	4
		Pruritus	4
		Inflammation, skin	3
		Ill	2
		Irritation, mouth/lips	2
		Application site pruritus	1
		Discharge, nose	1
		Oedema	1
		Oedema, eyes/lids	1
		Oedema, head/face	1
		Epiphora	1
		Hyperesthesia	1
		Infection, skin	1
		Irritation, eyes/lids	1
		Lesions, mouth/lips	1
		Lesions, skin	1
		Nail disorder	1
		Nausea	1
		Low platelets	1
		Pruritus, eyes	1
		Respiratory disorder	1
		Skin disorder	1
		Sweating	1
		Sweating, eyes/lids	1
		Sweating, head/face	1
		Taste abnormality	1
		Urticaria	1
		Wheezing	1

Table 15.2 (*Continued*)

Drug	*Route*	*Signs*	*Number of times Reported*
Tylosin *(cont.)*	Unknown	Injection site pain	2
		Injection site swelling	2
		Irritation, eyes/lids	2
		Irritation, pharynx	2
		Rash	2
		Urticaria	2
		Congestion, lungs	1
		Depression/lethargy	1
		Ear disorder	1
		Exfoliation, skin	1
		Inflammation, skin	1
		Nausea	1
		Pain	1
		High blood pressure	1
		Pruritus	1
		Skin, dry	1
		Swelling, feet/digits	1
		Taste abnormality	1
		Wheezing	1
	Various	Neurological disorder	1
		Respiratory disorder	1
		Taste abnormality	1
Virginiamycin	Topical	Skin disorder	2
Xylazine	Oral	Irritation, mouth/lips	2
		Somnolence	2
	Parenteral	Hypoesthesia	1
	Unknown	Bradycardia	1
Zeranol	Missing	Pain	1
	Parenteral	Injection site swelling	3
		Injection site inflammation	2
		Atrophy, testicles	1
		Congestion, skin	1
		Injection site bleeding	1
		Irritation, skin	1
	Unknown	Injection site inflammation	2
		Nausea	2
		Congestion, skin	1
		Dizziness	1
		Oedema	1
		Injection site pain	1
		Injury	1
		Irritation, skin	1
Zilpaterol	Inhalation	Dyspnoea	1
		Pain	1
		Sweating	1
		Vomiting	1

common and even ocular contamination is a quite frequent event. Several drugs such as clomipramine are associated with oral contamination. A number of drugs are associated with abnormal menstrual cycle events, including altrenogest, progesterone, dinoprost, gonadorelin and cloprostenol. Several drugs are associated with deaths, including suicides and these include albendazole, dexamethasone, neomycin and thiabendazole in combination, droperidol/fentanyl, isoflurane, ivermectin, roxarsone and selenium/vitamin D. The antimicrobial drug tilmicosin is associated with several deaths. Perhaps what is striking though is that although this table covers a period of approximately 25 years, there are so few adverse drug experiences in humans.

15.4 Conclusions

Veterinary medicinal products are widely used in sick companion and farm animals and, on the basis of the data reviewed here from the UK and the USA, the main adverse effects in humans appear to be needle stick injuries or other accidents involving self-injection. Adverse events, including those arising from the toxicity of the drug, either systemic or local, do occur, but considering the millions of doses used world wide, and especially in the two countries examined here, it must be concluded that they are relatively rare.

Of course, it is widely recognised that there is significant under-reporting of adverse drug reactions even in human pharmacovigilance, which has been operating in some form or another in most countries for several decades.[121–123] This was recognised with the UK veterinary reporting scheme as long ago as 1988 and efforts have been made to increase reporting by greater publicity.[124,125] These schemes very often only succeed with the provision of feedback to practitioners.[126] Nevertheless, they are worth pursuing to obtain the types of data discussed in this chapter. The fact that issues may arise such as the organophosphorus dip problems in the UK, that defy scientific interpretation, should not deflect from this aim. The most recent UK data (2011) shows a slight rise in the numbers of adverse reactions in humans, with needlestick injuries predominating. Seven reactions were considered to be serious and required hospital treatment.[127] Thus, the trends already discussed for UK adverse reactions to veterinary medicinal products in humans in the UK continue.

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CHAPTER 16

Veterinary Medicines and the Environment

16.1 Introduction

Human and veterinary medicines have the potential to produce adverse effects and robust adverse drug reporting schemes have developed around the globe to address, collate and document these effects and where necessary to ensure that appropriate regulatory actions are taken.[1–6] These efforts have assisted in the development of the discipline of pharmacovigilance, the structured study of all aspects of adverse reactions to human and veterinary drugs and vaccines.

Pharmacovigilance is essentially the continued monitoring of product safety, including environmental product safety, after marketing has commenced and so it can be seen as the process of evaluating and improving the safety of marketed medicines (see Chapter 2).[7] It includes data collection, information flow, knowledge of relevant regulations, product data, and the overall management of relevant information, along with required regulatory actions and responses where justified.[8–10]

Some of the concerns over human pharmaceuticals arise from their ability to penetrate and accumulate in the environment. As well as side-effects of drugs in treated animals and exposed humans, veterinary pharmacovigilance schemes frequently incorporate the reporting of adverse environmental effects.[4,11] This is important as many veterinary pharmaceutical products have the potential to exert harmful effects on the environment, as discussed later in this chapter. Moreover, there is growing realisation that human and veterinary drugs are penetrating the environment in increasing quantities.

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16.2 Human Pharmaceuticals

There is substantial evidence that human pharmaceuticals, neutraceuticals and cosmetic materials are entering the environment to an increasing degree and these are being found in sewage, other effluents, river water and sediments.[12–38] At high enough concentrations, some of these substances have the potential to exert harmful effects on the environment and on the organisms in it.[39–48] This may be exacerbated by mixtures of chemicals.[49,50] By 2007, some 1700 detections of over 100 human pharmaceuticals had been reported.[37] Substances found include amoxicillin, azithromycin, erythromycin, codeine, valsartan, acrivastin, fexofenadine, loratidine, desloratidine, simvastatin, ranitidine, cimetidine, naproxen, allopurinol, mebeverine, ceftazidime, ethinylestradiol, sulfamethoxazole, gemfibrozil, ibuprofen, diazepam, carbamazepine, ketoprofen, diclofenac, mefenamic acid, phenazone, propoxyphenazone, cloprostenol, doxycycline, oxytetracycline, fluoxetine, triclosan, trimethoprim, fluoroquinolones, macrolide antimicrobials, paracetamol (acetaminophen), salbutamol, salicylic acid, clofibrate, atenolol and furosemide, as well as X-ray contrast media and recreational drugs. Moreover, the geographic distribution is vast, with pharmaceuticals being found in the environment in most developed countries, including European countries, the USA, Canada, Australia, China and many Asian territories.[23,29,50–86] These may arise from hospital or domestic disposal, as well as from excretion by treated patients.[62,87–90] Some may have the potential to harm human health, even at the low levels found in the environment, possibly through exposure in drinking water.[15,79,91–107]

There is particular concern that the pharmacodynamic activities of some drugs may be expressed in consumers, and this concern has been paramount over the presence of hormonally active substances, and particularly those with the capacity to act as endocrine disrupters in humans or indeed, in environmental organisms.[96,98,108–117] There is a considerable body of concerns that include adverse effects on sexual, thyroid, adrenal and reproductive function in humans and other animals, as well as genital abnormalities (*e.g.* hypospadias) and effects on embryonic and foetal development.[118–171] This has led to the tighter regulation of human pharmaceutical products in a number of countries from the point of view of environmental effects and environmental assessment,[12,13,94,100,172–195] and the development of regulatory guidelines.[173,181]

16.3 Veterinary Pharmaceuticals

Veterinary medicines, including vaccines and other products derived from biotechnology, also have the capacity to enter the environment and these too are subject to regulation, risk assessment and guidelines, as discussed in Chapter 2; these too have the capacity to affect environmental and human health.[3,4,196–203] The recent withdrawal of cypermethrin-based sheep dips in the UK because of environmental contamination and potential adverse environmental effects serves as an example of what might happen – both from a scientific and regulatory view point.[204] This is an increasingly important area of veterinary pharmacovigilance.

Indeed, the issue of pharmaceuticals, including veterinary pharmaceuticals in the environment and their potential effects on humans and other organisms has led to the coining of the terms environmental pharmacology, ecopharmacology or pharmacoenvironmentology as an alternative to being merely seen as a branch of human or veterinary drug pharmacovigilance.[179,205,206]

16.3.1 Regulation of Veterinary Medicinal Products and Environmental Safety

As discussed in Chapter 2, in most countries, veterinary (and human) medicinal products are assessed on the basis of satisfactory quality, safety and efficacy. Safety refers to a range of potential hazards including safety to the patient, *i.e.* the animal, safety to the consumer of animal produce, *i.e.* safety of residues of veterinary medicines in food of animal origin, and safety to users and to others potentially exposed (veterinarians, veterinary nurses, children exposed to treated animals) as discussed in Chapters 3 and 4. It also refers to safety to the environment and to organisms in the environment potentially exposed to veterinary medicines as a result of their use, *e.g.* from run-off following topical treatments, from residues in urine and faeces during systemic treatments, and through accidental and deliberate direct contamination, *e.g.* through careless or illegal disposal of medicines into water courses or into the marine environment. If all of these aspects of safety are satisfactory, and the product is of the appropriate pharmaceutical quality and the data demonstrate that it does therapeutically or prophylactically what the drug sponsor claims, then a licence, approval or marketing authorisation, depending on local terminology, will be granted.

Although the majority of regulatory processes for the control of veterinary medicinal products are similar in considering these aspects, the actual regulatory schemes themselves differ widely in their requirements, bureaucracy and application. In most European Union (EU) countries these products are regulated through national government agencies (although some, and notably Germany have one agency for pharmaceuticals and a separate one for biological products). This is also true of the European Medicines Agency (EMA) and the products that come within its control. However, in the United States, conventional pharmaceuticals are controlled by the Food and Drug Administration's (FDA) Center for Veterinary Medicine, biological products by the US Department of Agriculture while ectoparasiticides are regarded as pesticides and regulated by the US Environmental Protection Agency.[206–210] All of this can cause confusion, especially to larger companies with global operations. A product may be a drug in Europe but a pesticide elsewhere, including the United States.

In the EU any safety violation for a veterinary medicinal product is a veterinary pharmacovigilance issue. Indeed, pharmacovigilance for veterinary medicinal products in the EU includes:

- Adverse drug reaction in the animal patient.
- Adverse drug reaction in exposed humans.

- Suspected lack of expected efficacy.
- Violation of maximum residue limits (see Chapter 3).
- Adverse environmental effects.

In the EU and elsewhere, the adverse environmental effects of veterinary medicines are assessed in two stages. In fact, under a previous EU Directive, Directive 81/851/EEC, as amended by Directive 92/18/EC, there was a requirement to conduct a two phase assessment. In the recently revised legislation, Directive 2001/82/EC as amended by Directive 2004/28/EC there is no reference to this two-phased approach but instead, in its rather lengthy introductory recitals, it requires that *the environmental impact should be studied and consideration be given on a case by case basis to specific provisions seeking to limit it*. Moreover, Article 12.3j requires the submission of studies with applications for marketing authorisations that include *tests assessing the potential risks posed by the medicinal product for the environment. This impact shall be studied and consideration be given on a case-by-case basis to specific provisions seeking to limit it*. Despite the requirement for a two phase approach no longer being present in the legislation, European Union guidelines make it clear that this is what is still expected. These two phases are:

- Phase I – an environmental impact assessment for the drug based on physico-chemical properties, its likely penetration into the environment and its metabolic profile in target patient animals and, if a certain trigger value is exceeded:
- Phase II – studies that might be conducted to investigate environmental hazards and thus eventually lead to risk mitigation measures that could, in the most extreme circumstances, lead to the product being refused a licence, marketing authorisation, approval *etc.*

The original EU guidelines for environmental risk assessment were published by what was then the EMEA in 1997. The guideline on Phase I assessment allowed the omission of certain products such as those intended for companion animals (cats, dogs, rabbits, small rodents) and required the establishment of the predicted environmental concentration (PEC) for soil and groundwater. If the trigger values mentioned earlier, the PEC for soil of $10\,\mu g\,kg^{-1}$ and for groundwater $0.1\,\mu g\,L^{-1}$, were exceeded, a Phase II assessment was required (Tait, in press). These original EMEA guidelines have now been superseded by those developed through the efforts of the International Cooperation on Harmonisation of Technical Requirements for the Registration of Veterinary Medicinal Products, VICH.

VICH has representatives of industry and regulatory authorities from Japan, the USA and the EU with observers from a number of other countries and organisations. It has developed a number of guidelines including those for areas of toxicity testing, residues analysis and pharmacovigilance which VICH-associated countries are meant to adopt.[211–215] VICH has developed two guidelines for environmental risk assessment, which have many

similarities with the original EMEA guidelines, including the Phase I and II approach:

- VICH Topic GL 6 Environmental Impact Assessment (EISs) for Veterinary Medicinal Products (VMPs) – Phase I
- VICH Topic GL 38 Environmental Impact Assessments for Veterinary Medicinal Products Phase II Guidance

Both of these have been adopted by the EMA to replace its existing guidelines and are available from the EMA or VICH websites (http://www.emea.europa.eu/ or http://www.vichsec.org/).

One of the outcomes of the harmonisation process was that the soil PEC trigger value was changed to 100 $\mu g\,kg^{-1}$. Some have seen this as a weakness, and have criticised the VICH guideline and the VICH processes.[216] However, some of those associated with the process have vigorously spoken out and supported it.[199] Both VICH guidelines are in current use within the EU.

A Phase I assessment involves a decision tree approach and a number of decision routes can lead to a halt in the process on the grounds that a more extensive environmental risk assessment is not required. As already mentioned, this includes the use of the product in companion animals. However, use in minor species, in individual animals or in a small number of animals in a flock, or where the drug is metabolised to innocuous materials, can also exempt the product/drug from the assessment process. The decision tree then divides into drugs used in the aquatic environment and those used in terrestrial animals. Even then, further assessment may be dispensed with if entry to the environment is prevented or if the PEC or other triggers, including those concerning aquatic environmental factors, are not exceeded and mitigation factors may also be taken into account, including the results of biodegradation in soils and manure.[217] However, should this not be the case, a Phase II assessment is likely to be required.[11]

While a Phase I assessment may be regarded as largely (but not exclusively) a paper-based exercise, Phase II is anything but this. It consists of scientific testing. Having said this, the studies required are divided into Tier A and Tier B, with A being the simpler (and cheaper) while B are more complex (and generally more expensive to perform). Moreover, guidance is provided for each of the three main areas of farming – aquaculture, intensive and pasture-based.

The Tier A studies include physico-chemical studies and environmental fate studies, some of which may have already been conducted to support the Phase I assessment. These are:

- Water solubility.
- Dissociation constants in water.
- UV visible absorption spectrum.
- Melting point/range.
- Vapour pressure.
- Octanol/water partition coefficient.

- Soil adsorption/desorption.
- Soil biodegradation.
- Degradation in aquatic systems.
- Photolysis.
- Hydrolysis.

In addition, Tier A may also require studies of aquatic effects. Examples include:

- Freshwater algal growth inhibition.
- Freshwater *Daphnia* immobilisation.
- Freshwater fish acute toxicity.
- Saltwater algal growth inhibition.
- Saltwater crustacean acute toxicity.
- Saltwater fish acute toxicity.
- Nitrogen transformation assay (28 day).
- Effects on terrestrial plants.
- Subacute toxicity/reproductive toxicology in earthworms.

Additional studies may be required for ectoparasiticides and endectocides:

- Studies of effects on dung fly larvae.
- Studies of effects on dung beetles.

The whole point of Tier A testing is to refine the PEC value based on a wider array of testing. If the PEC value trigger is not exceeded after Tier A testing and refinement, the assessment may, and usually does, halt at this point. However, if this or other triggers, including the Risk Quotient (RQ), a value based on the ratio of the PEC with the predicted no effect concentration (PNEC), PEC/PNEC, are exceeded, Tier B testing is likely to be required. Simplistically, Tier B testing is really intended to confirm that any worst fears arising from Phase I and Tier A testing are unfounded. The tests here are more complex but provide more details on environmental fate and effects. These include:

- Bioconcentration in fish.
- Freshwater algal growth inhibition.
- Effects on *Daphnia* reproduction – freshwater.
- Effects on early-life stage – freshwater fish.
- Invertebrate toxicity – freshwater.
- Saltwater algal growth inhibition.
- Crustacean chronic toxicity or reproduction – saltwater.
- Chronic toxicity – saltwater fish.
- Sediment invertebrate toxicity – saltwater sediment.
- Nitrogen transformation – 100 days.
- Effects on terrestrial plants (further species over Tier A).
- Effects on earthworms.

All of the Tier A and Tier B tests, as well as those which appear in Phase I should be conducted in accordance with internationally recognised test guidelines, and principally with those developed by the Organisation for Economic Cooperation and Development (OECD) or the International Organization for Standardization (ISO). All of the tests mentioned here have OECD (101, 102, 104–107, 111, 112, 117, 201–203, 210, 211, 216, 218-220, 305, 307, 308) or ISO (10253, 14669) Guidelines available and all tests must be conducted in accordance with the requirements of Good Laboratory Practice (GLP).

The requirements described above may well apply to products intended for use in aquaculture and intensive farming as well as to products used in pasture farming but further data, or at least different data, may still be required for products intended for use in aquaculture or in intensive farming. Consider, for example, a product used in aquaculture, specifically salmon farming, in the EU. In Europe, salmon farming is typically carried out in Scotland, Ireland, Norway and the Faeroe Islands. In Scotland, it tends to be sited in sea lochs *i.e.* lochs open to the sea and subject to tidal flushing. Sea lochs have their own natural inhabitants and any medicines used must be safe for these as well as for any other species that might be farmed in the same body of water *e.g.* oysters or mussels and to representative members of the food web. Assuming that the product is given with feed, the following studies for the drug, main metabolites and possibly for major degradation products are likely to be required to satisfy EU regulatory authorities:

- Product leaching from feed.
- Degradation as a function of pH.
- Photodegradation/photolysis.
- Dissipation and leaching in soil.
- Sorption and desorption, soil.
- Aerobic soil metabolism in different soils.
- Degradation in sea water and sediments.
- Concentration and persistence under fish cages.
- Deposition and persistence in marine sediments.
- Experimental dispersion studies in representative sea lochs.
- Toxicity to salmonids.
- Toxicity to representative fauna (crustacean *e.g.* *Corophium volutator,* polychaete worm *e.g.* *Arenicola marina*).
- Toxicity to representative plants *e.g.* the freshwater alga *Selenastrum capricornatum*.
- Toxicity to shrimp *e.g.* *Crangon septemspinosa* or *Crangon crangon* or mysids *e.g.* *Mysidopsis bahia*.
- Toxicity to prawns *e.g.* Dublin Bay prawn, *Nephrops norvegicus*.
- Toxicity/reproductive toxicity to *Daphnia magna*.
- Deposition and toxicity to oysters and their larvae.
- Deposition and toxicity to mussels and their larvae.
- Acute toxicity to several representative fish types.
- Acute toxicity to birds *e.g.* mallard duck.

The results of some of these studies, along with those of physico-chemical property studies, would form the basis of the Phase I assessment, while the remainder would be proscribed by the results of the Phase I assessment and the refinement in Tier A of Phase II.

Data generated in these studies may be relatively simple to understand, or their complexity may merely raise other issues and further questions. There is rarely any clear cut decision that can be made except for the basic regulatory question – authorise or not authorise? If the product raises so many doubts, questions or concerns that the regulatory decision is not to authorise (rare) then at least this has a degree of finality unless the sponsor decides to appeal the decision. If the decision is to authorise, then the regulatory authority, working with the sponsor, has to decide on how to take the results of these environmental studies into account. In most regulatory areas, for example food additives and user safety, this is covered by the approach of hazard identification and assessment (largely covered by conducting the studies), exposure assessment, risk assessment, risk management and risk communication.[218–221]

Exposure assessment is, at least in part, addressed as part of the assessments already described, although a sponsor might be asked to conduct post-marketing studies to assess the degree of environmental exposure once the product is in use. Risk assessment measures would be taken as part of the regulatory process and would at least include semi-quantitative assessments of the likelihood of any identified hazard *e.g.* phytotoxicity, being expressed when the product is in (normal) use. Once this has been done, risk management measures can be considered. Risk management measures include all those precautions required to reduce exposure, and thus to assuage concerns over any risks. These could include restrictions on doses administered to animals, restrictions on dosing frequencies and the need to store manure and farm waste for a sufficient period to ensure that drug residues have depleted to concentrations below those which give rise to concern, *e.g.* so that the manure may be safely spread on to pasture land. Risk communication generally takes the form of advice and regulatory phrases that appear on the label or in other product literature. These measures, as mentioned above, are designed to ensure that any hazards identified in experimental studies are not expressed as a result of normal therapeutic use. However, if adverse events occur through misuse or inadvertent contamination, or adverse effects occur which were not identified as part of the test programme, then veterinary pharmacovigilance should serve to identify these and where necessary, lead to measures to prevent recurrence.

16.3.2 Adverse Environmental Effects of Veterinary Medicinal Products

16.3.2.1 Avermectins

Compounds such as diazinon, an organophosphorus compound, are highly toxic to earthworms and other terrestrial organisms as well as to bees.[222,223] However, concern has been expressed over the toxicity of the macrocyclic lactone endectocides used widely in large animal veterinary medicine. These

include ivermectin and doramectin, and the related compound moxidectin (a milbemycin). Avermectins and milbemycins have given rise to this concern as they are voided from the animal in the faeces where they may continue to exert their insecticidal effects. Hence, there has been speculation as to whether they might cause major environmental problems, not only due to their potential effects on insect populations but also because they might prevent the biodegradation of animal dung.[224–229] The avermectins have low toxicity to a wide range of terrestrial invertebrates and they possess low phytotoxicity but they may be more toxic to particular organisms at concentrations likely to be encountered under agricultural conditions.[230,231] The results of a number of experimental studies have indicated adverse effects on dung fauna by avermectins such as ivermectin and abamectin, whereas levamisole, moxidectin, tiamulin, olaquindox, metronidazole and some of the benzimidazole anthelmintics, including fenbendazole and albendazole, appear to have no significant adverse effects; there is evidence to suggest that sustained release bolus formulations may offer greater risks than other modes of administration.[232–247] There is also some evidence to suggest that dung from treated animals may be less attractive to dung fauna, but the reasons for this are unknown.[248] Treatment with ivermectin might also contribute to reductions in phosphorus recycling but the evidence for this is limited. The concentrations of residues of ivermectin in dung pats are slow to decline.[240] Other investigators have found no or little evidence for adverse effects of avermectins on dung fauna or on dung pat degradation.[249–251]

The issue of avermectins and their environmental effects remains a controversial area.[252–257] The treatment of terrestrial animals for parasite control is seasonal, as is the breeding of dung fauna. The latter might be at less risk if the breeding season and the treatment seasons are separate, but there may be some degree of risk if coincidental, and the concentrations found may depend on a number of factors including the diets of the treated animals,[256,258–260] and not all cattle in a herd will necessarily be treated simultaneously.[261] Interestingly, the original environmental impact assessments of avermectins in the United States took into account patterns of use, their toxicity, metabolic characteristics, predicted environmental concentrations and behaviour in the environment but no consideration was given to effects on dung pat degradation or dung fauna.[262] There are some parallels with the treatment of cattle using deltamethrin. Depending on the time they are treated, and their frequency of drug administration, the effects on insects in cattle dung were either negligible or significant. For example, concentrations in faeces after a therapeutic treatment were sufficient to kill adult dung beetles.[263] Nevertheless, attempts to control parasitic flies by treating them with avermectins so that residues in dung exert a beneficial insecticidal effect have met with little or no success.[264–267] Fluazuron had no adverse effects on survival and reproduction in dung beetles.[268]

16.3.2.2 Diclofenac and Vultures

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 in other vulture species. In one area, the decline in the

Oriental white-backed vulture has been in the region of 95% since the 1990s.[269] 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 that had been treated with diclofenac prior to death. Diclofenac is available as an over the counter veterinary drug in Pakistan and is widely used.[270–274]

16.3.2.3 Aquaculture

Aquaculture or fish farming is one of the major developments in ensuring the availability of high quality protein while at the same time enabling conservation of natural fish stocks.[275–282] Fish suffer from a range of viral, bacterial and parasitic diseases and medicines are used systematically in aquaculture to combat these. These medicines generally take the form of antibiotics for bacterial infections, antifungal drugs, some of them unauthorised, and ecto-parasitic drugs to combat parasitic infections, particularly sea lice in farmed salmon.[283–292] The products used may be either liquid formulations or those integrated into fish feed. Either way, they are used in the aquatic environment such as rivers (*e.g.* in trout farming), sea lochs (*e.g.* in salmon farming), or in coastal waters (*e.g.* in cod farming). In all cases, excess medicinal product, either direct from liquid formulations or leachate from medicated feed, may spread out from the site of treatment.[293–295] In addition, materials arising from fish excreta also provide a pollution risk,[296–299] while environmental contaminants may accumulate in fish.[300,301]

One of the major economic and animal welfare problems associated with salmon farming is sea lice.[292,302] These are ectoparasitic copepods including *Lepeophtheirus salmonis* and *Caligus elongatus* which feed on the skin of Atlantic salmon, *Salmo salar*. They cause economic damage and welfare problems in farmed fish.[291,303] Several products have been used to treat this condition in the UK and elsewhere, including those containing azamethiphos, dichlorvos, hydrogen peroxide, emamectin benzoate, cypermethrin, teflubenzuron and diflubenzuron.[285–287,289–291,304–308] Prior to authorisation, these medicines have had to be tested for potential adverse environmental effects, but attempts have been made to monitor their impact once in use.[206,309] A recent report has identified two sea lice medicines as priorities for study – cypermethrin and emamectin benzoate at four fish farms in Lochs Craignish, Sunart, Diabaig and Kishorn. Major components of the ecosystem were examined including benthic meiofauna, inter-tidal organisms, sublittoral organisms, benthic macrofauna, zooplankton and phytoplankton. Thus far, the authors have not ''observed any catastrophic perturbation of the sea lochs'' studied.[310] Emamectin benzoate has been shown to have a favourable environmental profile while being extremely effective in controlling sea lice.[307,311]

16.3.2.4 Other Routes of Environmental Contamination

Environmental release of drugs from companion animals is likely to be minimal.[293] However, domestic users, farmers and veterinary practices could conceivably dispose of unused or unwanted medicines in such a way that the environment could be compromised, and some substances used in veterinary medicines are known to be toxic to aquatic animals and plants.[19–21,312] These include synthetic pyrethroids such as flumethrin and deltamethrin and organophosphorus compounds such as diazinon and chlorfenvinphos. Substances used as active ingredients in aquaculture products such as emamectin benzoate, hydrogen peroxide, azamethiphos and cypermethrin have been extensively studied as part of the authorisation process and although potentially hazardous, they should not cause any undue risks when used according to the label instructions.

16.3.3 Reporting of Environmental Adverse Events and Incidents with Veterinary Medicines

Since the 1980s, the UK's regulatory authority for veterinary medicinal products, the Veterinary Medicines Directorate (VMD) has operated a reporting scheme for adverse drug reactions, including adverse environmental effects.[3,4] For over twenty years, its findings have been reported regularly in the *Veterinary Record*, and adverse environmental effects or environmental incidents have been included in this since 1999.

The numbers of environmental incidents are shown in Table 16.1. The vast majority of these involved the deaths of aquatic vertebrates and fish, frequently because of contamination of water courses with sheep dip formulations. The ingredients in these are synthetic pyrethroids (*e.g.* cypermethrin) and, to a lesser extent, organophosphorus compounds, notably diazinon. The remaining incidents involved poisoning in birds of prey, possibly deliberately.[312–319]

Table 16.1 Environmental Incidents Reported to the UK's VMD.

Year	*Number of Incidents*
1998	39
2001	34
2002	6
2003	9
2004	11
2005	81
2006	62
2007	42
2008	4
2009	1
2010	7

The small decline in incidents noted in 2001 has been attributed to the introduction of a Certificate of Competence and suitable training for those involved in sheep dipping operations (see also Chapter 15). This was developed to ensure worker safety in the use of sheep dips but a beneficial side-effect may have been a better recognition of the dangers of environmental contamination and subsequent modifications of behaviour. Product labels for many products have also been modified to provide advice on good environmental practices. The pyrethroid dips were suspended by the VMD in February 2006 because of environmental concerns and events.[204,317,320] However, as is evident from Table 16.1, the number of environmental incidents has remained low since 2008. However, it is of concern that the single incident reported in 2009, and four of the seven incidents reported in 2010 involved the poisoning of birds of prey with the sheep dip ingredient diazinon.[320–323]

16.4 Conclusions

The active ingredients used in veterinary (and human medicines) clearly have opportunities and the means to enter the environment, particularly those used in food animals and in aquaculture. Many of these have the potential to exert effects on ecosystems, and notably on microfauna although many also have the potential for phytotoxicity including the fluoroquinolones, the tetracyclines and pleuromutilins such as tiamulin and valnemulin.[324] These effects underline the need for strict regulatory assessment prior to authorisation, and robust environmental monitoring following marketing. Moreover, the major concerns are not only direct entry into the environment, *e.g.* through use in aquaculture or *via* the urine and faeces of treated animals, but also from the use of contaminated manure and poultry litter in agriculture, and consequently, the indirect contamination of the environment.[325–329] There is also a considerable obligation on investigators to understand the degradation and fate of veterinary pharmaceutical products in the environment so that concerns over their potential effects can be mitigated and, where necessary, regulatory action taken, as occurred with the synthetic pyrethroids in the UK.[330–335] In understanding the potential environmental risks involved, the principal of ranking these may well have some utility for both veterinary and human pharmaceuticals.[45,61,152,175,195,335–337]

Risk management procedures and risk communication through recommendations for safe use, contraindications, restrictions and warnings, including where appropriate pictograms, must be clearly defined and clearly placed on the product labels and in product literature and in extreme cases, where environmental hazards and risks clearly outweigh therapeutics benefits, the only realistic and appropriate regulatory action may be to refuse to authorise a particular product or to suspend the authorisations for products already being marketed.

Much of what has been written here, and indeed much of what has been published on environmental issues relating to human and veterinary drugs, has focussed on the potential effects of pharmaceuticals on plants and animals in

the environment. However, an equally important if not more important problem that is frequently overlooked is what the effects of these substances might be on human health especially if they find their way into drinking water. Much of the emphasis on human health effects has, quite rightly, focussed on residues of drugs in food derived from treated animals, as was discussed in earlier chapters. The principles applied there, *i.e.* the calculation of acceptable daily intake values and the elaboration of limits akin to maximum residue limits, could probably be applied to the presence of veterinary and human drugs in drinking water. Concerns over some specific hazards and the magnitude of the associated risks are frequently difficult to address, and this is particularly true with the topic of endocrine disrupting chemicals, especially when these occur in drinking water although it must be stressed that not all of these are derived from either human or veterinary medicines.[25,45,61,77,103,104,106,107,109–111,114,120,122,124,127,128,131,132,135,139,142–147,175,195] Although some of these substances originate as medicines, *e.g.* contraceptive steroids and some antimycotic agents, others arise from personal care products and industrial chemicals.[338–349] This is a major area of concern for human reproductive health assessment, and one that deserves thorough investigation and consideration. Where these types of product are shown to have a veterinary origin, as is the case with the anabolic agents in countries where their use is permitted, then clearly measures to protect the environment and public health must be considered (see Chapter 13).

Other hazards from veterinary and human antibiotics in the environment are not toxicological but microbiological. These are the hazards and risks associated with the induction of antimicrobial resistance that may arise from the selection of resistant bacteria from populations of otherwise susceptible organisms. Many of the concerns in this area arise from the therapeutic use (and misuse) of antimicrobial drugs in human and veterinary medicine. Additionally, there are concerns over the induction of resistance among organisms present in the environment, subsequent transfer of resistance genes to human and animal pathogens and transport of resistant organisms through the environment.[75,350–368] Some of these issues are dealt with in Chapter 17.

It is clear that although the effects of veterinary and human drugs on environmental organisms is understood, at least at a basic level, as is their behaviour in the environment, the effects of these compounds on human health is less well defined and poorly understood. To say that the toxicological (and microbiological) potential of these compounds for humans requires further investigation is perhaps an understatement. Although the tiers of tests required to demonstrate the environmental safety of pharmaceuticals are relatively well characterised, similar tests for investigating the safety to humans of environmental pharmaceuticals are virtually non-existent, although, the EUSES model referred to in Chapter 4 may be used predictively to estimate effects on human health from environmental pollutants.[369]

If the state of the environment reflects the potential state of public health, then clearly the issues relating to contamination of the environment with pharmaceuticals, regardless of their origins, and the subsequent effects on

health, cannot continue to be disregarded. In particular, the health issues surrounding the endocrine disrupting agents, and their entry into and contamination of the natural environment needs further and urgent systematic and intensive investigation.

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CHAPTER 17

Potential Adverse Microbiological Effects of Antimicrobials

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17.1 Introduction

Despite much debate there still remains in some quarters some concern in relation to microbiological hazards to human health arising from the veterinary use of antimicrobial compounds. The major concern in the minds of some is that the use of antimicrobials in veterinary medicine may select for antimicrobial resistance among bacterial populations present in the target animal population including zoonotic pathogens and commensal flora; concerns have been expressed in relation to antimicrobial residue and therapeutic concentrations of drugs. Such resistant pathogens have the potential to infect susceptible persons through consumption of improperly handled food products. Additionally antimicrobial resistance determinants present among the non-pathogenic commensal foodborne 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.

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Whilst these concerns have given rise to regulatory guidance across the globe it must be emphasised 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. Furthermore it is crucial to consider the benefit of use of antimicrobials in veterinary medicine,[1] rather than just dwell on potential adverse effects; this will be discussed further at a later stage.

The links between the use of antimicrobials in veterinary medicine and resistance development in human medicine are somewhat tenuous,[2–6] although this has been contested by other workers.[7–10] Irrespective of whatever view is held, it is appropriate that all users of antimicrobial compounds examine the ways in which society uses these valuable resources. It is also important 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 must be viewed alongside the use of antimicrobials in humans, especially when one considers that there are no clinically important bacteria that have not developed some type of resistance to antibiotics.[11] The resistance problem is steadily increasing worldwide and 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 Organization figures state that there are >11 million deaths annually[†] 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[12] 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 and 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.[12–14] 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.

In this chapter I wish to consider two very different effects of antimicrobials, the first concerning antimicrobial resistance in *Campylobacter* and the second the ongoing developments concerning the regulation relating to the acceptable daily intake of antimicrobial residues. Before addressing either it is important to consider what is meant by antimicrobial resistance. This might seem a strange point to discuss but there is an increasing tendency to use epidemiological cut-off values rather than clinical breakpoints to define resistance. This matter has been dealt with by a number of authors,[15–17] and the arguments need not be repeated in full save to say that true clinical resistance can only be defined by using clinical breakpoints and not epidemiological cut-off values.

[†]World Health Organization. Shaping the future, World Health report 2003 http://www.who.int/whr/2003/en/whr03_en.pdf.

This was highlighted by Magiorakos *et al*., in their important paper proposing international standard definitions for defining multidrug resistant, extensively drug-resistant and pan drug-resistant bacteria.[18] For those not familiar with the arguments it is important to note that in Europe, the national antimicrobial resistance surveillance schemes do not all define resistance in the same way. This means that it is not possible to simply compare resistant rates from different surveillance schemes as they are not measuring the same parameter. Indeed, even within national surveillance schemes, methods of analysis have changed over time such that the percentage resistance values may not be comparable. There are two fundamental reasons for this being the case: (i) the trend for 'resistance' to be defined by the epidemiological cut-off value rather than by the long-established clinical breakpoint; and (ii) no standardisation on how to define the epidemiological or wild-type cut-off value. Whilst the use of epidemiological cut-off values might be important for the detection of decreased susceptibility, it is inappropriate to use this value to determine the percentage clinical resistance.[16] Additionally, whilst it is intuitive that decreased antimicrobial susceptibility may in time lead to clinical resistance, the hypothesis has not been tested until now. Indeed one of the proponents of using epidemiological cut-off values has reported that an isolate might, through mutations or gene transfer, develop reduced susceptibility but still have a sufficiently low MIC to allow successful therapy.[19] A change from clinical break points to epidemiological cut-off values when determining the percentage resistance does matter depending, of course, on the antimicrobial class and bacteria of interest. One example that illustrates the point relates to *Salmonella* and fluoroquinolones. In MARAN 2004,[20] ciprofloxacin resistance in all *Salmonella* ($n = 2195$) was reported to be 0.3% applying a clinical breakpoint of $> 2\,\mathrm{mg\,L^{-1}}$. In MARAN 2005, ciprofloxacin resistance in all *Salmonella* ($n = 2238$) was reported to be 10.1% as the epidemiological cut-off value of $> 0.06\,\mathrm{mg\,L^{-1}}$ was used, yet there was no change in the population susceptibility distribution. The reader may be misled into believing that clinical resistance has increased to 10.1%. Here, the differentiation between decreased susceptibility and clinical resistance is important. The changes introduced by the respective surveillance schemes occur largely as a result of the evolution in thinking relating to antimicrobial susceptibility distributions for wild-type bacterial populations, *i.e*. those strains not carrying acquired antimicrobial resistance determinants. These changes raise the question as to how epidemiological cut-off values are determined; as yet there is no consensus.

17.2 Antimicrobial Resistance and *Campylobacter* Species

17.2.1 Why is *Campylobacter* Important?

Scallan *et al*. published[21] updated estimates of foodborne diseases acquired in the United States showing a lower overall incidence than previously thought.[22]

Scallan and colleagues estimated that in the US there are 9.4 million foodborne illnesses per year, of which 5.5 million are caused by viruses, 3.6 million by bacteria, and 0.2 million by parasites. The pathogens that caused the most illnesses were norovirus (5.5 million), non-typhoidal *Salmonella* spp. (1.0 million), *Clostridium perfringens* (1.0 million), and *Campylobacter* spp. (0.8 million). The figures for *Campylobacter* are much lower than have been previously estimated,[23] and contrast with European data where a mean notification figure of 47 cases per 100 000 population has been reported[‡] although notification rates differ markedly, ranging from zero per 100 000 population in Romania to 95 per 100 000 population in the United Kingdom in 2007. In Germany the reports for 2009 totalled, 62 807 equating to 79 per 100 000 population.[§]

Human campylobacteriosis generally arises from the ingestion of contaminated foods of animal and poultry origin (or by cross-contamination from these foods), ingestion of contaminated water, and by direct contact with infected animals.[24] Whilst an important risk factor of *Campylobacter* spp. infection in industrialised countries is considered to be the consumption and handling of *Campylobacter* infected chicken meat,[25] it is well accepted that foreign travel is a major risk factor; [6] person-to person transmission is thought to be uncommon.[26] *Campylobacter* infection typically results in an acute, self-limiting gastrointestinal illness, characterised by diarrhoea, fever, and abdominal pain.[25] The incubation period is usually 2–5 days, with a range of 1–10 days, depending on dose ingested.[26]

As already stated, most infections are self-limiting although antibiotic treatment may be required in cases of prolonged or severe illness. Following diagnosis and where treatment is necessary a macrolide will be the drug of choice.[¶,27,28] Ternhag *et al.* have argued that this can prove problematical because erythromycin appears to be effective only if it is given early in the course of illness.[29] If treatment is empirical then fluoroquinolones are generally preferred as they will also be effective against *Enterobacteriaceae*[27] although it has been well documented that *Campylobacter* resistance to quinolones has emerged as an important problem.[30,31] There has been much debate as to whether quinolone resistant *Campylobacter* infection is associated with adverse human health consequences.[3,6–10] The largest study to date, from the United Kingdom,[32] found no difference in the duration of illness between cases due to quinolone- resistant *Campylobacter* strains and those due to quinolone-susceptible *Campylobacter* strains. Evans and colleagues made the point that none of these previously mentioned studies undertook any patient follow-up to examine whether quinolone-resistant strains might be associated with adverse medium-term outcomes.[33] These last-mentioned workers performed a

[‡]European Centre for Disease Prevention and Control (ECDC). Annual epidemiological report on communicable diseases in Europe 2009. Stockholm: ECDC; June 2010 (revised edition). Available from: http://www.ecdc.europa.eu/en/publications/Publications/ 0910_SUR_Annual_Epidemiological_Report_on_Communicable_Diseases_in_Europe.pdf

[§]Robert Koch Institute (RKI). SurvSTAT@RKI. [Internet]. Berlin: RKI.[Accessed 11Aug 2010]. Available from: http://www3.rki.de/SurvStat

[¶] The Sanford Guide to Antimicrobial Therapy.

Table 17.1 Ciprofloxacin resistance (Clinical Breakpoint $\geq 4\,\mu g\,mL^{-1}$) among *Campylobacter jejuni* isolates from humans, retail meats, and chickens, by year, 1998–2009 (data from NARMS).

Year		*2002*	*2003*	*2004*	*2005*	*2006*	*2007*	*2008*	*2009*
No. isolates tested	Humans	329	303	320	791	709	992	1046	1355
	Chicken Breasts	198	325	510	403	426	332	329	403
	Chickens	525	374	508	567	228	166	78	117
Ciprofloxacin Resistance (% and *n*)	Humans	20.7%	17.2%	18.1%	21.5%	19.5%	25.8%	22.4%	23.0%
		68	52	58	170	138	256	234	312
	Chicken Breasts	15.2%	14.5%	15.1%	15.1%	16.7%	17.2%	14.6%	21.1%
		30	47	77	61	71	57	48	85
	Chickens	18.6%	14.7%	21.3%	15.0%	8.8%	21.7%	32.1%	19.7%
		98	55	108	85	20	36	25	23

case-comparison study of ciprofloxacin resistant and ciprofloxacin-susceptible *Campylobacter* infection to compare disease severity, duration of illness, and medium term clinical outcomes. They found no evidence of more-severe or prolonged illness in participants with quinolone-resistant *Campylobacter* infection and no evidence of any adverse medium term consequences. As stated by Evans, this challenges the view that there is a substantial health burden associated with quinolone-resistant *Campylobacter* infection and suggests that the clinical and public health significance of quinolone resistance in *Campylobacter* infection may have been overestimated.

The issue, however, is real because it was considered of such magnitude that the US Food and Drug Administration banned the use of the fluoroquinolone, enrofloxacin, for use in poultry in 2006 because of the supposed links with fluoroquinolone resistance in *Campylobacter jejuni* infections in man. It is interesting to note that this ban seemingly has had no effect on fluoroquinolone resistance in *Campylobacter jejuni* isolated from human cases of infection nor from isolates from chickens, as detailed by the NARMS 2009 Executive Report (Table 17.1).[34]

The National Antimicrobial Resistance Monitoring System – Enteric Bacteria (NARMS) is a national public health surveillance system in the United States that tracks changes in the susceptibility of certain enteric bacteria to antimicrobial agents of human and veterinary medical importance. The NARMS program was established in 1996 as a collaboration among three federal agencies: the U.S. Food and Drug Administration (FDA), the Centers for Disease Control and Prevention (CDC), and the U.S. Department of Agriculture (USDA). NARMS monitors antimicrobial susceptibility among enteric bacteria from humans, retail meats, and food animals. Monitoring is conducted for several enteric pathogens, including *Campylobacter*. The primary objectives of NARMS are:

- To monitor trends in antimicrobial resistance among enteric bacteria from humans, retail meats, and animals.

- To disseminate timely information on antimicrobial resistance to promote interventions that reduce resistance among foodborne bacteria.
- To conduct research to better understand the emergence, persistence, and spread of antimicrobial resistance.
- To provide data that assist the FDA in making decisions related to the approval of safe and effective antimicrobial drugs for animals.

17.2.2 What Do We Know About Resistance Development in *Campylobacter*?

Bacteria acquire antibiotic resistance by mutations and by horizontal gene transfer. Mutations in the genes that code for the antibiotic targets allow bacteria to become resistant to antimicrobial activity. Horizontal gene transfer is mediated by transduction, conjugation, and natural transformation.[35] Transduction involves bacteriophage, conjugation is mediated by self-replicating plasmids requiring cell-to-cell contact and natural transformation involves the uptake of free DNA from the microbial environment, the DNA being incorporated into the recipient chromosome. Most bacteria possess at least one of the mechanisms for horizontal gene transfer.[36]

There are many publications that show transfer of resistance genes from almost all antimicrobial classes, between bacterial species within a laboratory setting,[37,38] there is much less data, however, directly supporting the transfer of resistance genes between hosts.

It has been known for many years that *Campylobacter* species develop *in vivo* resistance during fluoroquinolone therapy. This has been demonstrated in man[39,40] and in animals.[41,42] Resistance to fluoroquinolones is normally accepted as developing in one of five ways,[43] (i) due to alterations in drug permeability, (ii) drug efflux, (iii) target enzyme mechanisms, (iv) gyrase-protecting proteins and (v) plasmid mediated quinolone resistance. Until the relatively recent reports of plasmid mediated fluoroquinolone resistance, a central feature of fluoroquinolone resistance was that it develops in a step-wise fashion with changes considered to arise spontaneously within large bacterial populations after which the mutants are selectively enriched if therapeutic drug concentrations are not high enough to kill the resistant mutants. Fluoroquinolone resistance is thus characterised by gradual accumulation of mutations that result in lower intracellular drug concentration and/or sensitivity of the target DNA topoisomerases, these mutations being almost without exception chromosomal. The source of such resistance will normally be a result of errors in DNA replication and repair, and mutants will normally be present in bacterial populations containing $>10^7$ cells.[43] It becomes clear that fluoroquinolones are not primarily the cause of the mutation but they merely select for the mutation within a population thereby facilitating resistance development. More recently there have been increasing reports of plasmid mediated quinolone resistance in the *Enterobacteriaceae*, largely attributed to efflux pumps resulting in marginal increases in MIC values.[44,45]

Whilst not well documented, macrolide resistance can also develop during therapy. Lindow and colleagues recently described the first documented evidence of *in vivo* acquired macrolide resistance in a person infected with *C. jejuni*, however, it must be emphasised that this was in a controlled human infection as opposed to a natural infection.[46] There are many reasons why this type of data must be viewed with caution, indeed surveillance data shows us that macrolide resistance in *C. jejuni* in man is not an issue, suggesting that there are many barriers to resistance development in natural infections.

Selection of spontaneous mutants is different to gene transfer between bacteria. *Campylobacter* is capable of conjugation and natural transformation.[47,48] Jeon and colleagues showed that in *C. jejuni*, horizontal transfer of resistance determinants is mediated by natural transformation, but this does not play a major role in the emergence of fluoroquinolone-resistant *Campylobacter* strains during treatment with fluoroquinolone antimicrobials.[49] De Boer and others made the point that despite the wide acceptance and theoretical considerations, direct *in vivo* experimental evidence that horizontal transfer of DNA generates genetic diversity among bacteria in their natural habitat is sparse.[50] The event requires the simultaneous presence of multiple strains at a distinct niche and active mechanisms that allow DNA transfer and integration into the chromosome. These workers stated that *C. jejuni* appears to fulfil these criteria, as multiple strains are frequently isolated from the same host, and several *C. jejuni* strains have been demonstrated to be naturally competent for DNA uptake.[50] In an experimental infection model in chickens they provided direct experimental evidence for horizontal DNA transfer among *C. jejuni* strains in their natural *in vivo* habitat although this does not appear to be prevalent in natural infections. They also commented that the genome of a *C. jejuni* strain is potentially unstable and that under natural conditions, the generation of genetic diversity can be very rapid even in the absence of selective pressure. Despite the instability of the *Campylobacter* genome it has been shown that the molecular mechanisms responsible for resistance to antimicrobial classes such as fluoroquinolones, macrolides, lincosamides and tetracyclines, with rare exceptions, correlate well with the current monitoring clinical breakpoints that are used in surveillance programmes such as NARMS;[51] this will not necessarily be the case for some of the drug classes and the European surveillance programmes, for further discussion see Silley *et al.*, 2011.[17]

In an excellent review, Belanger and Shryock made the general point that the use of macrolide and lincosamide antibiotics in food animals may select for macrolide-resistant *Campylobacter*,[52] but the extent to which this selection occurs depends on the animal species and macrolide antibiotic use in question. Examples were cited showing studies where macrolide resistance increased as a result of macrolide use,[53–55] and where resistance did not change[54,56] emphasising the comprehensive body of data that has been generated, particularly in Denmark and the US as part of government-sponsored research studies since the late 1990s. Although absolute numbers vary, similar trends are seen in Denmark and the US: the prevalence of erythromycin resistance is low in the *C. jejuni* isolated from cattle and chickens and higher in the *C. coli* isolated

from swine and chickens. Belanger and Shryock comment that the most notable difference between the two countries is that the prevalence of macrolide resistance in the *C. coli* of Danish swine has declined over time presumably because of discontinued use of macrolide antibiotics as growth promoters in Denmark in 1998. This same impact is not evident for the *C. jejuni* isolated from Danish cattle and chicken where macrolide resistance has been maintained at low levels throughout the sampling period.

It is worth considering the arguments put forward by Belanger and Shryock concerning the post-harvest contamination of meat by *Campylobacter* species. The point is made that meat is contaminated with *Campylobacter* when faecal matter is inadvertently released from the intestinal tract of a slaughtered animal during processing. The likelihood of this occurring is not the same for all food animal species as intestinal *Campylobacter* loads and processing methods vary. Chickens are most likely to become contaminated with *Campylobacter* in the slaughter plant. The concentration of *Campylobacter* in the intestinal contents of the chicken may be as high as 10^9 cfu g^{-1},[57,58] and the accidental spillage of this material during mechanical evisceration may lead to significant contamination levels on the meat as will the use of common chill tanks during processing. In contrast to the situation with poultry, the concentration of *Campylobacter* in the intestinal contents of cattle and swine is much lower than that of chickens, 10^2–10^5 cfu g^{-1},[58–60] and manual evisceration, carcass washes and individual air-chilling of beef and pork carcasses effectively minimise *Campylobacter* contamination.[58,61] Belanger and Shryock go on to point out that the levels of *Campylobacter* contamination on meat in the packing plant does not necessarily reflect those that a consumer may encounter because bacterial load on the meat may decline after additional refrigeration or freezing.[58] Notwithstanding this, they show that *Campylobacter* loads on poultry are higher than those of other food animals and can be high enough to cause disease.

It is not surprising when one considers the issues addressed by Belanger and Shryock that of the risk assessments carried out to date, there is little evidence that antibiotic use in food animals gives rise to resistance that subsequently prejudices antimicrobial use in treatment of human disease. It is well established that whilst Campylobacteriosis is an important foodborne illness, antibiotic treatment is usually not required. When treatment is called for, erythromycin, a macrolide antibiotic, is often recommended for the treatment of severe cases. Hurd and Malladi assessed the risk that food animal use of macrolides will lead to resistant infections in man and compromised human treatment.[62] They used a retrospective approach; estimating the number of campylobacteriosis cases caused by specific meat consumption utilising the preventable fraction and then determined the number of cases with macrolide resistant *Campylobacter* spp. based on a linear model relating the resistance fraction to on-farm macrolide use. In the publication of the risk analysis they considered the uncertainties in the parameter estimates, utilised an elaborate model of resistance development and separated *C. coli* and *C. jejuni*. As there are no published data for the probability of compromised treatment outcomes

due to macrolide resistance, they used estimates of compromised treatment outcomes based on data for fluoroquinolone-resistant infections. The conservative results showed the human health risks to be extremely low. For example, the predicted risk of suboptimal human treatment of infection with *C. coli* from swine was only 1 in 82 million; with a 95% chance it could be as high as 1 in 49 million. Risks from *C. jejuni* in poultry or beef were even less. It has already been argued that Denmark has a rich data set. In 2006, macrolides were withdrawn from the list of antibiotics recommended for veterinary treatment of diarrhoea in Danish pigs. The motive was to lower the antibiotic consumption in general and to mitigate the risk related to human infection with macrolide-resistant *Campylobacter*. Alban and co-workers subsequently conducted a risk assessment following international guidelines to address the risk for human health associated with usage of macrolides in Danish pigs.[63] The conclusions were consistent with those reported by Hurd and Malladi[62] and the authors stated, "In general, human cases of campylobacteriosis are self-limiting, and it is questionable whether there is any excess risk related to infection with macrolide resistant *Campylobacter* compared to sensitive *Campylobacter*. In conclusion, the risk associated with veterinary use of macrolides in Danish pigs for the human health of Danes seemed to be low". Other macrolide risk assessments have come to similar conclusions,[64,65] as have risk assessments for fluoroquinolones[64,66] and for virginiamycin.[67] A recent review from the Netherlands on quinolone resistant *Campylobacter* and other resistant bacteria in the food chain has concluded that "there are no indications that the disease burden has increased as a consequence of quinolone resistance and the healthcare costs are similar to those for susceptible *Campylobacter* infections".[68]

The conclusion can be drawn that resistance issues arising from use of antimicrobials in veterinary medicine have limited impact upon resistance in human medicine. It is, however, beyond doubt that food-producing animals can act as a reservoir for antimicrobial drug-resistant genes but there is little direct evidence that this reservoir serves as a source for the transfer of these genes to bacteria causing infection in man. Indeed, Mather *et al*. have examined long-term surveillance data on antimicrobial resistance, not in *Campylobacter*, but in another important zoonotic organism, *Salmonella typhimurium* DT104 (DT104) isolates from concurrently sampled and sympatric human and animal populations in Scotland.[69] Using novel ecological and epidemiological approaches to examine diversity, and phenotypic and temporal relatedness of the resistance profiles they showed that the ecological diversity of resistance phenotypes was significantly greater in human than in animal isolates. They concluded that, while ecologically connected, animals and humans have distinguishable DT104 communities, differing in prevalence, linkage and diversity. Furthermore, they inferred that the sympatric animal population is unlikely to be the major source of resistance diversity for humans. This suggests that current policy emphasis on restricting antimicrobial use in domestic animals may be overly simplistic. This is of course a separate issue to the *Salmonella* themselves that clearly do cause zoonotic infection in man.

The conclusions above are not made in isolation. Following a request from the European Commission, the European Food Safety Authority (EFSA) Panel on Biological Hazards was asked to deliver a scientific opinion on *Campylobacter* in broiler meat production, looking at control options and performance objectives and/or targets at different stages of the food chain. EFSA commissioned the development of a quantitative microbiological risk assessment (QMRA) model which has been used to estimate the impact on human campylobacteriosis due to the presence of *Campylobacter* species in broiler meat. *C. jejuni* and *C. coli* were considered equivalent for the purpose of risk assessment in this opinion because there is no information on variability between these two species with respect to their behaviour in the food chain, impact of interventions or virulence for humans. The report made the important point that there are no indications that *Campylobacter* strains with antimicrobial resistance behave differently in the food chain than their sensitive counterparts. The report stated that handling, preparation and consumption of broiler meat may account for 20% to 30% of human cases of campylobacteriosis, while 50% to 80% may be attributed to the chicken reservoir as a whole (broilers as well as laying hens). The transmission routes from chickens to humans, other than handling, preparation and consumption of broiler meat, are not well understood, and related public health benefits cannot currently be quantified. The public health benefits of controlling *Campylobacter* in primary broiler production are expected to be greater than control later in the chain as the bacteria may also spread from farms to humans by other pathways than broiler meat. There is, however, very little information about these pathways and quantifying the impact of interventions at farm level was only done for broiler meat-related cases. Strict implementation of biosecurity in primary production and of GMP/HACCP during slaughtering is expected to reduce the level of colonization of broilers with *Campylobacter*, and the contamination level of carcasses and meat from colonised flocks. The effects of such implementation cannot be quantified because they depend on many interrelated local factors. Nevertheless, their impact on public health risk reduction may be considerable.

When considering antimicrobial resistance data and the drivers behind resistance development, be they residue or therapeutic concentrations of drug, we must be mindful of the findings reported by Sommer and colleagues who have functionally characterised the resistance reservoir in the microbial flora of healthy individuals.[70,71] Most of the resistance genes they identified using culture-independent sampling have not been previously identified and are evolutionarily distant from known resistance genes. By contrast, nearly half of the resistance genes they identified in cultured aerobic human gut isolates were identical to resistance genes harboured by major pathogens. They considered that the immense diversity of resistance genes already present in the human microbiome could contribute to future emergence of antibiotic resistance in human pathogens. They also argued that the data clearly demonstrates that the antibiotic resistance reservoir of the large fraction of the human

microbiome recalcitrant to culturing is severely under sampled; more important, and this is probably the most significant issue, is that the data suggests that barriers exist to lateral gene transfer between these bacteria and readily cultured human pathogens, otherwise these newly identified novel resistance genes would be prevalent within the cultured bacterial population. This is not the case and must give us some confidence that there are many, as yet undefined, mechanisms that protect bacterial pathogens from taking up even more resistance genes, be they selected by residue or therapeutic concentrations of drug.

17.3 Acceptable Daily Intake of Antimicrobial Residues

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); this 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 is necessary to determine a toxicological, pharmacological and microbiological ADI (see Chapter 3). In recent years, there has become an increasing awareness of the potential impact of residues on the gastrointestinal flora, this was reviewed in 2007[72] and will be brought up to date in this chapter. The impact of therapeutic antimicrobials on human gut flora is well established.[73–76] 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 formation and work of VICH, a trilateral (EU–Japan–USA) programme aimed at harmonising technical requirements for veterinary product registration has been a welcome development in recent years. 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;

(1) 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 a wider international harmonisation.
(2) 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.

(3) 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. 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.

The VICH Task Force recognized 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.[77–79] 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.[80] This flora provides important functions to the host playing an important role in maintaining human health by preventing colonization by pathogens, degrading dietary and *in situ* produced compounds, producing nutrients and shaping and maintaining the normal mucosal immunity. It is also widely accepted that ingested antimicrobial drugs can potentially alter the ecology of the intestinal flora reaching the colon because of incomplete absorption or being absorbed, circulated and excreted *via* bile or secreted through the intestinal mucosa.[73,74] 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 colonization 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 which were previously sensitive or to a relative increase in the proportion of organisms that are already less sensitive to the drug.

The current guideline is as an attempt to address the complexity of the human intestinal flora[79] and reduce uncertainty when determining microbiological ADIs; it is not, however, the purpose of this chapter to review the ecology of the human gastro-intestinal 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 article of Cerniglia and Kotarski.[81]

The guideline requires the determination of two distinct microbiological ADI values, the essence of which is summarized in the five steps outlined below.

17.3.1 Steps in Determining the Need for a Microbiological ADI

When determining the need for a microbiological ADI, the following sequence of steps is recommended. The data may be obtained experimentally from the published literature or other sources.

Step 1

Are residues of the drug, and (or) its metabolites, microbiologically active against representatives of the human intestinal flora?

Recommended data:

a. Minimum inhibitory concentration (MIC) data from the following relevant genera of intestinal bacteria (*Escherichia coli*, and species of *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Enterococcus*, *Eubacterium* (*Collinsella*), *Fusobacterium*, *Lactobacillus*, *Peptostreptococcus*/*Peptococcus*).
b. It is recognized that the understanding of the relative importance of these micro-organisms is incomplete and that the taxonomic status of these organisms can change. The selection of organisms 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:

a. Absorption, distribution, metabolism, excretion, 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. 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:

a. 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 colon content of animals.

If the answer to any of 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 colonization 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 no-adverse effect concentration/no-adverse effect levels (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 colonization 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.

17.3.2 How the Data are Handled – Colonization Barrier

The guideline is relatively new and only came into effect in Europe and the USA in May 2005 and there are few antimicrobial compounds that have been fully evaluated for which a microbiological ADI has been agreed and for which the data is in the public domain. In an attempt to understand how the microbiological data is handled, examples of typical data will be taken from old data that is in the public domain. Step 1 requires the determination of MIC data

Table 17.2 MIC_{50} of enrofloxacin against bacterial species of human intestinal origin at an inoculum level of 10^7 cfu mL^{-1} (from JECFA, 1997).

Genus	*n*	$MIC_{50}/\mu g\ ml^{-1}$
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

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 17.2.[72]

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 there any residual antimicrobial activity remains after residue concentrations of antimicrobial compound have interacted with the human digesta. Currently there is no such data in the public domain but data is currently under review for a range of classes of antimicrobials and 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%. This data is sufficient to allow the calculation of the ADI_{micro} for colonization 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, this seems somewhat out of line with the objectives of VICH.

17.3.3 Calculations

The ADI with respect to disruption of the colonization barrier is calculated according to the formula detailed in Guideline CVMP/VICH/467/03-FINAL:[83]

$$\mathrm{ADI}(\mu g/kg^{-1}\ bw) = \frac{MIC_{calc} \times \text{mass of colonic contents}}{\text{Fraction oral dose available} \times \text{weight of human}}$$

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:

$$\text{lower } 90\%\text{CL} = \text{Mean } MIC_{50} - \frac{\text{StdDev}}{\sqrt{n}} \times t_{0.10.df}$$

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 17.2 suggests 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 $32\,\mu g\,mL^{-1}$ 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 being used in veterinary medicine. The following MIC_{50} values can be used to determine MIC_{calc}.

Bacteroides fragilis $4\,\mu g\,mL^{-1}$; Other *Bacteroides* spp. $4\,\mu g\,mL^{-1}$; *Bifidobacterium* spp. $0.25\,\mu g\,mL^{-1}$; *Clostridium* spp. $0.125\,\mu g\,mL^{-1}$; *Enterococcus* spp. $2\,\mu g\,mL^{-1}$; *Escherichia coli* $4\,\mu g\,mL^{-1}$; *Eubacterium* spp. $0.5\,\mu g\,mL^{-1}$; *Fusobacterium* spp. $0.5\,\mu g\,mL^{-1}$; *Lactobacillus* spp. $32\,\mu g\,mL^{-1}$; *Peptostreptococcus* spp. $2\,\mu g\,mL^{-1}$.

From this input data, the MIC_{calc} can be calculated to be $0.74\,\mu g\,mL^{-1}$ for "Superkill". This value will subsequently be used to calculate the microbiological ADI with respect to disruption of the colonization barrier.

It is accepted in regulatory circles that the mass of colon contents is agreed to be 220 g and the weight of a human is 60 kg. The fraction of oral dose available is described in the Guideline, "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^{-1} and fraction available as 5% then the microbiological ADI with respect to the colonization barrier can be calculated.

$MIC_{calc} = 0.74\ \mu g\, mL^{-1}$
Fraction available = 0.05
The equation constants are: Mass of colonic contents = 220 g
Weight of human = 60 kg

$$ADI = \frac{0.74 \times 220}{0.05 \times 60} = 54.27\ \mu g\, kg^{-1} bw$$
$$= 3.26\ mg\ per\ 60\ kg\ person$$

17.3.4 How the Data are Handled – Resistance Development

It is important that before any work commences with regard to resistance development that there is agreement as to which group 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 faecal samples.*"

One question that arises is whether the normal human intestinal 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[72] 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.

17.3.5 Guideline Revision

VICH GL 36 has been implemented since 2005 to address the impact of antimicrobial drugs on the microorganisms in the human gastrointestinal tract. Having gained experience in working with the guideline, regulators from all VICH regions considered that additional guidance and clarity were needed regarding *in vivo* and *in vitro* testing methods to determine the fraction of the oral dose available to microorganisms. The endpoint of interest was the microbiological activity of bioavailable drug. As a result of extensive discussion by a VICH Expert Working Group, additional guidance has been added, as Appendix D, to the original document.[82] The VICH Expert Working Group reviewed new data generated specifically to help provide clarity. The group also reviewed data in the scientific literature and information from disclosed sponsor submissions. Some of these data have subsequently been published.[83] As a result, the Appendix contains three sections: a table of examples of test systems, methodological aspects of their implementation and a description of how they could be applied for determining the fraction of an oral dose available to microorganisms. In essence, the Expert Working Group called for additional chemical data as an input factor to validate the microbiological data, despite the fact that the objective of this guidance document is to determine a microbiological ADI. Concerns were expressed that a microbiological assay methodology was not sufficiently sensitive; whilst it is clear that a chemical assay has major advantages in this respect, it is debateable whether such an approach is necessary. If a microbiological assay cannot determine a microbiological effect then it may be assumed that there is not a microbiological issue arising from the said residue. It is accepted of course that the challenge is whether the chosen microbiological assay has been appropriately validated. The flow chart summarising how Appendix D might work in practice is shown in Figure 17.1.

17.4 Concluding Thoughts

If we are to properly understand the risk factors associated with antibiotic use, arising directly from therapeutic or indirectly through residue concentrations, the two of course being related, it is fundamental that we have accurate data capturing the type, amounts and duration of antibiotics used in respective settings. Unfortunately, as pointed out by Halpern, exact quantities of antibiotics used in human and animal medicine are not known and estimates vary significantly with the source of the data.[84] Ideally, for antimicrobial-use data to have relevance to resistance-development patterns, these data should be recorded on the farm, along with the indication for treatment, the route of administration, the dose and duration and other relevant data, such as prevailing disease patterns and incidence. Only when such data are provided can

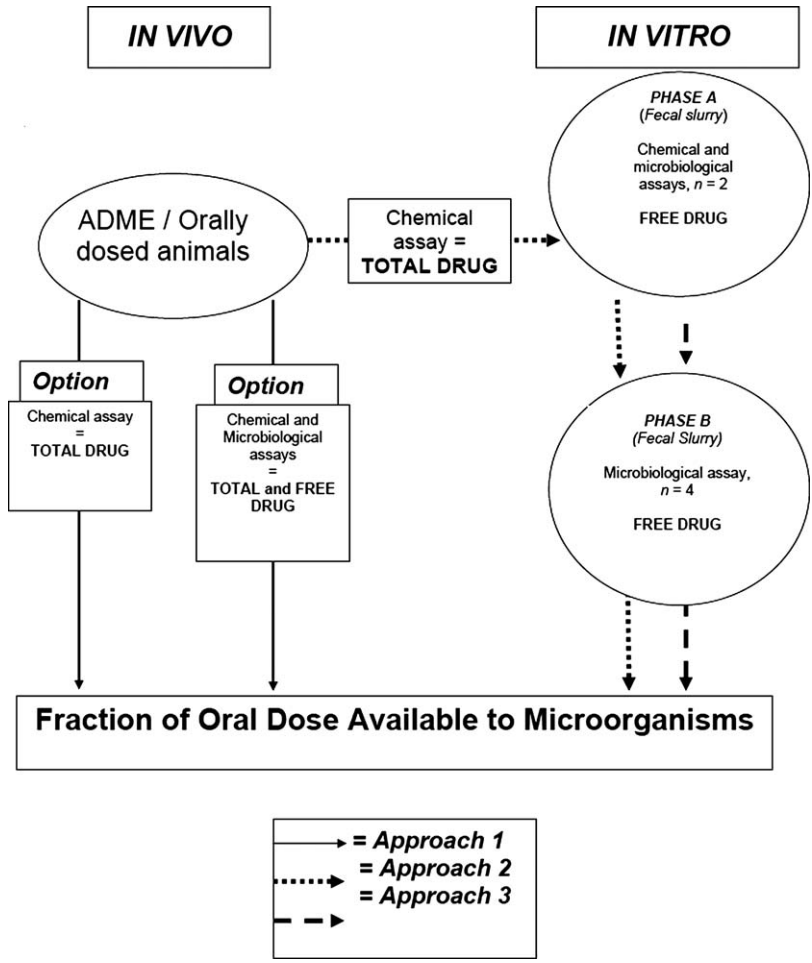

Figure 17.1 Schematic representation of test systems to determine the fraction of oral dose available to microorganisms (from VICH GL 36).

information on the use of antimicrobials be used to assess cause and effect with any great accuracy. However, the collection of such data is understood to be a challenge because of the resources it would require. If data on antimicrobial use could be collected in a consistent manner so helping interpretation of resistance monitoring and resistance development these data could then be used as some of the inputs for science-based risk assessment before considering risk management options.

Even then there are challenges, as pointed out by Halpern, as once the risk assessment model is chosen and the data used for analysis is reliable and robust, there must be agreement about the quantity and quality of factors to be considered. Halpern argues that a simplistic model that allows only for the

consideration of human health risks cannot provide an understanding of the overall impact of antibiotic use in food animals. For an adequate understanding, variables affecting human and animal risk and benefits must be considered. The health status of food animals destined to enter the human food supply chain is an important, although often overlooked factor in predicting the risk of human foodborne infections,[1] and without antibiotic use this will be compromised. Halpern raises the challenge as to whether the risk of increased microbial carcass contamination is greater than the risk of exposure to a smaller number of potentially resistant pathogens as a question that must be captured in the analysis and considered by policy-makers.[84]

I am in full agreement with Halpern's conclusion that the preservation of the effectiveness of antibiotics is essential to protect the health of animals and humans and that insufficient evidence currently exists to support prohibitions on the use of antibiotics in food animals. The advantages provided to both human and animal populations from continued use of antibiotics in food animals outweigh the minimal risk to humans currently documented. I similarly believe that there is a lack of documented evidence that antimicrobial residues significantly contribute to adverse human health outcomes.

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